European Food Safety Authority

XF ACTORS

3rd European Conference on Xylella fastidiosa and XF-ACTORS final meeting

Optimizing molecular assays to support early detection of *Xylella fastidiosa* in host plants

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INTRODUCTION & OBJECTIVES

- Efficient sampling approaches combined with reliable, fast testing procedures are crucial factors in plant surveillance for early detection of *Xylella fastidiosa* (Xf).
- In the frame of the European project 'XF-ACTORS', work was performed on developing effective sampling methods to support reliable detection of Xf, particularly when plants are asymptomatic.

The **objectives** of this work were:

- to evaluate the efficiency of two methods for DNA extraction from plant material (1g), employing two commercial kits: one silica-column-based and one magnetic-bead-based, in combination with automated DNA-extraction platforms.
- to test the performance of both above-mentioned DNA extraction methods in pooled (10g) plant samples.
- to evaluate the sensitivity of an isothermal DNA amplification and detection system applied on crude plant material (homogenate) without DNA purification, using a portable fluorometer (AmpliFire, Douglas Scientific).

MATERIALS & METHODS

Detection method: qPCR

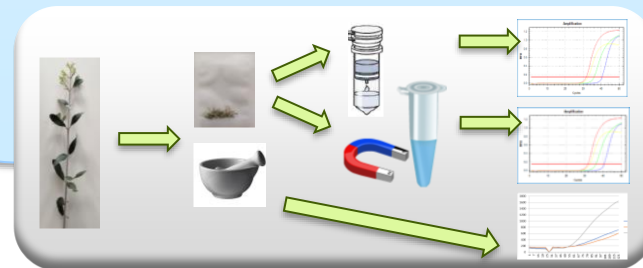
- **Plant matrix:**
  Olea europaea, *Prunus ceracifera*, *Ficus carica*, *Lavandula* sp., *Nerium* sp., *Pelargonium* sp., *Pistacia* sp., *Juniperus* sp. and *Tagetes* sp. (*i.e.* hosts of high economic importance & wide distribution in Greece, or major role in Xf epidemiology): 1g or 10g
- **Bacterial inoculum:**
  Inactivated Xf cells (CFBP 8477) were added in the plant matrix (spiked samples) at 10^6, 10^5, 10^4, 10^3, 10^2 cfu/ml
- ***Sample preparation***
  DNA extraction by:
  - DNeasy Mericon™ Food Standard Protocol [EPPO PM7/24(4)] using Qiacube platform (Qiagen)
  - Maxwell® RSC PureFood GMO and Authentication Kit AS1600 using Maxwell® RSC platform (Promega)
- DNA extracts from Xf spiked plant matrix were assessed in regard to their:
  - quality (A_{260}/A_{280})
  - quantity (ng/μl)
  - performance in qPCR: Harper *et al.* (2010; erratum 2013); Francis *et al.* (2006)

Detection method: isothermal DNA amplification

- **Plant matrix:**
  Polygala myrtifolia (indicator host for monitoring the presence of insect vectors of Xf) and *Myrtus microphylla* (species with strong inhibition in conventional PCR)
- **Bacterial inoculum:**
  Inactivated Xf cells (CFBP 8477) were added in the plant matrix (spiked samples) at 5 to 1250 cells/reaction
- ***Sample preparation***
  - Crude plant homogenate with no DNA extraction
  - Kit of AmplifyRP XRT, using the portable fluorometer (AmpliFire, Douglas Scientific)

Conditions for testing:
- 'standard' (ST), recommended by manufacturer
- ST+incubation of homogenate in AMP1 buffer for 15 min
- ST+incubation of homogenate in AMP1 buffer for 60 min
- ST+low speed centrifugation of homogenate (5130 rpm/5 min)

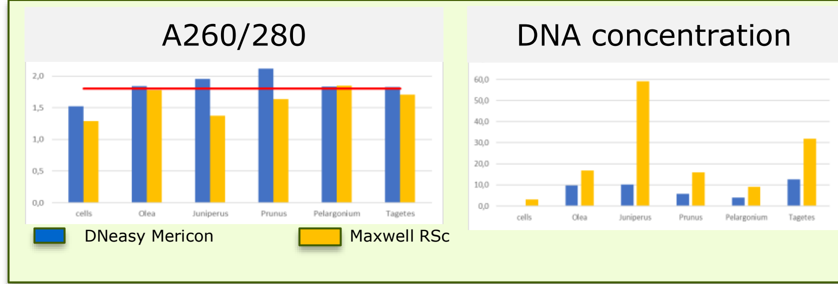
Positive control: Xf cells (inactivated; 625 cells/reaction, *i.e.* 10^7 cfu/ml)
Negative control: healthy *Myrtus* sp. tissue



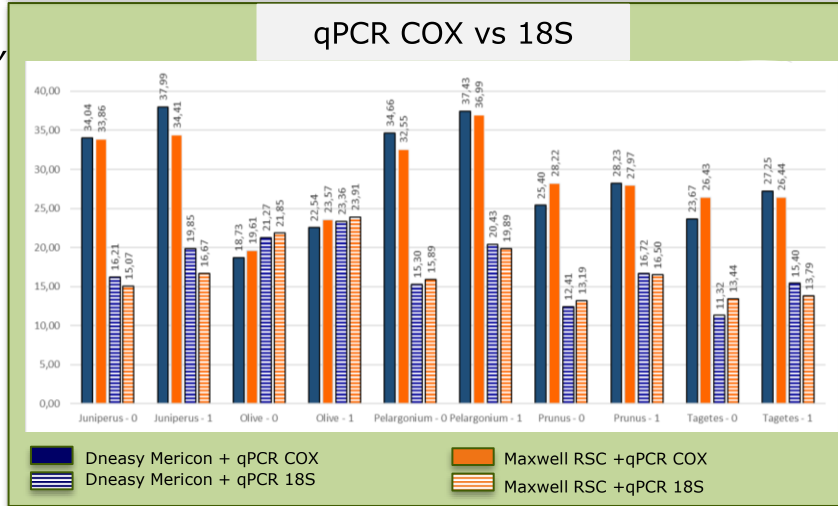
RESULTS

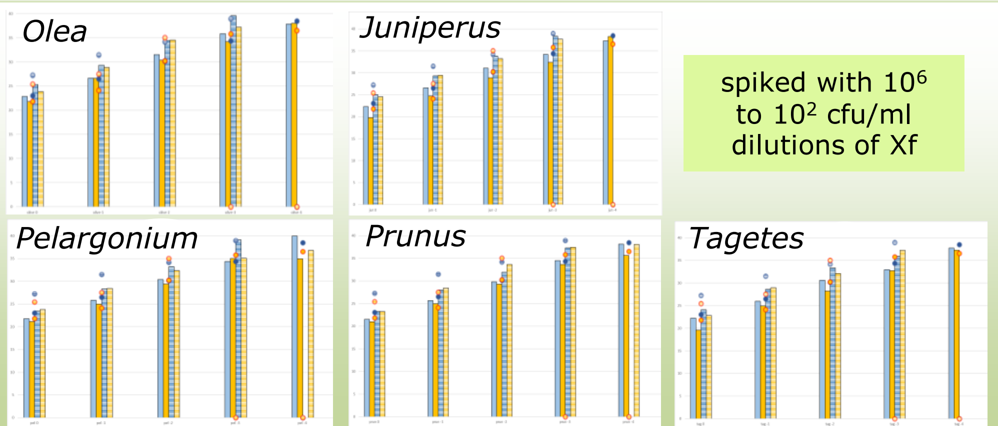
For spiked plant tissue of 1g

- Concentration: DNeasy Mericon < Maxwell RSC
  A260/280: DNeasy Mericon > Maxwell RSC

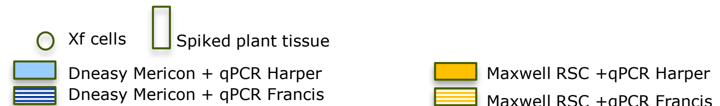


- Cqs for target **COX** ≈ Cqs for target **18S**, for *Olea*, for both kits
  Cqs for target **COX** > Cqs for target **18S**, for *Juniperus, Pelargonium, Prunus, Tagetes*, for both kits
- Cqs by qPCR **Harper** *et al*.< Cqs by qPCR **Francis** *et al*. for all hosts, and both kits
- qPCR **Harper** *et al*.:
  Cqs after DNeasy **Mericon** > Cqs after **Maxwell** RSC, for all hosts spiked at 10^6 to 10^4 cfu/ml. The opposite was true for *Pelargonium* 10^3 cfu/ml, *Olea* and *Juniperus* 10^2 cfu/ml





- qPCR **Francis** *et al.*:
  Cqs after DNeasy **Mericon** varied among host plants and inoculum level from higher to lower compared to Cqs after **Maxwell** RSC

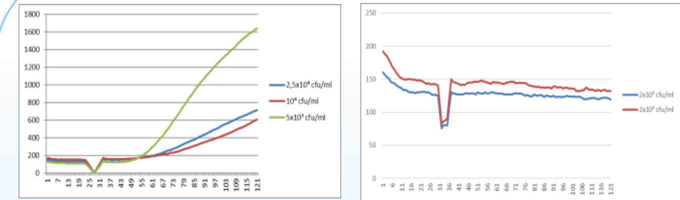


RESULTS

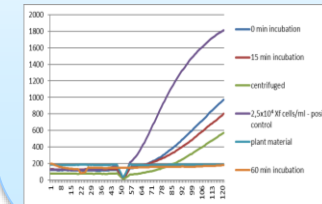
For spiked plant tissue of 10g

- Concentration: DNeasy Mericon < Maxwell RSC (especially *Lavandula* sp.)
  A260/280: DNeasy Mericon > Maxwell RSC only for *Ficus* and *Lavandula*
- Cqs for target **COX** > Cqs for target **18S** (all hosts, both kits)
  Cqs by qPCR **Harper** *et al*.<Cqs by qPCR **Francis** *et al*. (all hosts, both kits)
- qPCR **Harper** *et al*.:
  Cqs after DNeasy **Mericon** > Cqs after **Maxwell** RSC, for *Ficus* and *Pistacia*
  Cqs after DNeasy **Mericon** varied among host plants and inoculum level from higher to lower compared to Cqs after **Maxwell** RSC
- qPCR **Francis** *et al*.:
  Cqs after **DNeasy Mericon** > Cqs after **Maxwell** RSC, for *Ficus* and *Pistacia*
  Cqs =0 after Maxwell for *Lavandula*, while detection possible after DNeasy Mericon

Isothermal DNA amplification



The sensitivity of the AmpliFire system was estimated at approximately $5x10^3$ cfu/ml.



Modifications did not improve sensitivity. The best results were obtained with the use of the immunological cassette post amplification.

MAIN CONCLUSIONS & ACKNOWLEDGEMENTS

- No single DNA extraction protocol can be recommended for detection of Xf in all plant species. The choice of method depends also on the amount of plant tissue to be analyzed.
- Harper *et al*. (2010; erratum 2013) qPCR protocol performed well with DNA extracts from large amount of tissue, which is significant for pooling samples together.
- Targeting eukaryotic 18S rDNA as internal control in qPCR is more efficient than targeting COX.
- Isothermal amplification system (AmpliFire) provided promising results for onsite Xf detection.

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