

## Pinewood nematode induced changes to the photosynthetic pigment levels of *in vitro* pine shoots

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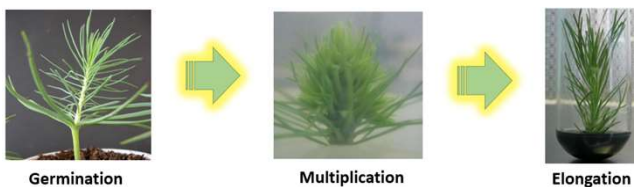
### INTRODUCTION

The pinewood nematode (PWN) *Bursaphelenchus xylophilus* causes ecological damages to pine forests, leading to high economic losses. PWN infection damages pine vasculature and resin canals leading to affected photosynthesis and promoting the accumulation of reactive oxygen species (ROS).

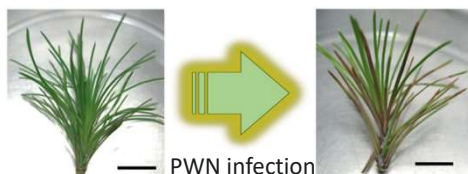
Tracing fine biochemical changes is hindered by the changeability of natural conditions and pine genetic variability. *In vitro* cultures offer a reliable alternative to analyse biochemical changes associated with PWN infection. In this preliminary study chlorophyll, carotenoids and anthocyanin were quantified through spectrophotometry on *in vitro* pine shoots infected with the PWN.

### METHODS

- Seeds were surface sterilized with **ethanol (96%)** and germinated.
- Seedling shoots were transferred to **Schenk and Hildebrandt (SH)** medium supplemented with 0.1 mg/L **IBA** and 0.5 mg/L **BAP** for **multiplication** [1].



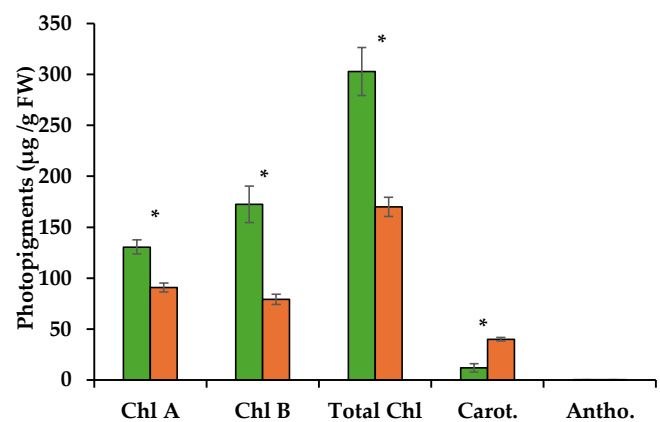
- Microshoots were transferred to SH medium with 3 g/L **activated charcoal**, for shoot **elongation**.
- Ca. 5 cm long shoots were selected for infection with 100 sterilized PWNs and infection progression was followed for 30 days.



- Infected and uninfected shoots were ground in liquid nitrogen.
- 100 mg of macerate were added to 1 mL of methanol, kept for 48 h at -20 °C, centrifuged at 12000 x g, and the supernatant collected.
- Absorbance was read at 665.2, 652.4, 600, 536 and 470 nm [2,3]

### RESULTS & DISCUSSION

Uninfected *P. pinaster* microshoots (**green**) were compared to PWN infected *in vitro* microshoots (**orange**)



Infection with the PWN resulted in:

**40** µg /g FW less **chlorophyll a** (Chl a),

**93** µg /g FW less **chlorophyll b** (Chl b),

**133** µg /g FW less **total chlorophyll** (total Chl),

**28** µg /g FW more **carotenoids**, and

**0.03** µmol /g FW less **anthocyanins**

Photopigments	PWN
Chlorophyll A	31 % decrease (statistically significant)
Chlorophyll B	54 % decrease (statistically significant)
Total Chlorophyll	44 % decrease (statistically significant)
Carotenoids	70 % increase (statistically significant)
Anthocyanins	9 % decrease (not statistically significant)

### CONCLUSION

- PWN infection influences photosynthesis by decreasing photosynthetic pigments in *P. pinaster* microshoots
- Increase in carotenoids and low influence on anthocyanins suggests PWN-induced oxidative stress

### REFERENCES

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- Hodges *et al.* 1999, doi:10.1007/s004250050524