

Oral presentation

Optimisation of sampling and testing procedures for detecting *Xylella fastidiosa* in large lots of plant for planting and nursery stocks

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Abstract: Inspections and diagnostic tests for *Xylella fastidiosa* are mandatory on consignments/place of production for the most susceptible species listed in the EU Decision 2017/2352, as well as for the movement of 'specified plants' produced in nurseries located in the so-called demarcated areas. Although inspections and sampling are carried out in accordance with the ISPM31 standards, at laboratory level such requirements imply the manipulation of a large amount of material (leaves/shoots/cuttings). Attempts have been made to experimentally optimise a protocol suitable for the concentration of the bacterial cells starting from large volume of plant homogenate, prior to being tested with the protocols commonly adopted for detecting the presence of *X. fastidiosa* in plants. Diagnostic sensitivity of serological and molecular tests was assessed by using infected plant materials (*X. fastidiosa* subspecies *pauca*, ST53) spiked at different ratios with non-infected plant materials. Portions (single leaves or pieces of stems) of infected plants were individually tested to establish the distribution of the bacterium in a given infected symptomatic/asymptomatic plant. Briefly, petioles and/or stem portions recovered from infected plants of *Polygala myrtifolia*, *Nerium oleander*, *Olea europaea*, *Lavandula stoechas*, and scraped xylem tissue from infected cuttings of *Prunus avium* were pooled at different ratios with healthy materials up to 40 g/sample. Indeed, tests included plantlets from the species of the Brassicaceae and Solanaceae families (non-host plants of the ST53 strains) upon spiking the pooled samples with stem portions of infected periwinkle. After grinding the pooled samples, plant homogenates were centrifugated and the recovered pellets resuspended in the appropriate extraction buffer and processed using the standard serological or molecular protocols. The data gathered for sampling (minimum number of portions/aliquot) and for testing (maximum size of the pool) provide useful guidance when processing a large number of samples, satisfying sample size requirements while keeping the diagnostic tests technically and economically affordable.

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