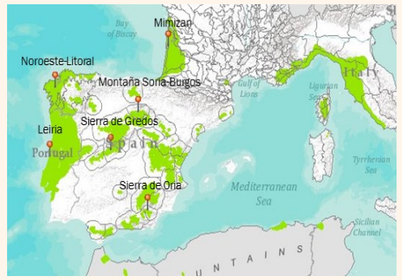
2. TEST ON *P. Pinaster* PROVENANCES

Objective:

After identifying maritime pine as a species susceptible to PWN, and given the great genetic variability of the species, we studied variation in susceptibility to PWN among five *Pinus pinaster* provenances (four Spanish, one Portuguese) of the Iberian Peninsula and one French provenance, to determine the degree of susceptibility of the Galician population of maritime pine compared to other provenances.



Main results:

The provenances with the most damage, and therefore the greatest mortality, were precisely those in which PWN is present: the Portuguese provenance of Leiria and the coastal Galician 1a Northwest-Litoral provenance. This indicates that the Galician forest stands could be significantly affected by Pine Wilt Disease if damage in natural conditions resembles what occurred with seedlings in the greenhouse.

3. TEST ON FAMILIES IN THE *P. pinaster* AND *P. radiata* GENETIC IMPROVEMENT PROGRAMS AT LOURIZÁN FOREST RESEARCH CENTER

Objective:

These tests evaluated genetic variation in susceptibility among families included in the Galician breeding programs for maritime pine and insignis pines. The aim of these tests was to determine if tolerance to PWN could be included as a new selection trait in the breeding programs, as this constitutes a primary means of fighting the disease in the countries most affected by it.

Ninety-one maritime pine families and 55 insignis pine families were evaluated to date.

Main results:

Some maritime pine families had notably greater stability and resistance to PWN, with moderately high heritability values. Definitive results for insignis pine have not yet been obtained, but everything points to the existence of some resistant or tolerant families that are stable over time.

If these results are confirmed in the field, it may be possible to obtain resistant or tolerant plants for future reforestation in Galicia.

INDITEX Centro de Investigación Forestal LOURIZÁN

INGACAL INSTITUTO GALEGO DA CALIDADE ALIMENTARIA

galicia

CONCLUSIONS

P. sylvestris, *P. pinaster* and *P. radiata* forest stands in Spain could be severely affected by Pine Wilt Disease in places where the climate favors its development.

Conventional measures to control and manage the PWD act on the main organisms involved in its development, the vector, the pathogen and the host, but have not proved sufficient to control the disease to date.

Some of the best solutions for protecting forest stands against PWD involve the selection and production of resistant or tolerant pines. Our results show genetic variation among the families included in the Galician breeding program for maritime pine, as well as high estimates of family heritability for wilting and mortality. With these results in mind, and as we wait for confirmation of similar results for insignis pine, tolerance to PWN will be included as a new trait in the breeding programs for both species.

D.L.: C 2018 – 2017

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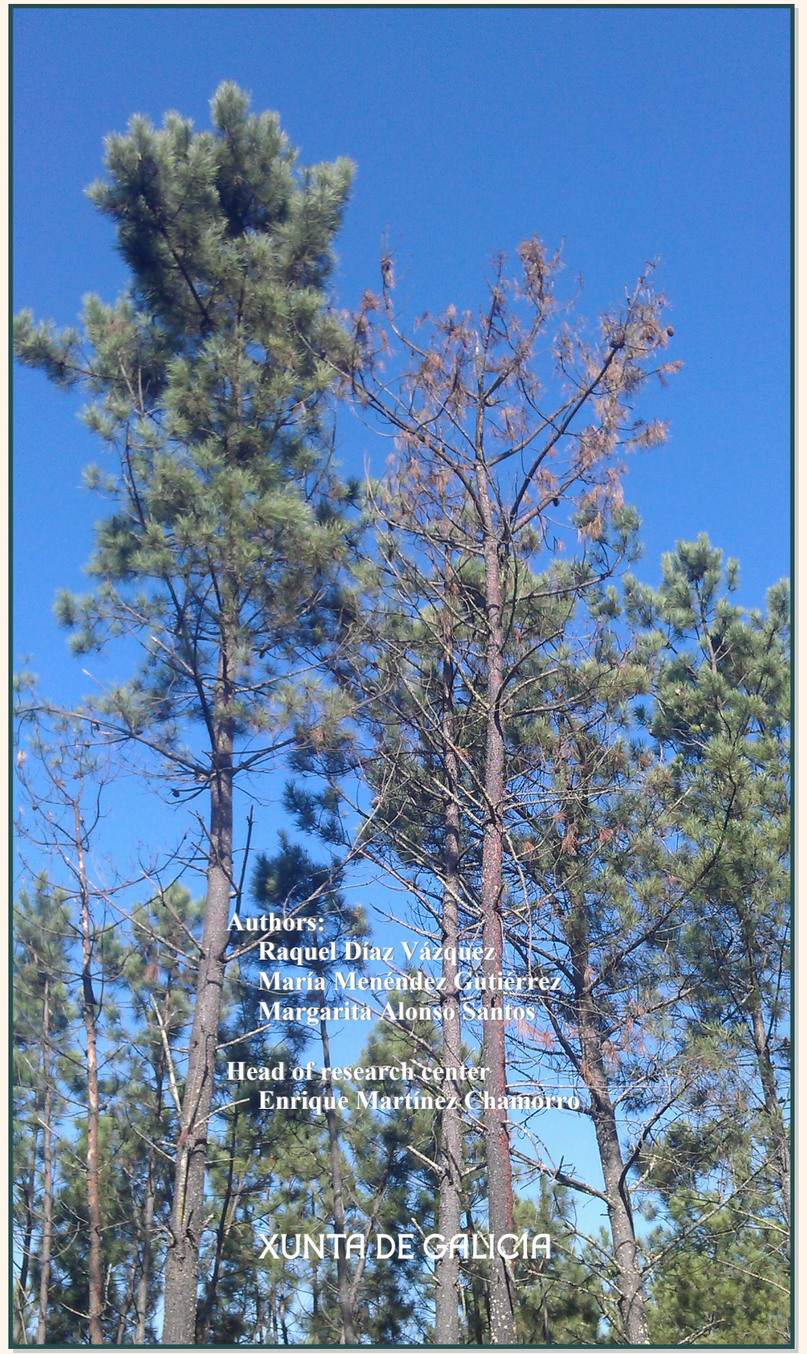
Contact: Centro de Investigación Forestal de Lourizán
Estda. De Marín, km. 3,5. 36153 Pontevedra
Teléfono: 986 805000
www.lourizán.xunta.gal

XUNTA DE GALICIA

GENETIC BREEDING AGAINST PINE WOOD NEMATODE (*Bursaphelenchus xylophilus*) IN THE LOURIZÁN FOREST RESEARCH CENTER



Authors:
Raquel Díaz Vázquez
María Menéndez Gutiérrez
Margarita Alonso Santos

Head of research center
Enrique Martínez Chamorro

XUNTA DE GALICIA

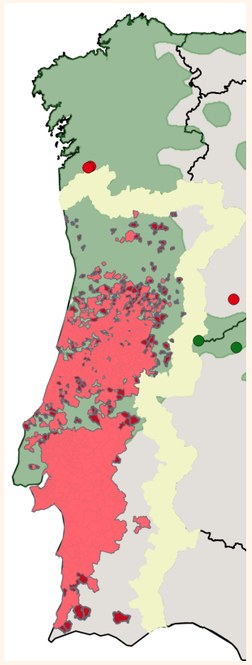
THE PINE WOOD NEMATODE (*Bursaphelenchus xylophilus*)

Pine wood nematode, *Bursaphelenchus xylophilus* (PWN), is the causal agent of Pine Wilt Disease (PWD) and affects many conifers by provoking wilting and death.

Native to North America, this organism has created heavy economic and ecological damage in the countries where it was introduced (Japan, China, Korea, Taiwan and Portugal). In Spain, the first outbreak was declared in 2008, and to date only four other foci have been declared. The infection has already been eradicated in two of the five sites. Though the disease seems to be evolving slowly in Spain, it is important to note that in the countries most affected by this disease, evolution was slow at first but caused enormous damage a few years later. In Korea, for example, the disease remained in the Busan area for 8 years following the first positive report. Soon after, it was detected more than 80 km away and currently it advances at a rate of 14 km per year (Kyeong-Hak, 2016). In Portugal, PWD was initially detected in the Setúbal Peninsula and remained there for 9 years before appearing 200 km away. Today, all of Portugal has been declared demarcated area and more than 50 million euro have been spent on controlling it (Bonifácio, 2016).

Affected countries are using diverse control strategies to fight against PWD (primarily through chemical or biological treatment of the affected stands and the vector), though to date none of them has been able to completely stop the disease. Since the 1980s, countries like Japan have invested strongly in genetic breeding and have found tree individuals of Asian pine tree species that are tolerant to the disease. In recent years, hundreds of thousands of tolerant seedlings have been produced for reforestation there (Hoshi, 2016).

The problem in Galicia



There is a clear risk of PWD propagation from Portugal and/or the active Spanish foci. Currently, the nematode is spread throughout virtually all of Portugal and is advancing rapidly. In fact, De la Fuente et al. (submitted) have forecast that the disease will begin to spread naturally through Spain in 2022. Maritime pine (*Pinus pinaster*) and insignis pine (*Pinus radiata*) occupy extensive land in Galicia and have high ecological and economic value (over 3 million m^3 harvested in 2015; CONFEMADERA, 2015) and have demonstrated susceptibility to *B. xylophilus* in forests. In light of these realities, we should prepare ourselves for what could be a serious threat to the sector.

GENETIC BREEDING AGAINST PINE WOOD NEMATODE in the Lourizán Forest Research Center

With the aim of controlling pine wilt disease, the Lourizán Forest Research Center began work to adapt its facilities in 2011 and started doing genetic breeding research against PWN in 2013. The objective is to develop maritime and insignis pine plants that are resistant or tolerant to the disease.

Because it is a quarantine organism, research must be done under strict safety conditions. The greenhouse was fitted with a HDPE storage tank for cleaning water, a double entry door, drip irrigation, and 2 x 2 mm light mesh in the ventilation systems.

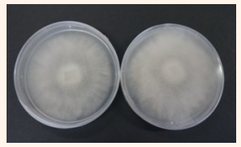
General research lines related to pine wood nematode

- Evaluate the development of PWD and variation in susceptibility to this disease among different *Pinus* species and several maritime pine provenances.
- Study the genetic variation of the tree families in the Galician breeding programs of maritime and insignis pines, for selection and improvement of PWN-tolerant material.
- Determine the chemical, physiological, and morphological factors involved in the tolerance mechanisms of different host trees against PWN attack, to prevent both the development of the disease and the death of the host.

Test procedures

A. ARTIFICIAL *B. xylophilus* INOCULATION IN SEEDLINGS

The *B. xylophilus* nematode is reared and maintained on the *Botrytis cinerea* fungus, which was cultivated in PDA (Potato Dextrose Agar) medium at 25ºC in dark conditions.



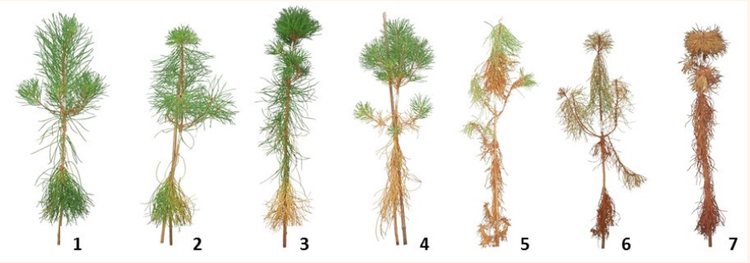
The inoculation procedure consists of the following steps:

- Elimination of needles and sterilization of the inoculation area.
- Making a 2-cm longitudinal cut and eliminating the bark to expose the cortex .

- The wound is covered by a strip of bandage and parafilm® is used to form a funnel around the wound.
- The bandage is saturated with the nematode suspension and parafilm® funnel is closed.

B. MEASUREMENTS

- Periodic evaluation of wilting symptoms using a 7-level scale (1, healthy plant; 7, dead plant).



- Measurement of morphological variables (height, diameter, number of branches).
- Measurement of hydric potential to evaluate hydric status.
- Measurement of chlorophyll fluorescence to determine damage to the photosynthetic apparatus.
- Analysis of chemical compounds to determine their relation to PWD resistance or tolerance.
- Quantification of nematodes extracted from the plants.

Research done at CIF Lourizán

1. TEST ON SPECIES OF THE *Pinus* GENUS

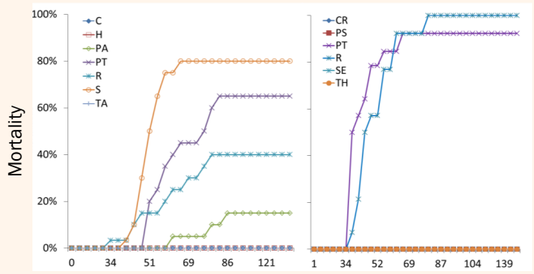
Objetive:

To determine the degree to which pine species could be affected by PWN in Spain, we studied interspecific variation in susceptibility to PWN in seven pine species of particular importance in Spain: *P. canariensis, P. halepensis, P. pinaster, P. pinea, P. sylvestris, P. radiata* and *P. taeda*. Additionally, we evaluated other conifer species (*Pseudotsuga menziesii, Thuja plicata, Sequoia sempervirens,* and *Cryptomeria japonica*) which might be considered as alternative species for future plantations due to their forestry value.

Main results:

Classification of species based on their susceptibility:

- Susceptible: *P. sylvestris* (highly susceptible), *P. pinaster* and *P. radiata*
- Slightly susceptible: *P. pinea*
- Tolerant: *P. taeda, P. halepensis, P. canariensis, T. plicata, S. sempervirens and C. japonica*



Notably, the nematodes could migrate from the point of inoculation to the roots in all species; however, they only multiplied in susceptible species.