Sarniguet C et al., *Bursaphelenchus xylophilus* identification, from literature to routine analysis: how to make morphological analysis reliable. In: Schröder, T. (ed.), Pine Wilt Disease Conference 2013, pp. 96-97, Braunschweig, ISSN: 1866-590X

# (101) *Bursaphelenchus xylophilus* identification, from literature to routine analysis: how to make morphological analysis reliable.

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Official surveys on *Bursaphelenchus xylophilus* are conducted in a compulsory manner since 2000 in European Union including in France. Considering the Portuguese situation and the dispersal of *Bursaphelenchus xylophilus* throughout its territory and recent occurrences in Spain, the reliability of sampling and analysis undertaken is a critical issue to early detect any further dispersal.

The implementation of quality assurance for official analysis also led to evaluate the reliability of identification methods, based on morphology or on molecular principles.

Published morphological identification keys to *xylophilus* group (Braasch *et al*. 2009) and to *B. xylophilus* species levels (EPPO 2009) were considered for evaluation. The keys were submitted to a panel of slides including *B. xylophilus* individuals, males and females. Conclusions were drawn about the possibility to observe specific criteria, such as lateral lines number, shape of spicules, number of caudal papillae and presence of vulval flap for the group level (Table 1), and the position of excretory pore for species level (Table 2). Some criteria were shown not to be reliable for routine use: number of caudal papillae and excretory pore position.

Consequently, reliable identification keys for *B. xylophilus* were designed (Table 3) and validated according to a standardized process and taking into account recommendations from EPPO protocol (EPPO 2010).

Additional results are available in Sarniguet *et al*. (2013).

## REFERENCES

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Table 1. Results of evaluation of the *xylophilus* group key according to Braasch *et al*. (2009): number of conform observation/total number of observations

| Observed criteria | Females | Males |
|---|---|---|
| Lateral lines | 23/60 | 41/60 |
| Spicules | NA | 60/60 |
| Caudal papillae | NA | 0/60 |
| Vulval flap | 46/60 | NA |

NA: not applicable

Table 2**.** Results of evaluation of the EPPO (2009) *B. xylophilus* identification key: number of times the criteria is observed/ total number of observations

| Features to be observed | Results |
|---|---|
| Excretory pore not observed | 29/60 |
| Excretory pore not at expected place | 11/60 |
| Position of the excretory pore conform to *B. xylophilus* | 20/60 |

Table 3. Key designed for *Bursaphelenchus xylophilus* species identification from female individuals

| | | |
|---|---|---|
| **1** | Female with conical or slender tail with or without mucro | not *B. xylophilus* |
| | Female with sub-cylindrical tail …… | **2** |
| **2** | Female with sub-cylindrical tail, rounded end without mucro | *B. xylophilus* |
| | Female with sub-cylindrical tail with a terminal mucro | not *B. xylophilus* or *B. xylophilus* mucronate form (1) |

(1) molecular identification needed