

The results obtained from the Madeira isolates suggest a high genetic diversity of *B. xylophilus* population on the island. Isolates BxMad4SV and BxMad3F are distinct from all other isolates and show low similarity values, which suggests new introductions in the island. The same insect vector, *M. galloprovincialis*, is known to occur on the island for more than 15 years, at least since 1996, but possibly much longer (Erber & Aguiar, 1996). This could explain the easy spread of the nematode, unnoticed for several years until its recent detection (Fonseca *et al.*, 2010). The other isolate from Madeira, BxMad22C, groups with isolate BxPt74SCD with a similarity of 82%, suggesting that at least part of the Madeira population is related to the mainland population.

Previous studies on *B. xylophilus* populations have shown intraspecific variability between isolates from different geographic origins using ISSR (Metge & Burgermeister, 2006), revealing a significant degree of genetic divergence according to geographic distribution. Two major clusters were identified: one including the North American isolates (Canada and USA) displaying a high level of genetic diversity, and a second cluster including Asian and Portuguese isolates at a lower genetic diversity level. Another study, using AFLP, suggested that China was affected directly from North America or from neighbouring countries such as Japan (Cheng *et al.*, 2008). Other studies with Korean isolates using microsatellites and AFLP (Jung *et al.*, 2010a, b), suggest that the high genetic diversity found among some Korean populations is due to the gene flow between China, Japan and Korea. The present results support previous reports (Metge & Burgermeister, 2006; Vieira *et al.*, 2007), namely IGS- and ISSR-based grouping of most Portuguese isolates with Asian isolates.

Until now, little information about *B. xylophilus* markers has been available, but the recent release of the entire genome sequence of *B. xylophilus* (Kikuchi *et al.*, 2011) will be a challenge and opportunity to discover novel suitable markers to study intraspecific variability, determine pathways of disease spread and follow the evolution of introduced isolates. The increase in global trade has an important impact on natural ecosystems, making the invasion of *B. xylophilus* a serious ecological and economic problem. *Bursaphelenchus xylophilus*, in particular, damages conifers (*Pinus* spp.) making pine wilt disease one of the most serious problems of forests worldwide. The understanding of the disease and its spread will be of paramount importance to draw up new measures to avoid the dissemination of PWN to other countries. Future re-

search on *B. xylophilus* must continue to follow the evolution and spread of this biological invader in pine forests.

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