# Methods for diagnosis of *Xylella fastidiosa in the UK*

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# Reference isolates

| NCPPB # | Identification as received | Notes |
|---|---|---|
| **4339** | *Xylella fastidiosa* subsp. *multiplex* | Grows on PW supplemental media at 28°C. |
| **4431** | *Xylella fastidiosa* subsp. *multiplex* | |
| **4432** | *Xylella fastidiosa* subsp. *fastidiosa* | 16s rRNA shows as *Xylella fastidiosa* subsp. *piercei* |
| **4473** | *Xylella fastidiosa* subsp. *fastidiosa* | |
| **4588** | *Xylella fastidiosa* subsp. *multiplex* | = LMG 9063 |
| **4589** | *Xylella fastidiosa* | = LMG 15098 |

- *X. f.* subsp. *pauca* and variants not yet included

# Interlaboratory method comparisons (Anses)

- 3 x FR (Anses, INRA), IT (Bari), NL (NVWA), NZ (Auckland) and UK (Fera)
  - Xfp (coffee & citrus)
  - Xff (grapevine)
  - Xfm (olive and peach)
- ELISA data too eratic to analyse
- Real-time PCR  best method
- Conventional PCRs strain specific
- LAMP promising but needs further validation

# Routine diagnostic methods

- Fastidious isolation media (PW & BYCE)

- DNA Extraction:
  - 0.3g plant material per sub-sample
  - Usually 2 sub-samples f symptomatic leaf material.
  - A slightly modified CTAB extraction method
    - Based on Doyle, J. J. and Doyle, J. L. 1990 Isolation of plant DNA from fresh tissue. *Focus* 12: 13-5.
    - No further DNA clean-up.

# Routine diagnostic methods

- Real-time PCR (Harper *et al*., 2013)
  - Samples tested in duplicate using the protocol as described in the ring test.
  - Standard COX assay used as internal control.
  - Inhibition problems reduced by also diluting extracts (1:10) and adding BSA to mastermixes (6 µg per reaction)
- LAMP (Harper *et al*., 2013)
  - Successfully used to confirm positive *Coffea* sample but not yet used routinely

# Routine identification methods

- MLST sequence identification of subsp.
  - **Rodrigues *et al*., 2003** Detection and diversity assessment of *Xylella fastidiosa* in field-collected plant and insect samples by using 16S rRNA and gyrB sequences. Appl. Environ. Microbiol. 69:4249–4255.
  - **Schuenzel *et al*. 2005**. A multigene phylogenetic study of clonal diversity and divergence in North American strains of the plant pathogen *Xylella fastidiosa.* Appl. Environ. Microbiol. 71: 3832–3839.
  - **Yuan *et al*., 2010**. Multilocus Sequence Typing of *Xylella fastidiosa* causing Pierce's disease and oleander leaf scorch in the United States. Phytopathology 100 (6), 601-611
  - **Parker *et al*. 2012**. Differentiation of *Xylella fastidiosa s*trains via multilocus sequence analysis of environmentally mediated genes (MLSA-E). Appl. Environ. Microbiol. 78: 1385–1396
  - **Nunney et al. 2013**. Recent Evolutionary Radiation and Host Plant Specialization in the Xylella fastidiosa Subspecies Native to the United States. Appl. Environ. Microbiol. 79: 2189–2200
- http://pubmlst.org/xfastidiosa/

# Strain specific PCR assays

- **Firrao, G. and C. Bazzi. 1994**. Specific identification of *Xylella fastidiosa* using the polymerase chain reaction,Phytopathologia Mediterranea,; 33: 90-92.
- **Minsavage GV *et al*. 1994**. Development of a polymerase chain protocol for detection of *Xylella fastidiosa* in plant tissue, Phytopathology 84: 456-461.
- **Rodrigues *et al*., 2003** Detection and diversity assessment of *Xylella fastidiosa* in field-collected plant and insect samples by using 16S rRNA and gyrB sequences. Appl. Environ. Microbiol. 69:4249–4255.
- **Hernandez-Martinez *et al*. 2006.** Differentiation of strains of *Xylella fastidiosa* Infecting grape, almonds, and oleander using a multiprimer PCR assay. Plant Disease 90 (11), 1382-1388.
- **Guan *et al*. 2015.** Specific detection and identification of American mulberry-infecting and Italian olive-associated strains of *Xylella fastidiosa* by polymerase chain reaction. PLoS ONE 10(6): e0129330. doi:10.1371/journal.pone.0129330

# Risks

**_X. fastidiosa_-affected countries from which host plants are known to have entered the UK in the last 10 years (PHSI data).**

| Country of origin | *X. fastidiosa* status in country | Host plants inspected on arrival |
|---|---|---|
| **Brazil** | Present, restricted distribution | *Lantana, Nicotiana* |
| **Canada** | Present, few occurences | *Lonicera* |
| **Costa Rica** | Resent, no details | *Artemisia, Bidens, Nicotiana, Pennisetum, Veronica, Vinca* |
| **Italy** | Present, restricted distribution | *Acacia, Acer, Aesculus, Alnus, Canna, Citrus, Coprosma, Cotoneaster, Cyprus, Cytisus, Daucus, Fraxinus, Fuchsia, Hedera, Hydrangea, Juglans, Koelreuteria, Lactuca, Lantana, Lonicera, Malus, Mentha, Morus, Nerium, Pelargonium, Pittosporum, Platanus, Populus, Prunus, Pseudotsuga, Pyracantha, Quercus, Rhus, Rosa, Rosmarinus, Salix, Sambucus, Syringa, Ulmus, Vaccinium, Vitis* |
| **Mexico** | Present, no details | *Helianthus, Pelargonium* |
| **USA** | Present, widespread in some states | *Fragaria, Helianthus, Malus, Nicotiana, Rosa, Veronica* |

# Risks – potential UK vectors

10 species of xylem feeding insects commonly found in the UK, 7 of which feed on known hosts of *Xylella fastidiosa*:

- *Cicadella viridis* (*Rosa* & *Vitis*)
- *Aphrophora alni* (*Alnus*, *Fraxinus*, *Populus* & *Salix*)
- *Aphrophora major* (*Salix*)
- *Aphrophora pectoralis* (*Salix*)
- *Aphrophora salicina* (*Salix* & *Populus*)
- *Neophilaenus exclamationis* (*Salix*)
- *Philaenus spumarius** (*Olea* & *Rosa*)

Most are polyphagous on unspecified woody plants

# Planned survey activities

- Monitoring/inspection

  Started with small numbers of entire plants for intensive sampling and testing

  - Tracing back *Coffea* plants from known infected consignments
    - 1 of 2 samples positive
  - Newly arriving *Polygala myrtifolia* with suspect symptoms
    - Up to 30 samples (2015)
  - Other known hosts with suspect symptoms from imports/nurseries (*Acer, Nandina, Nerium, Olea, Quercus, Salix, Vaccinium, Vitis*,)
    - No findings
  - Sticky traps in tree nurseries for testing of potential vectors (2016?)

# Future research

- Infection of potential reservoir plants under controlled climatic conditions
  - e.g. *Vinca*, *Poa*
  - *Xff, Xfm, Xfp*
- Transmission to other high risk hosts
  - Mode of feeding - Electro Penetration Graphics (EPG)
  - Transmission times & efficiencies
  - Systemic colonisation and symptom expression in hosts
- Validation of LAMP assay