

# The pinewood nematode, *Bursaphelenchus xylophilus*, in Portugal: possible introductions and spread routes of a serious biological invasion revealed by molecular methods

Vera VALADAS<sup>1</sup>, Marta LARANJO<sup>2,3</sup>, Pedro BARBOSA<sup>1</sup>, Margarida ESPADA<sup>1</sup>,  
Manuel MOTA<sup>1,\*</sup> and Solange OLIVEIRA<sup>2</sup>

<sup>1</sup> NemaLab-ICAAM & Departamento de Biologia, Universidade de Évora, Évora, Portugal

<sup>2</sup> Lab. Microbiologia do Solo-ICAAM & Departamento de Biologia, Universidade de Évora, Évora, Portugal

<sup>3</sup> IIFA (Instituto de Investigação e Formação Avançada), Universidade de Évora, Évora, Portugal

Received: 15 December 2012; revised: 14 February 2012

Accepted for publication: 17 February 2012

**Summary** – The pine wood nematode (PWN), *Bursaphelenchus xylophilus*, the causal agent of pine wilt disease (PWD), is a major world-wide pathogen and pest of pine, with impacts on forest health, natural ecosystem stability and international trade. In Portugal, PWN was first diagnosed in 1999, the first occurrence also for Europe. The disease was recently detected on the island of Madeira and in northern Spain. In an attempt to search for more reliable and robust molecular markers that enable the study of intraspecific variability of *B. xylophilus* from different geographic locations, the intergenic spacer (IGS) region of the 5S rRNA gene and inter-simple sequence repeats (ISSR) analysis were used to determine the genetic relationships among 43 *B. xylophilus* isolates from Portugal, China, Japan, South Korea and USA. IGS sequence analysis showed that this region can only be used to establish interspecific relationships, since no differences were detected among Portuguese isolates from different geographic locations. Fingerprints obtained with ISSR show high genetic variability among Portuguese isolates, except for the ones obtained prior to 2008. The ISSR dendrogram suggests the spread of the disease inside continental Portugal and to Madeira. Until 2008, *B. xylophilus* populations found in continental Portugal showed low genetic diversity, pointing to a single introduction, probably from Asia, whereas recent populations from continental Portugal (2009–2010) and Madeira show high genetic diversity, suggesting multiple introductions from different origins.

**Keywords** – genetic diversity, IGS ISSR, intraspecific variability, ITS, molecular marker, pine wilt disease.

The pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934; Fuchs, 1937), is indigenous to North America and is widespread in natural coniferous forests in Canada and USA (Sutherland & Peterson, 1999). At present, *B. xylophilus* is considered one of the most important pests and pathogens in the world (Webster & Mota, 2008). The general fear of establishment of PWN, the causal agent of pine wilt disease (PWD), into countries where conifer forests assume great importance, stems from the devastating damage caused by this nematode to pine forests (Mamiya, 2004; Shin & Han, 2006). The introduction of PWN into non-native areas (outside of North America) is primarily associated with trade and the global flow of forest products (Bergdahl & Halik, 1999; Webster, 2004). Non-manufactured wood, especially in raw log form, has been identified as one of

the most high-risk pathways of biological invasions, carrying forest insects and pathogens into new environments (Evans *et al.*, 1996; Tkacz, 2002). Many *Bursaphelenchus* species, including the PWN, have been routinely intercepted in packaging and wood products in several countries, *e.g.*, Austria (Tomiczek *et al.*, 2003), China (Gu *et al.*, 2006), Finland (Tomminen *et al.*, 1991) and Germany (Braasch *et al.*, 2001). Furthermore, recent detection of PWN in packaging wood imported from countries considered free of this pest, due to the repeated use and circulation of this type of wood material, *e.g.*, Brazil, Belgium, Italy and Spain (Gu *et al.*, 2006), undoubtedly underlines the importance of trade globalisation for the potential entry/establishment of this pathogen into endemic forests worldwide.

\* Corresponding author, e-mail: mmota@uevora.pt