The role of induced defenses in *Pinus* spp. against the pine wood nematode (*Bursaphelenchus xylophilus*)

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Introduction

The pine wilt disease (PWD) is caused by the nematode *Bursaphelenchus xylophilus* and affects mainly *Pinus pinaster* trees, leading to the cut down of thousands of symptomatic and asymptomatic trees every year, imposing great economical and environmental challenges. So far, no effective solution has been developed.

Jasmonic acid is a plant hormone involved in various physiological processes such as growth, photosynthesis, disease signaling and induced resistance. It has been demonstrated that its synthetic derivative methyl jasmonate (MeJa) can be used to stimulate the defense mechanisms of several plant species, including conifers, protecting plants against a variety of herbivores and pathogens.

The aim of this work was to evaluate whether the exogenous application of MeJa could enhance the defense mechanisms of four susceptible pine species against the pine wood nematode (PWN).

Methods

- 60 two-year old plants (*P. pinaster*, *P. pinea*, *P. radiata* and *P. sylvestris*) were kept under natural light and temperature conditions and watered every two days (Fig. 1).
- 0, 25 or 50 mM MeJa were sprayed on the foliage of 20 plants of each species.
- *B. xylophilus* 65 GO strain was grown on barley seeds with *Botrytis cinerea* (Fig. 2) at 26 ºC, in the dark, and extracted using Baermann funnel technique (Fig. 3).
- 15 plants of each species and MeJa treatment were inoculated with ca. 1000 nematodes and 5 were inoculated with water as control (Fig. 4).
- Two months after inoculation, plants were harvested and the size of the nematode population, stem water, soluble phenolics and lignin contents and total chlorophyll were analyzed.


Fig. 1


Fig. 2


Fig. 3


Fig. 4

Results

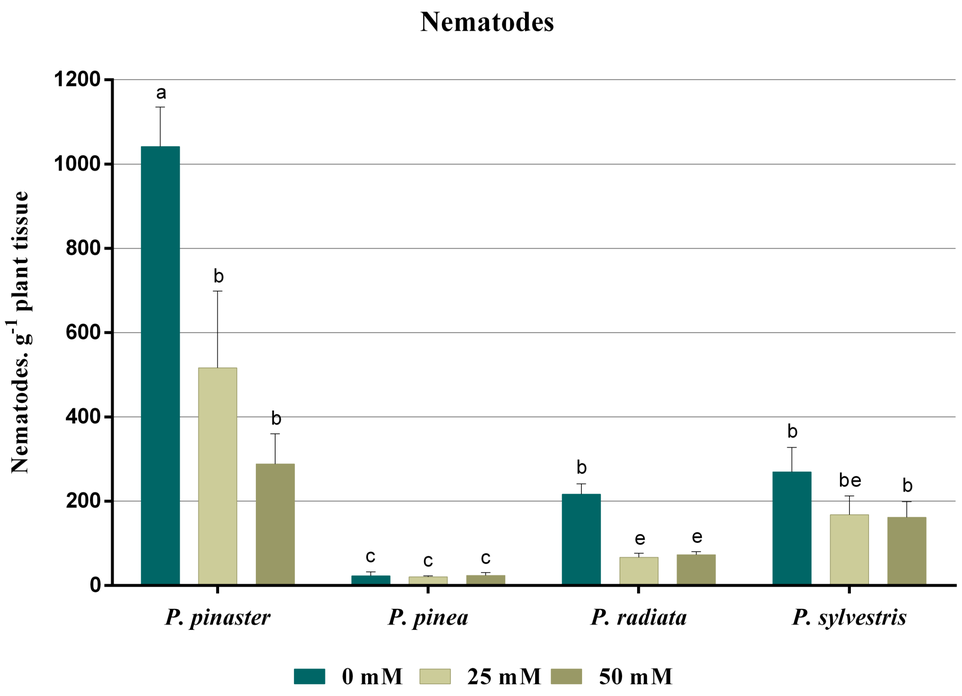

Figure 5 – Number of nematodes per gram of stem tissue in *P. pinaster*, *P. pinea*, *P. radiata* and *P. sylvestris* plants treated with 0, 25 or 50 mM MeJa, 2 months after inoculation . Vertical bars represent the standard error (n = 10). Bars with the same letter are not significantly different ($p < 0.05$, Students' T-test).

- The nematode population (Fig. 5) was significantly lower in *P. pinaster* and *P. radiata* plants treated with both MeJa concentrations.
- Water loss (Fig. 6A) and chlorophyll degradation (Fig. 6B) were significantly reduced in *P. pinaster* plants treated with MeJa.
- Total soluble phenolics (Fig. 6C) and lignin (Fig. 6D) biosynthesis is very distinct between the four species, especially after MeJa treatment.
- *P. pinaster* and *P. radiata* plants treated with 25 mM MeJa showed increased soluble phenolics biosynthesis and reduced lignin concentration (Fig. 6D) in stems, especially in PWN-inoculated plants.

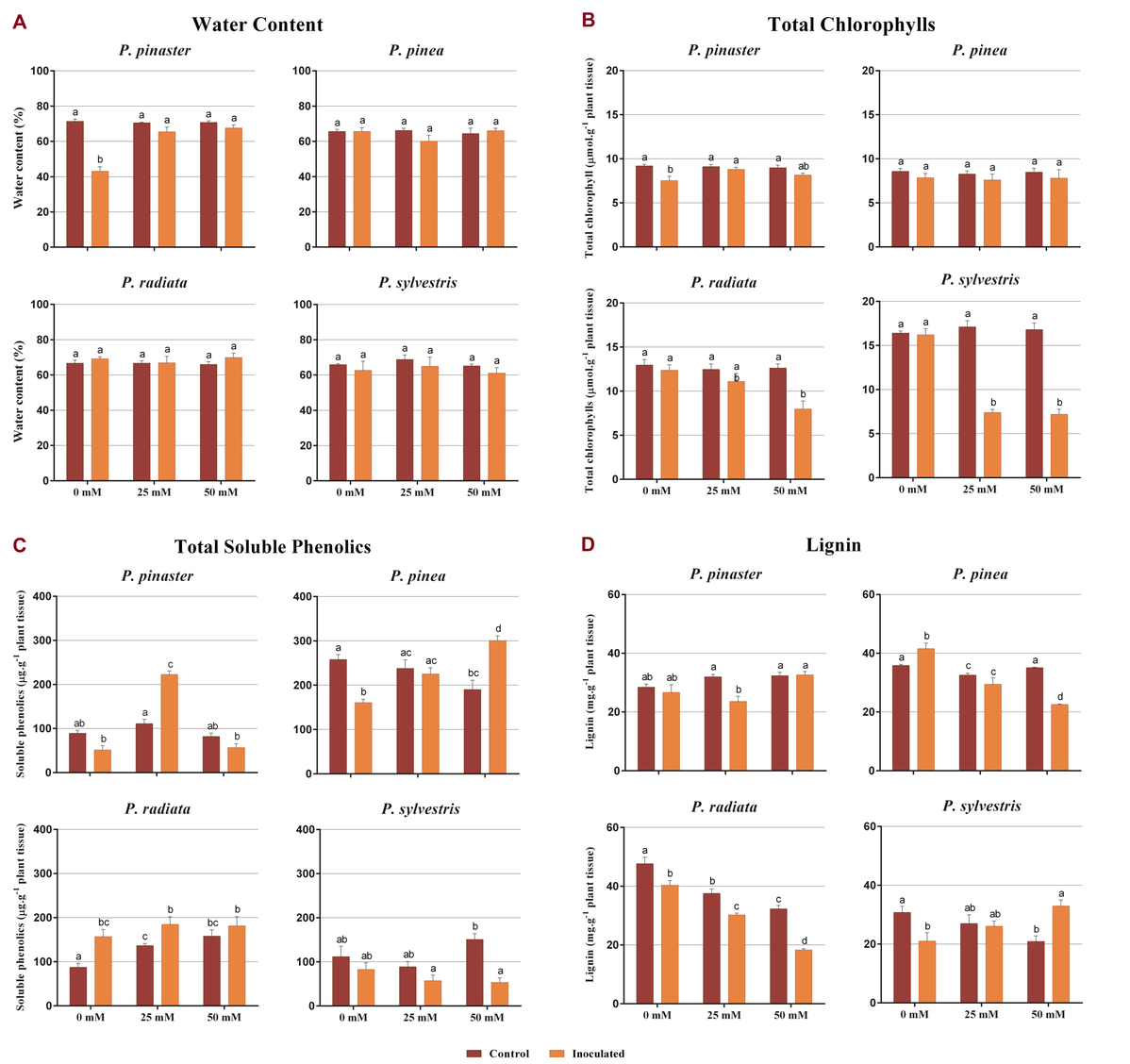

Figure 6 – Water content (A), total chlorophyll (B), total soluble phenolics (C) and lignin (D) in *P. pinaster*, *P. pinea*, *P. radiata* and *P. sylvestris* inoculated and control plants treated with 0, 25 or 50 mM MeJa, 2 months after inoculation . Vertical bars represent the standard error (n = 5). Bars with the same letter are not significantly different ($p < 0.05$, Students' T-test).

Conclusions

Foliar application of MeJa seems to impair PWN reproduction rate, especially in *P. pinaster* trees, which are the most susceptible species to the disease. In addition, MeJa treatment prevented water loss in stems and total chlorophyll decrease in needles in *P. pinaster* infected plants, proving to be protective against the development of the disease. In general, MeJa mobilized cell wall-bound phenolics into their soluble form in infected plants, probably to improve plants defense mechanisms. The induction of *Pinus* spp. immune system through exogenous application of MeJa could be a valuable strategy to control PWD. Further research will involve genomic and metabolomics analysis of MeJa treated plants.

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