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Development of a reverse transcription loop-mediated isothermal amplification (RT-LAMP) method to detect living pinewood nematode, *Bursaphelenchus xylophilus*, in wood.

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Current molecular techniques for the detection of PWN rely on the presence of genomic DNA and thus cannot differentiate between living and dead PWN. The detection of dead nematodes could lead to unnecessary trade disruption. We have developed a reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay, which specifically identifies living PWN in wood by detecting the presence of expansin mRNA as a viability marker. This diagnostic method was found to be more sensitive, faster, more cost-effective, and allows for simpler visual detection as compared to PCR. We chose an expansin gene, because it had been sequenced for both PWN and its closest related species, *B. mucronatus,* (Kikuchi et al., 2009), and because it contains an intron, which is present only in genomic DNA. In order for an RT-LAMP method to be a reliable indicator of viability, it was important to ascertain that only cDNA transcribed from mRNA was amplified, and to eliminate the possibility that any genomic DNA (gDNA) could be amplified. We designed 6 LAMP primers that recognize 8 distinct regions in the target sequence (Notomi *et al.*, 2000). One of the primers was positioned at an exon-exon junction of the expansin gene, so that genomic DNA could not be amplified. When testing gDNA samples and cDNA from different *Bursaphelenchus* species, we found exclusive amplification of cDNA from PWN. Positive samples were detected with HNB (hydroxynaphthol blue) by a change of colour from violet to blue (Goto *et al*., 2009). The sensitivity of the RT-LAMP diagnostic to detect living PWN was higher than that obtained by a conventional PCR diagnostic method, and similar to a real time RT-PCR

assay. We modified an RNA extraction protocol (Chomczynski and Sacchi, 1986) in order to improve extraction quality from wood. We have optimized the RT-LAMP assay not only on nematodes from pure isolate cultures, but also on samples directly isolated from 4 g of PWN-infected wood. This assay was used to test the presence/absence of living PWN in wood that had been heat treated according to ISPM 15 (FAO 2009). From the results obtained, we found that all heat-treated wood samples were free of living PWN, and thus the heat treatment applied to these wood samples was an effective treatment to kill PWN. We will be using this method to verify the efficacy of other wood treatment such as sulfuryl fluoride and phosphine**.** This method will help resolve disputes over the detection of PWN by clarifying whether any PWN present in wood is alive or dead. It can also be used to evaluate the efficiency of wood treatment procedures.

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