

Enhancing the UK diagnostic capabilities for *Xylella fastidiosa*



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

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HARMONISATION AND PROTOCOL IMPROVEMENTS

Laboratories involved:

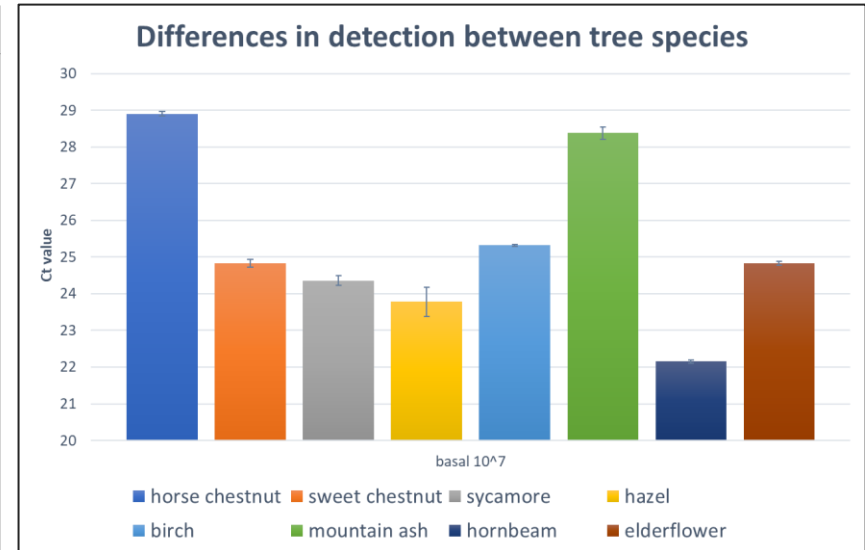
- Fera, FR, SASA and AFBI
- Informal proficiency test of the front-line qPCR test
- Achieving same sensitivity on Ct values

Improving detection in tree hosts

- Testing included: different tree species, tissue types, reduced lysis step and two real-time PCR assays (Francis et al., 2006; Harper et al., 2013)

	Fera		FR		SASA		AFBI	
Dilution	Ct mean	Result	Ct mean	Result	Ct mean	Result	Ct mean	Result
neat	19.02	Positive	22.84	Positive	20.34	Positive	18.2	Positive
1 in 10	22.55	Positive	24.63	Positive	23.66	Positive	20.83	Retest
1 in 100	25.88	Positive	27.85	Positive	27.11	Positive	25.05	Positive
1 in 1000	29.25	Positive	31.76	Positive	30.47	Positive	27.6	Positive
1 in 2000	30.16	Positive	33.14	Positive	31.3	Positive	29.18	Positive
1 in 4000	31.26	Positive	35.06	Retest	32.46	Positive	29.29	Positive
1 in 8000	32.23	Positive	34.68	Retest	33.36	Positive	30.47	Positive
1 in 16000	33.18	Positive	36.13	Retest	34.22	Positive	31.51	Positive
1 in 32000	34.7	Positive	37.62	Retest	35.44	Positive	31.68	Positive
1 in 64000	35.18	Positive	38.22	Retest	37.04	Inconclusive	34.01	Positive
1 in 128000	36.25	Inconclusive	39.74	Retest	37.65	Inconclusive	34.54	Positive
1 in 256000	36.22	Inconclusive	38.48	Retest	38.07	Inconclusive	-	Negative
1 in 512000	36.82	Inconclusive	-	Negative	38.68	Inconclusive	34.84	Positive
1 in 1024000	40	Negative	-	Negative	40	Negative	36.63	Inconclusive
1 in 2048000	40	Negative	-	Negative	40	Negative	-	Negative
1 in 4096000	40	Negative	-	Negative	40	Negative	-	Negative

Latest positive diagnostic result
 Latest detection
 First negative



■ Improved SOPs will be circulated between laboratories

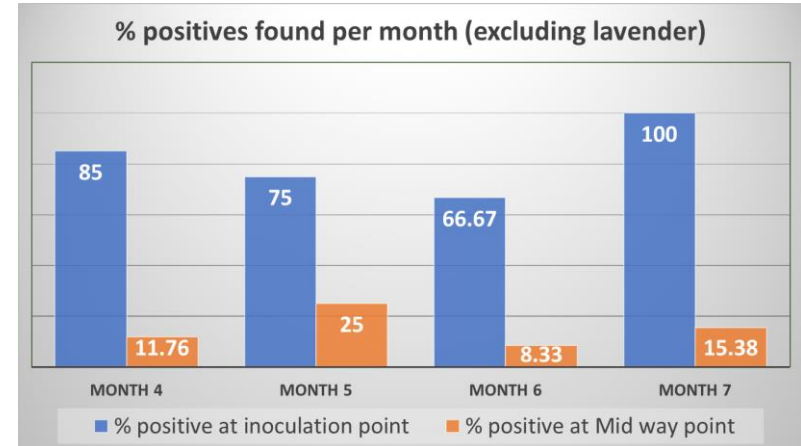
DISTRIBUTION AND COLONIZATION

Three high risk host species (lavender, oleander, coffee) were inoculated with three isolates (representing different subspecies) of *X. fastidiosa*

- Plants were initially kept in a glasshouse compartment at 25°C and two months later half of the plants were transferred to an ambient temperature (not heated) compartment
- Symptoms were monitored monthly and samples were collected for qPCR testing
- Results:
 - Symptoms were difficult to assess and often not associated with positive qPCR samples
 - Oleander and coffee were successfully inoculated
 - There was evidence of movement beyond the area close to the inoculation point on some oleander plants



Number of plants that tested positive for the presence of <i>Xylella</i> by real-time PCR (seven months after artificial inoculation)						
Plant Host	Plants inoculated with <i>Xylella fastidiosa</i>					
	subsp. <i>fastidiosa</i>		subsp. <i>multiplex</i>		subsp. <i>pauca</i>	
	Non-heated glasshouse	Heated (25°C) glasshouse	Non-heated glasshouse	Heated (25°C) glasshouse	Non-heated glasshouse	Heated (25°C) glasshouse
<i>Lavandula dentata</i>	0	0	0	0	0	0
<i>Nerium oleander</i>	2	4	3	2	0	2
<i>Coffea arabica</i>	4	4	3	2	1	1
Total	6	8	6	4	1	3
Total per subsp.	14		10		4	



VOLATILE DETECTION USING MONOTRAPS

- Two groups of five coffee (*Coffea arabica*) plants were kept in two CE rooms
 - five plants were inoculated with *X. fastidiosa* subsp. *pauca*
 - five plants were mock inoculated
- Two types of Monotrap (RGC1 and RGPS) were analysed by GC-HRMS 4 and 16 weeks after inoculation
- Results:
 - Large amount of volatile organic compounds (VOCs) were retained in Mono Traps
 - A number of compounds were different between the two sample sets, i.e. potential early markers of infection
- VOCs can potentially be used as diagnostic markers of infection that might contribute to early detection



Number of compounds from inoculated plants and not found in controls

Time point (weeks)	MT-RGC18	MT - RGPS	Total
4	14	38	52
16	140	140	280

A CORE-GENOME MLST SCHEME FOR *X. FASTIDIOSA*

