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Molecular detection of *B. xylophilus* in complex DNA backgrounds

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Dozens of *Bursaphelenchus* species are associated with pine trees (*Pinus spp.*), most of them being harmless, as they feed exclusively on fungi associated with stressed or dying pine trees. *Bursaphelenchus xylophilus* is exceptional as it is a facultative, mostly lethal parasite of vital pine trees. Morphological identification of *Bursaphelenchus* species is predominantly based on spicule characters and depends on the availability of adult males. Invariably, microscopic identification of *B. xylophilus* in nematode suspensions is a time-consuming specialist job, and this largely limits sample throughput.

To control the spreading of *B. xylophilus* through Europe, fast and high throughput detection tests are required. DNA-based screening assays allow for the sensitive screening of large numbers of nematode samples of any kind, and do not depend on the developmental stage of the nematodes under investigation. Several diagnostic methods for molecular detection of *B. xylophilus* have been published. Some of them are designed against a framework with representatives from most *Bursaphelenchus* groups (e.g. Burgermeister *et al.* 2009) but are laborious as they include enzymatic amplicon digestion followed by gel-based fragment analysis. On the other hand, a relatively fast satellite DNA-based TaqMan assay has been developed with verified contrast against a limited number (n=10) of congeneric species (Francois *et al.* 2007).

On the basis of a framework of ~ 2,800 full length nematode SSU rDNA sequences (Van Megen *et al.* 2009), we have developed a new molecular assay for the quantitative detection of *B. xylophilus*. Using this framework, we identified unique DNA motifs that enable 'blind' identification of *B. xylophilus* in complex DNA backgrounds. This *B. xylophilus* specific test was developed using 35 SSU rDNA sequences of *B. xylophilus* and 113 SSU rDNA sequences of 44 non-target *Bursaphelenchus* species (including 13 sequences of *B. mucronates*). SYBR Green-based detection assays (similar to Rybarczyk *et al.* 2012) allow for reliable and cost-effective molecular screening of large numbers of

nematode samples in any (inspection) laboratory. Results will be presented on the validation of this new test and include a comparison with the performance of the *B. xylophilus* test (Francois *et al.* 2007) from the EPPO Standard PM 7/4 (3).

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