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Validation of a real time PCR assay for the detection of *Bursaphelenchus xylophilus* in targeted matrices in the framework of national survey

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Surveys on *Bursaphelenchus xylophilus* need detection methods that are reliable, sensitive, that allow high throughput analysis and can be applied on any matrix tested, such as wood material or insect for a direct detection.

Many molecular tests, especially real time PCR tests, were recently published but none of them was fully validated to fulfill all the previous requirements.

From a selection of three real time PCR assays, François *et al.* (2007), Leal *et al.* (2007), Cao *et al.* (2005), a validation process was designed based on EPPO recommendation PM7/98 (EPPO, 2010) and applied for the evaluation of the following performance criteria: sensitivity, specificity, repeatability, reproducibility or robustness.

The three real time PCR assays tested proved to be very sensitive as they all detect one individual of *B. xylophilus*, even if the reaction profile is clearly different from one assay to another probably due to the target gene. They also are repeatable and reproducible.

The specificity of the three tests is different especially when analysing wood: some tests gave false positive results with routine wood samples. With some tests, false positives were obtained from non target *Bursaphelenchus* species, especially in case of large amount of DNA, with Ct values that could lead to confusion with *B. xylophilus*.

Based on its performance, the real-time PCR assay developed by François *et al.* (2007) was considered as the most adapted for our routine use. So, this test was coupled with universal primers and probe as internal control, targeting 18S gene which is present in plant and insects cells (Ioos *et al.*, 2009). The performance of the *B. xylophilus* specific assay was not affected by the addition of this universal primer set, whatever the sample, wood or insect. This duplex real time PCR enabled the detection of one single *B. xylophilus*.

Consequently, the detection scheme applied for French national survey includes a first step of screening using real time PCR assay from François *et al.* (2007) for wood and insect samples. If any positive result is obtained for wood, it would be confirmed by morphological analysis and complementary molecular approach on a compulsory basis. For insect sample, any positive detection would lead to further investigations on site.

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