

3-4-13. Development of an Efficient PASA-based Diagnosis Protocol for the Identification of the Pinewood Nematode, *Bursaphelenchus xylophilus*

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Pine wilt caused by the pinewood nematode, *Bursaphelenchus xylophilus*, has been a serious problem in the southern regions of Korea. Efficient diagnosis of *B. xylophilus* from infected pinewood specimens is critical for the management of this pest. Traditional microscopic examination, however, often results in an erroneous identification because of the presence in the specimens of the closely related nonpathogenic species, *B. mucronatus*, having a great degree of morphological similarity to *B. xylophilus*. As an attempt for searching reliable genetic markers, we have PCR-cloned the 5s rRNA genomic DNA fragments containing both coding and inter-transcribed spacer (ITS) regions from *B. xylophilus* and *B. mucronatus*. Two species-specific genomic DNA fragments, 550-bp and 520-bp fragments, were amplified from *B. xylophilus* and *B. mucronatus*, respectively, through homology probing PCR strategies. Sequence analyses revealed that coding sequences of the 5s rRNA genes from the two species are almost identical (97.5% homology) but the ITS sequences are substantially different between the species. Based on the ITS sequence differences, we designed species-specific primer sets and developed a bi-PASA-based diagnosis protocol for the identification and discrimination of the two nematode species on a gene level.