Pine wilt disease (PWD) caused by pinewood nematode (PWN) is considered one of the most important threats to European coniferous forests. Some Pinus spp., P. sylvestris and Pinus pinaster among them, are the most susceptible hosts. However, resistance mechanisms against PWN are still unclear. In Spain five new B. xylophilus outbreaks have been reported in 2019.

This study aim to:
- Determine interspecific variation of constitutive compounds levels among groups and species, on three tissues (needles, stem bark-phloem, and stem xylem) and their relationships with nematode multiplication and mortality.

**INTRODUCTION**

Two-year-old seedlings of seven pine species were inoculated with B. xylophilus, and three different groups were established: non-susceptible (P. canariensis, P. taeda, P. halepensis, and P. pinea); susceptible (P. pinaster, P. radiata), and highly-susceptible (P. sylvestris).

Chemical compound data were obtained for needles, branches, stem xylem and stem bark tissues from trees harvested prior to inoculation. Water content, condensed tannins, total polyphenols, lipid-soluble substances, macronutrients (N, P, K, Ca, and Mg) and some micronutrient (Fe and Mn) levels were determined as described Menéndez-Gutirrez et al. (2018). Soluble carbohydrate and starch analysis, non-described before, were determined as Chow and Landhanssier (2004) and Dubois (1956), with some modification. Sugars were extracted in ultraturrax with (E10H2O: H2O) (80:20), centrifuged, and soluble carbohydrates were analyzed in extract by Dubois method as glucose, after ethanol was eliminated in rotary evaporator. Residue contained starch was hydrolyzed with H2SO4, and then, colorimetrically analyzed in the same manner as soluble carbohydrates. Results were expressed in mg glucose, g−1 lyophilized tissue.

Data analysis

Differences among susceptibility groups for the studied constitutive chemical compounds on three different tissues (needles, stem bark and phloem), and stem xylem were analyzed using a nested ANOVA following the model: X = μi + Ri + SPPj + cijkl, Where R (group of susceptibility), with three levels (NS=non-to slightly susceptible (P. canariensis, P. pinea, P. halepensis and P. taeda), S= susceptibility (P. pinaster and P. radiata), and HS=highly susceptible (P. sylvestris); SPP (species, nested to R, with seven species. Different letters in every tissue shows significant differences among susceptibility groups.

**RESULTS**

Spearman’s correlation among LS Means values of seedling mortality (M), wilting symptom development (W), median number of nematodes (NEM) and nematode multiplication; Higher P on the three tissues was also correlated with less water and more Nitrogen, Potassium, Iron, and starch than the other groups.

Needles of non-susceptible group had significantly less water and more Nitrogen, Phosphorus, Magnesium, and starch and less Potassium, Calcium, Iron, total polyphenols, condensed tannins and liposoluble substances than the highly susceptible group.

Xylem of non-susceptible group had more Nitrogen, Phosphorus, Magnesium, total polyphenols, and starch than the other groups.

Higher levels of constitutive N and/or starch in any tissue was related to less mortality and nematode multiplication; Higher P on the three tissues was also correlated with less nematode multiplication. Moreover, liposoluble substances, soluble carbohydrates and condensed tannins concentrations on the needles were negatively correlated with nematode multiplication. On the contrary, needles water and K were positively correlated with mortality and nematode invasion.

**CONCLUSIONS**

- Needles of non-susceptible group had significantly less water and more Nitrogen, Potassium, Iron, and starch than the other groups.
- Cortex + phloem of non-susceptible group had more Nitrogen, Phosphorus, Manganese, and starch and less Potassium, Calcium, Iron, total polyphenols, condensed tannins and liposoluble substances than the highly susceptible group.
- Xylem of non-susceptible group had more Nitrogen, Phosphorus, Magnesium, Manganese, total polyphenols, and starch than the other groups.
- Higher levels of constitutive N and/or starch in any tissue was related to less mortality and nematode multiplication; Higher P on the three tissues was also correlated with less nematode multiplication. Moreover, liposoluble substances, soluble carbohydrates and condensed tannins concentrations on the needles were negatively correlated with nematode multiplication. On the contrary, needles water and K were positively correlated with mortality and nematode invasion.

**MATERIALS AND METHODS**

Non-parametric Spearman correlation among average values of constitutive chemical compounds by species (n=7) and later wilt, mortality and nematode invasion were performed on every tissue. Principal components analysis of constitutive chemical compounds on different seedling tissues were performed.

Principal components analysis of constitutive chemical compounds on different seedling tissues.

**References**