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

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Pine wilt disease, caused by the pinewood nematode (PWN) *Bursaphelenchus xylophilus*, was first detected in Portugal in 1999, being the first report of the presence of this disease in a native conifer within the European Union. In Portugal the PWN is spread by an insect vector - *Monochamus galloprovincialis* - and, once infected, most plants cease resin production and show symptoms of needle chlorosis, usually dying in just a few months. In this work we aimed at determining if *Picea abies* and *Cupressus lusitânica* are susceptible species to the PWN.

One year old seedlings were maintained under controlled conditions at 80% humidity and 8h(26°C) light/16h(24°C) darkness cycles. *B. xylophilus* (HF strain) was grown on barley seeds with *Botrytis cinerea* at 26°C in the dark. Fifteen seedlings of each plant species were inoculated with 1800 nematodes in a sterile water suspension and monitored during 14 days. Fifteen control seedlings of each plant species were mock inoculated with sterile water. Several parameters were evaluated at 1, 7 and 14 days post inoculation: external symptoms appearance, chlorophyll content, nematode quantification, total phenolics and lignin content. Also, the morphology of both species was visualized with electron microscopy and compared with the morphology of the known PWN susceptible host (*Pinus pinaster*).

It was found that 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. Both in control and infected 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. In inoculated plants of both species nematodes could be found in the stem 1, 7 and 14 days post inoculation. Nematodes were more concentrated in the inoculation site, than above and below that area. P. abies presented a larger amount of nematodes than C. lusitânica, probably because is a member of the Pinaceae family, and so is best related morphological and physiologically to pine species (the preferred host for the PWN). This similarity of morphological characteristics between P. abies and P. pinaster was confirmed by visualizing stems of these species under electron microscopy. After quantification of total phenolics, no significant differences were found between control and inoculated plants of either species. However, differences were found in lignin content between control and inoculated plants. Further investigation on activation of defenserelated genes is under way.

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