Constitutive chemical compounds in different tissues of seven pine species and their relationship with susceptibility to pine-wood nematode (*Bursaphelenchus xylophilus*).

Margarita Alonso Santos, María Menéndez-Gutiérrez, Raquel Díaz Vazquez.

Centro de Investigación Forestal de Lourizán. Dirección Xeral de Ordenación Forestal. Consellería do Medio Rural. Xunta de Galicia. Ctra Marín, km 4. 36153 Pontevedra.

email: margarita.alonso.santos@xunta.gal

XUNTA DE GALICIA
CONSELLERÍA DO MEDIO RURAL
AGACAL
Axencia Galega
da Calidade Alimentaria
Centro de Investigación Forestal de Lourizán

INTRODUCTION

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.

MATERIALS AND METHODS

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

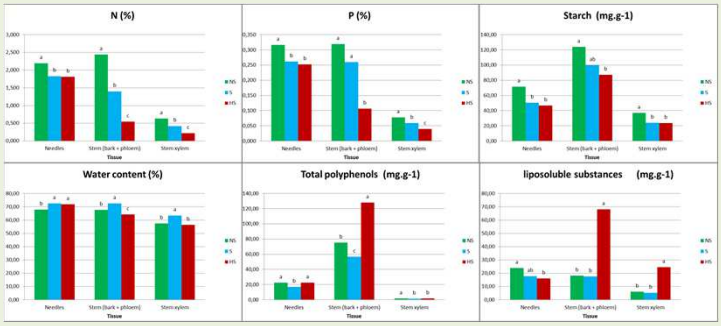
Chemical compound data were obtained for needles, branches, stem xylem and stem bark tissues from trees harvested prior to inoculation. Water content, condensed tannins, total polyphenols, lipid-soluble substances, macronutrients (N, P, K, Ca, and Mg) and some micronutrient (Fe and Mn) levels were determined as described Menéndez-Gutiérrez et al. (2018). Soluble carbohydrate and starch analysis, non-described before, were determined as Chow and Landhansser (2004) and Dubois (1956), with some modification. Sugars were extracted in ultraturrax with (EtOH: H_2O) (80:20) (v : v), 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 H_2SO_4 5N, and then, colorimetrically analyzed in the same manner as soluble carbohydrates. Results were expressed in mg glucose. g^{-1} lyophilized tissue.

Data analysis

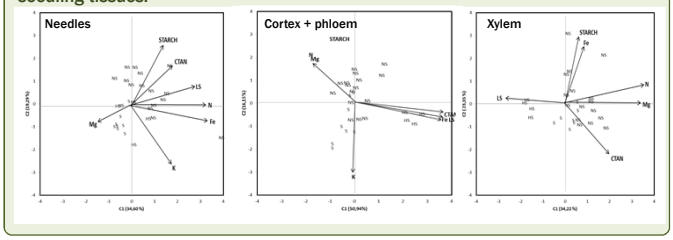
Differences among susceptibility groups for the studied constitutive chemical compounds on three different tissues (needles, stem (bark and phloem), and stem xylem) were analyzed using a nested ANOVA following the model: $X = \mu_i + R_j + SPP_{k(j)} + \xi_{l(ijk)}$, where R (group of susceptibility), 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, mentioned before. Duncan test was performed to determine differences among groups of susceptibility to every chemical compound analyzed in different tissues. Non-parametric Spearman correlation between 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.

RESULTS

Graphics shows the most remarkable results obtained from nested ANOVA : $X = \mu_i + R_j + SPP_{k(j)} + \xi_{l(ijk)}$, R (group of susceptibility), with three levels (NS= non-to slightly susceptible (*P. canariensis*, *P pinea*, *P. halepensis* and *P. taeda*), S= susceptible (*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.



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



Spearman's correlation among LS Means values of seedling mortality (M), wilting symptom development (W), median number of nematodes (NEM) recovered at four dates (13, 28, 42 and 142 days after inoculation (DAI)) and on different tissues (total plant, root, wood) and constitutive chemical compounds on needles, phloem+cortex and xylem (N: nitrogen, %; P: phosphorus, %; K: potassium, %; Ca: calcium, %; Mg: magnesium, %; Fe: iron, ppm; Mn: manganese, ppm; LS: lipid-soluble substances, mg·g^{-1}; TPOL: total polyphenols, mg·g^{-1}; CTAN: condensed tannins, mg·g^{-1}; SCARB: soluble carbohydrates, mg·g^{-1}; STARCH, mg·g^{-1}).

		M	W	NEM_{plant}	NEM_{root}	NEM_{wood}	NEM_{plant}	NEM_{root}	NEM_{wood}	NEM_{plant}	NEM_{root}	NEM_{wood}	NEM_{plant}	NEM_{root}	NEM_{wood}
Dai post inoculation					13			28			42			142	
Needles	WC	0,852*	0,929**	ns	ns	ns	0,821*	0,768*	0,714[a]	ns	0,704*	ns	0,757*	ns	0,757*
	N	-0,852*	-0,821*	ns	ns	ns	-0,786*	ns	-0,679[a]	ns	ns	ns	-0,757*	ns	-0,757*
	P	ns	-0,75[a]	ns	ns	-0,75[a]	-,821**	-0,67[a]	ns	ns	ns	ns	-0,757**	ns	-0,757**
	K	,852**	0,571	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Ca	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Mg	ns	ns	ns	ns	ns	0,679[a]	ns	ns	ns	ns	ns	ns	ns	ns
	Fe	-0,704*	-0,786**	ns	ns	ns	-0,929**	ns	-0,929**	-0,786**	-0,704*	ns	ns	ns	ns
	Mn	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	LS	-0,741*	-0,679[a]			-0,679[a]	-0,857**	ns	-0,75[a]	-0,857**	-0,815**	-0,685[a]	-0,802**	ns	-0,802**
	TPOL	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	CTAN	ns	-0,75[a]	ns	ns	ns	-0,75a	ns	-0,786**	ns	ns	ns	ns	ns	ns
	SCARB	-0,704*	-0,786**	ns	ns	ns	-0,786**	ns	-0,893**	-0,786**	-0,852**	-0,811**	ns	ns	ns
	STARCH	-,852**	-0,714*	ns	ns	ns	ns	ns	-0,679[a]	-0,714*	-0,852**	-0,703*	ns	ns	ns
Cortex + Phloem	WC	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	N	-0,778**	-0,929**	ns	ns	ns	-0,929**	-0,67[a]	-0,929**	ns	ns	ns	ns	ns	ns
	P	ns	-0,857**	ns	ns	ns	-0,857**	-0,867**	-0,714	ns	ns	ns	ns	ns	ns
	K	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Ca	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Mg	ns	ns	ns	ns	ns	-0,821**	-0,867**	ns	ns	ns	ns	ns	ns	ns
	Fe	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Mn	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	LS	0,741*	ns	ns	ns	ns	ns	0,67[a]	ns	ns	ns	ns	ns	ns	ns
	TPOL	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	CTAN	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	SCARB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	STARCH	-,778**	ns	ns	ns	ns	-0,75[a]	ns	-0,75[a]	-,821**	-,778**	ns	ns	ns	ns
Xylem	WC	ns	ns	ns	ns	ns	ns	ns	ns	0,714*	ns	ns	ns	ns	ns
	N	-,778**	-,929**	ns	ns	ns	-,929**	-0,67[a]	-,929**	ns	ns	ns	ns	ns	ns
	P	-,767**	-,955**	ns	ns	-0,414	-,955**	-,865**	-,811**	ns	ns	ns	-,809**	ns	-,809**
	K	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Ca	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Mg	ns	-0,714	ns	ns	ns	-0,75[a]	ns	-,821**	ns	ns	ns	ns	ns	ns
	Fe	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Mn	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	LS	ns	ns	ns	ns	ns	0,729*	ns	ns	ns	ns	ns	ns	ns	ns
	TPOL	-,778**	-,857**	ns	ns	ns	-0,75[a]	ns	-0,75[a]	ns	-,778**	-0,721*	-0,679[a]	ns	ns
	CTAN	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	SCARB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	STARCH	-,889**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-,757**	ns	-,757**

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).
a Correlation is significant at the 0.1 level (2-tailed).
N=7

CONCLUSIONS

- Needles of non-susceptible group had significantly less water and more Nitrogen, Potassium, Iron, and starch than the others 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.

References:

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