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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, the causal agent of pine wilt disease (PWD), is a serious pest and pathogen of forest tree species. It was first reported from Japan in 1915, where it became the major ecological catastrophe of pine forests. In 1999, the PWN was first detected in the European Union (EU), in Portugal, and immmediately initiated several government (national and EU) actions to assess the extent of the nematode's presence, and to contain *B. xylophilus* and its insect vector (*Monochamus galloprovincialis*) to an area with a 30km radius in the Setúbal Peninsula, 20 km south of Lisbon. The origin of the population of PWN found in Portugal remains unknown. Several hypotheses may be considered regarding pathway analysis, basically from two general origins: North America or the Far East (Japan or China). In Portugal, no studies have yet been made on the pathogenicity of the isolates collected from the affected area. These isolates, nearly one hundred collected so far, are now being studied for their genetic diversity. There is a need to understand the pathogenicity of these isolates, and to compare them with isolates from other geographical regions, such as Japan. The purpose of this research was to investigate 2 Portuguese isolates (T and HF) and compare them with 2 Japanese isolates (S-10 and C14-5) when inoculated onto Japanese red pine *P. densiflora*. The main aspects investigated were pathogenicity, crossability, (nematode propagation and tree mortality ratio) and DNA sequencing of the rDNA region.

## COMPARATIVE STUDY OF ENTOMOPATHOGENIC NEMATODES ISOLATION WITH TWO LEPIDOPTERA INSECT HOSTS AS BAITING TRAMP TECHNIQUE

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The entomopathogenic nematodes (EPNs) occurrence in five hundred soil samples from one hundred sites of natural areas and crop field edges from La Rioja (Northern Spain) were assessed using as insect hosts: *Galleria mellonella* (Lepidoptera: Pyralidae) and *Spodoptera littoralis* (Lepidoptera: Noctuidae). The two insect hosts isolated *Steinernema feltiae, S. kraussei and S. carpocapsae* species, although *Galleria mellonella* was more efficient isolating EPNs from 14% sites and 5.4% soil samples than *S. littoralis* with 8% and 2.6% respectively. Moreover *G. mellonella* was able to isolate EPNs in both habitats and *S. littoralis* was more successful in crops field edges. The EPNs recovery frequency and abundance obtained with *G. mellonella* were not statistically different between natural and agricultural associated areas (recovery frequency = 0.056 and 0.053, and abundance = 0.116 and 0.158, respectively). When we used *S. littoralis* as insect bait, the recovery frequency in natural areas (0.005) and crop field edges (0.045) were statistically different. The abundance values obtained with *S. littoralis* were 0.023 in natural areas and 0.123 crop field edges. Significant statistically differences between two insect hosts were observed in recovery frequency of natural areas. Although larval mortality percentage and EPNs density (n° IJs/100 $cm^3$) were not significantly different between hosts, *G. mellonera* was more sensible than *S. littoralis* to EPNs infection. The use of *S. littoralis* in entomopathogenic nematodes surveys is discussed.