5.17 Molecular detection of *Philaenus spumarius* DNA in predators gut

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Abstract: *X. fastidiosa* is one of the most destructive pathogens of olive orchards. Its recent arrival to Europe can cause important losses, with possible drastic consequences for the sector. *Philaenus spumarius* (Linnaeus 1758) was identified as vector of *X. fastidiosa* in Europe and finding candidate natural enemies of *P. spumarius* is essential to design environmentally friendly control strategies against *X. fastidiosa*. Generalist predators, such as spiders, arise as important potential biocontrol agents. In this context, a set of species-specific primers for detecting the presence of *P. spumarius* DNA in the gut of predatory arthropods is being developed.

All primers will be tested for checking cross-reactive amplification of arthropods DNA and evaluated in heterospecific mixes of nucleic acids. Subsequent feeding trials will be conducted using *Synemaglobosum* (Fabricius 1775), an important spider species in olive groves from Mirandela, Portugal. These trials will allow determining the detection efficiency of the primers designed and also they will be the starting point to detect predation in field trials. These primers therefore provide a very useful tool for screening the gut contents of potential predators of *P. spumarius*, and can thus reveal candidate species for this species biological control.

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Session 6 – Detection and identification

6.1 Internal controls and digital PCR supporting reliable detection of *X. fastidiosa*

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Abstract: Official detection of *X. fastidiosa* is done since 2005 using qPCR as screening tests (Schaad *et al.* 2002, Francis *et al*. 2006, PM 2016, Dreo *et al.* 2006). Since then the range of matrices has significantly extended. To assure the reliability of testing several critical issues were identified including (i) utilization and preparation of spiked controls with defined concentrations of the target and (ii) inhibition of amplification in some matrices. In total more than 130 spiked controls were analyzed in 2016 representing *Olea* (31), *Nerium* (24), *Rosmarinus* (24) and *Coffea* (13), and 46 samples of 21 other plant genera and insects. DNA was extracted using automated Quick Pick Plant Kit (Pirc *et al.* 2009) and tested undiluted, gave negative result in 19 (14 %) and 24/138 (17 %) of spiked controls in Shaad's and Francis' qPCR respectively. Samples with most inhibition were of *Rosmarinum*, *Lavandula*, and *Origanum* (14), *Prunus* (2), *Coffea* (2) and individual samples of *Nerium*, *Olea*, *Rubus* and *Hedera* plants. While these controls were positive when tested in dilutions (1:10) the results nevertheless identify matrices for which improvements of sample preparation and/or DNA extraction are necessary. COX amplification (Körner *et al.* 2017) also failed in 10/24 samples making it a relatively good predictor. Digital PCR allowed us to identify (i) its comparable sensitivity to qPCR and (ii) absolute quantification of extracted DNA as affected the matrices. Digital PCR has been reported to improve sensitivity and to have higher resistance to inhibition in plant samples [Dreo *et al.* 2014, Rački *et al.* 2014, Gutiérrez-Aguirre et al. 2015).

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