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

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INTRODUCTION

The occurrence of new recent B. xylophilus outbreaks in Spain and 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 (Akibe 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 used in the controlled assays for breeding tolerant or resistant genotypes.

MATERIALS AND METHODS

B. xylophilus isolates

<table>
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<th>Experiments</th>
<th>Inoculation</th>
<th>Rearing</th>
<th>Nematode quantification</th>
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<tr>
<td>1 Reproduction on B. cinerea: Cultured on 25 ml PDA medium for 4 days.</td>
<td>Inoculum dose: 1000 Bx/50 µl. 18 replicates per isolate</td>
<td>7 days at 25°C in the dark</td>
<td>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.</td>
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<tr>
<td>2 Reproduction in branch sections: One-year-old P. pinaster and P. radiata branches</td>
<td>Inoculum dose: 1000 Bx/50 µl. 9 replicates per isolate and species.</td>
<td>10 days at 25°C in the dark</td>
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<td>3 Migration in branch sections: Two-year-old P. pinaster branches</td>
<td>Inoculum dose: 200 Bx/50 µl. 10 replicates per isolate</td>
<td>24 h at 25°C in the dark</td>
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<td>4 Inoculation into pine seedlings: Two-year-old P. pinaster and P. radiata seedlings</td>
<td>Inoculum dose: 3000 Bx/500 µl. Randomized block design: 13 blocks, 2 treatments and 2 species.</td>
<td>109 days, mean day 0² 27.7°C</td>
<td>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.</td>
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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 ($\chi^2 = 42.34$ p < 0.0001). Nematode multiplication was greater for the isolate USA745, and the isolate K4 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 ($\chi^2 = 17.80$ p < 0.0068), but not in P. radiata ($\chi^2 = 6.94$ 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 K4. 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.