

Pathogenicity, reproduction and survival of axenic *Bursaphelenchus xylophilus*

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Pine wilt disease (PWD) is the most serious tree epidemic which causes vast catastrophic damage to pine forests. For a long time, the pine wood nematode (PWN), *Bursaphelenchus xylophilus* was believed to be the only pathogenic agent causing the disease. More recently, it has been hypothesized that some bacteria associated with *B. xylophilus* may play a crucial role on pine wilt. The role of nematodes and associated bacteria in PWD development remains to be further studied. Here, we focused on the biology of axenic *B. xylophilus*.

The pathogenicity of axenic *B. xylophilus* was tested by inoculating greenhouse-grown 4-year-old seedlings and 6-month-old axenic microcuttings of *P. densiflora* with aseptic PWNs and non-aseptic PWNs. Seedlings were inoculated with 5,000 PWNs. Microcuttings were inoculated under axenic conditions with 200 nematodes. After 20 days, the microcuttings inoculated with aseptic PWNs and non-aseptic PWNs wilted, and the wilting ratios were 90% and 80%, respectively. The average numbers of recovered nematodes were (364 ± 355) and (66 ± 52) per microcutting, respectively. Similarly, after 38 days, both of aseptic PWNs and non-aseptic PWNs wilted 80% of greenhouse-grown seedlings, with (34733 ± 34162) and (25057 ± 21410) nematodes per seedling, respectively. To compare the reproduction of aseptic PWNs and non-aseptic PWNs, 100 nematodes were transferred into a PDA plate with *Botrytis cinerea* and cultured at 25°C. One week later, the nematodes were isolated from the plate and aseptic and non-aseptic nematodes were counted. The results showed that there was no significant difference in the number between them. Furthermore, the survival of aseptic PWNs and non-aseptic PWNs under axenic conditions was studied. Five thousand PWNs were maintained in flasks containing 2 ml sterile water and incubated at 25°C. After 36 days, the survival rate of non-aseptic PWNs was lower than 5%, on the other hand, the survival rate of aseptic PWNs was about 50%.

Based on our research, it can be concluded that aseptic *B. xylophilus* does not lost its pathogenicity character. Also, it was amazing to see them live longer than non-aseptic one under axenic condition.