Migration and multiplication of pathogenic *Bursaphelenchus xylophilus* isolates of diverse geographic origins

XUNTA DE GALICIA
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AGACAL
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LOURIZÁN

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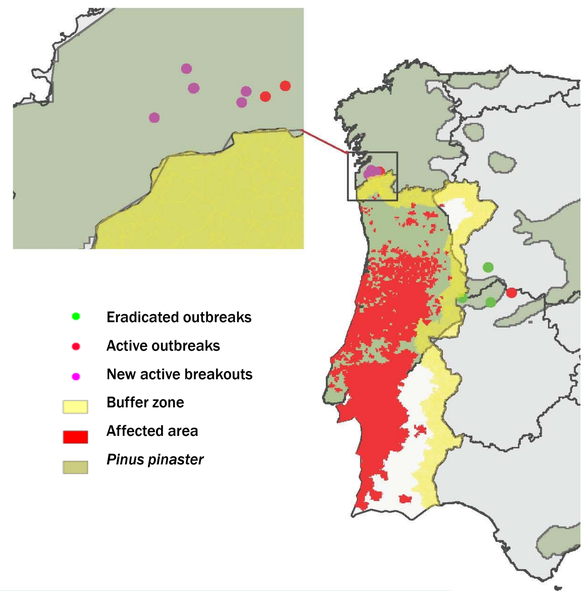


INTRODUCTION

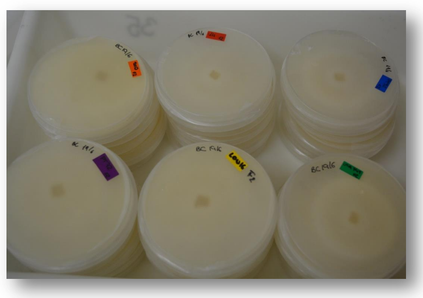
The occurrence of new recent *B. xylophilus* outbreaks in Spain and the adverse disease expansion forecasts require a rapid advance in genetic breeding against this pathogen (Ikegami and Jenkins 2018; de la Fuente et al., 2018). The optimum for breeding more PWD resistant trees is to use the most virulent isolates in the inoculation assays (Akiba et al., 2012).

This study aims to:
- Determine differences in virulence among 7 isolates of different geographic origins.
- Determine the most virulent isolate to be use used in the controlled assays for breeding tolerant or resistant genotypes.

MATERIALS AND METHODS

B. xylophilus isolates → Experiments → Inoculation → Rearing → Nematode quantification

Pt52T, Pt72CH — Portugal
USA745 — United States
Ka4, S10 — Japan
SpPO1, SpSA 1 — Spain



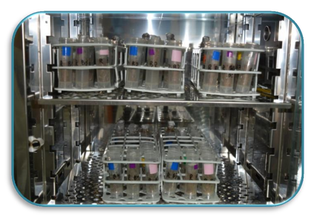
1 Reproduction on *Botrytis cinerea*: Cultured on 25 ml PDA medium for 4 days

Inoculum dose: 1000 Bx/50 µl. 18 replicas per isolate

7 days at 25°C in the dark

2 Reproduction in branch sections: One-year-old *P. pinaster* and *P. radiata* branches

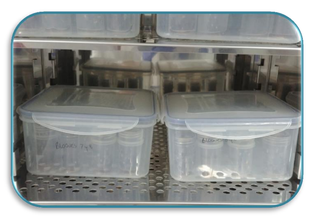
Inoculum dose: 1000 Bx/50 µl. 9 replicas per isolate and species.



10 days at 25°C in the dark

3 Migration in branch sections: Two-year old *P. pinaster* branches

Inoculum dose: 200 Bx/50 µl. 10 replicas per isolate



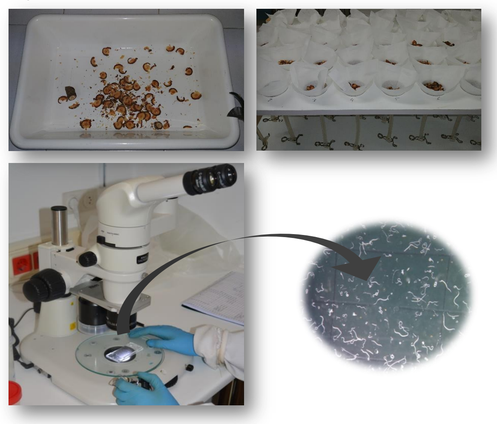
24 h at 25°C in the dark

4 Inoculation into pine seedlings: Two-year old *P. pinaster* and *P. radiata* seedlings

Inoculum dose: 3000 Bx/300 µl. **Randomized block design:** 33 blocks, 2 treatments and 2 species.

109 days, mean day tª 27.7°C

Agar dishes, branch sections and seedlings sectioned into 1-2 mm thick pieces were placed in funnels over 48 h at 25°C for nematode extraction and quantification.

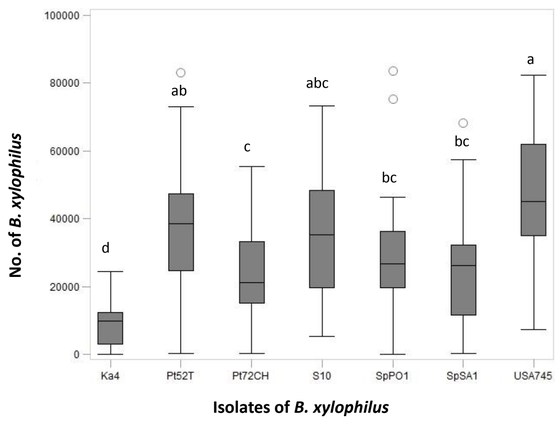


In the seedling inoculation assay external wilting symptoms were also assessed from the onset of the symptoms until the end of the experiment, using a seven-level scale.

RESULTS

1. BX REPRODUCTION ON FUNGAL MAT

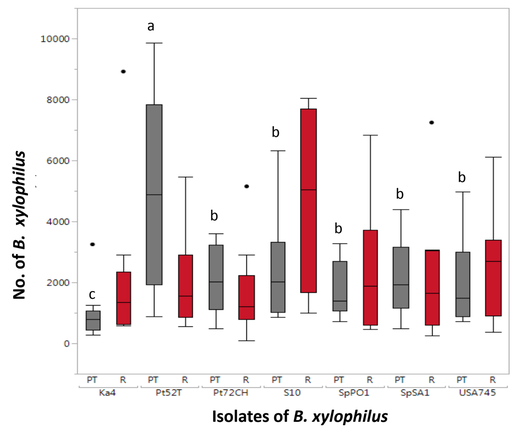
Nematode multiplication occurred in all isolates after 7 days on a culture of *B. cinerea* in PDA.



The number of nematodes differed significantly among *B. xylophilus* isolates (χ^2 = 42.34 p <0.0001).
Nematode multiplication was greater for the isolate USA745, and the isolate Ka4 showed the lowest nematode multiplication per Petri dish.

2. BX REPRODUCTION IN BRANCH SECTIONS

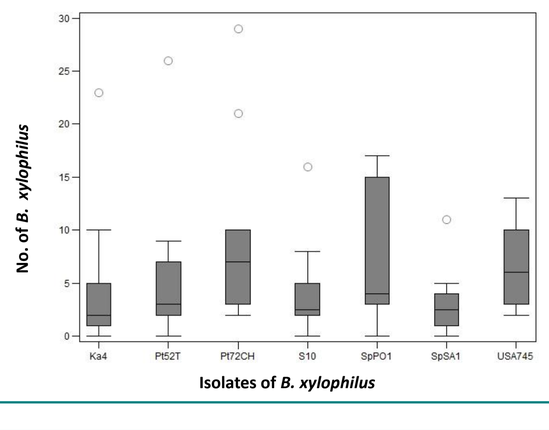
Nematodes of all isolates multiplied in *P. radiata* and *P. pinaster* branch sections after 10 days.



The number of nematodes multiplied in *P. pinaster* branch sections was significantly different among isolates (χ^2 = 17.80 p <0.0068), but not in *P. radiata* (χ^2 = 6.94 p <0.64).
The isolate with the significantly higher multiplication was Pt52T, whereas Ka4 showed the lowest multiplication.

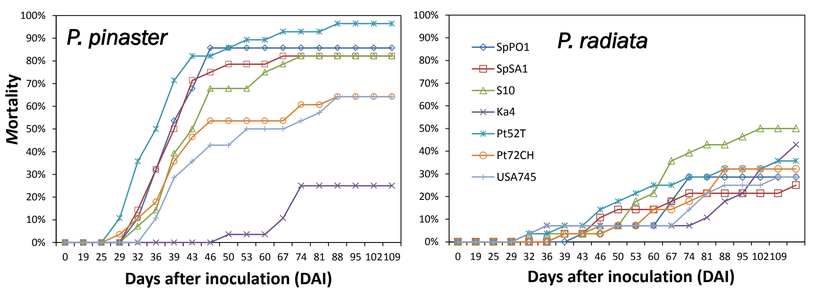
3. BX MIGRATION IN BRANCH SECTIONS

Isolates did not significantly differ in the number of nematodes passing through *P. pinaster* branch sections.



4. BX INOCULATION INTO PINE SEEDLINGS

P. pinaster and *P. radiata* seedlings were susceptible to all *B. xylophilus* isolates inoculated. Mean mortality and wilting symptoms were lower for *P. radiata* seedlings.



Differences among species (p <0.0001), isolates (p <0.001) and their interaction (p <0.0001) were found for mortality and wilting symptoms. The Spanish isolate SpPO1 and the Portuguese Pt52T caused significantly higher mortality in *P. pinaster* than Pt72CH, USA745 and Ka4. However, *P. radiata* seedlings inoculated with the Japanese isolate S10 had significantly higher mortality than the two Spanish isolates and the USA745 isolate.

CONCLUSIONS

- ❑ Significant differences among the virulent *B. xylophilus* isolates of different origins were detected.
- ❑ The virulence classification of the studied isolates was not the same for *P. pinaster* and *P. radiata.*
- ❑ The significant correlation found between the number of nematodes multiplied in branch sections and the virulence level established by the *P. pinaster* seedling inoculation test will allow a faster and a time-saving method for virulence evaluation of new isolates.
- ❑ The isolates Pt52T and SpPO1 were the most virulent ones for *P. pinaster* so any of them should be used as the “test isolate” for future assays when searching for resistant or tolerance genotypes.