Detection of *Xylella fastidiosa* in the frame of surveillance activities in Austria

Richard A. Gottsberger

Institute for Sustainable Plant Production

Dept. for Molecular Diagnostics of Plant Diseases

Where do we get the samples from?

- Suspicious plants are sampled by the inspectors of the PP services of the provinces and send to the lab (in the frame of the the EU monitoring, 2012/87/EU)
- Plants for import are sampled at the airports by the PPS officers and tested in the lab
- Other MS requested testing for *Xf* in our lab
- EUROPHYT reports on interception of *Xf* in MS > in case plants of concern were delivered to Austria information to national PPS, which informs the PPSs in the provinces. Samples of suspected plants are taken and send to the lab for testing. If positive detection, EUROPHYT notification from national PPS.

Samples analyzed for *Xf* in Austria 2015

- in the frame of the the EU monitoring: 4 *Prunus* sp. and 1 Oleander
- Import samples: 4 *Acer* sp., *Hamamelis* sp., *Cercis* sp. and *Cornus* sp.
- MS testing requested: 3 Olive samples
- EUROPHYT reports on interception of *Xf* in MS: 80 *Coffea* sp. samples received from trading companies. In 10 samples *Xf* detected. (all older plants)
- *Xylella fastidiosa* ssp. *sandyi* identified with molecular methods (subsp. specific PCRs and amplicon sequencing)
- Isolation attempts were not yet successfull. Probably very low bacterial titer!
- In Autumn 2014 MS testing requested for *Coffea* sp. samples: *Xylella fastidiosa* ssp. *pauca* identified.

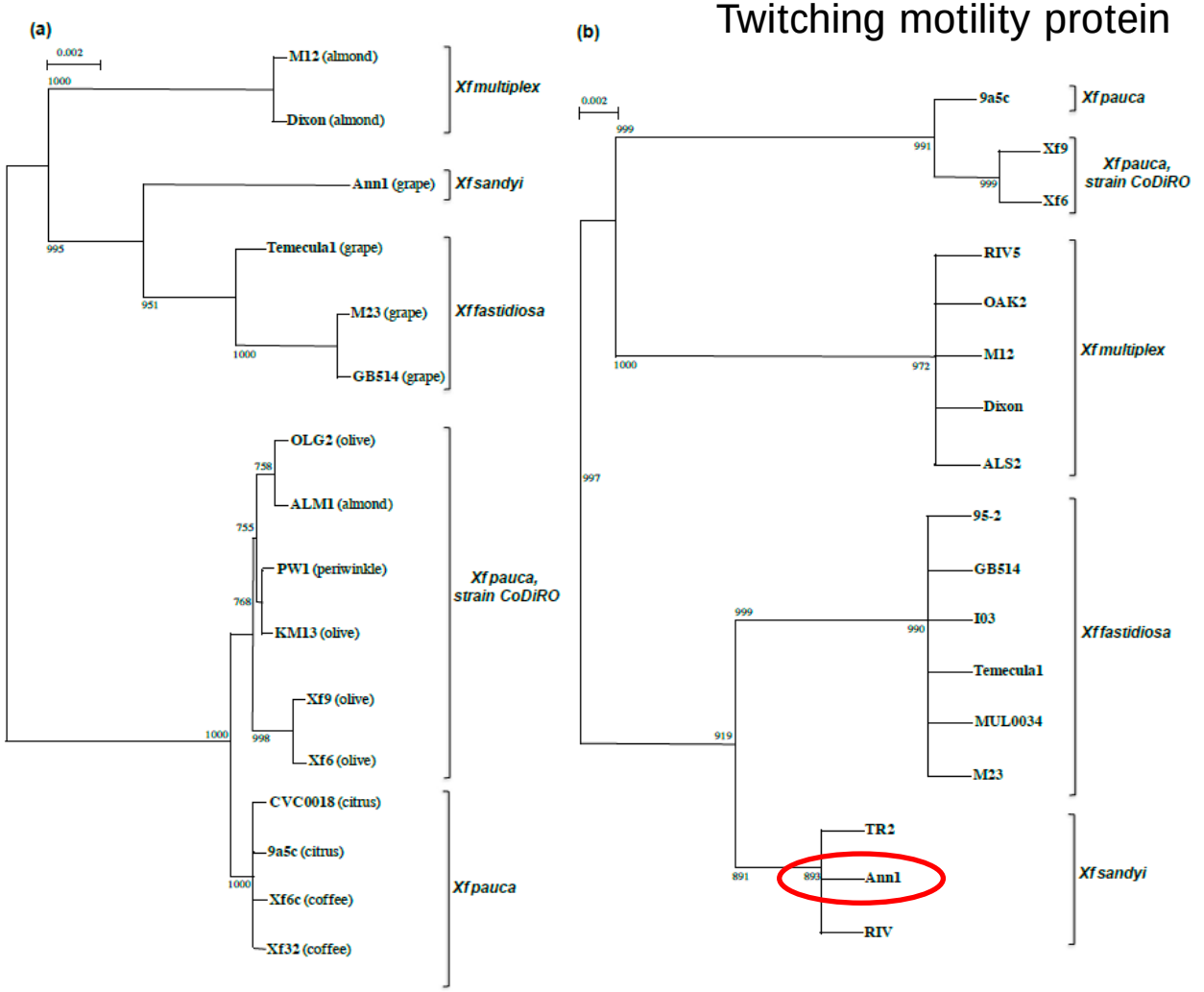
Procedure for detection of *Xf*

(not yet accredited)

- Midrips… …hloem + xylem).
- Bioreba…
- Always…

T. Elbeaino et al.

pilU
Twitching motility protein



Xylella fastidiosa subsp. sandyi Ann-1, cor	Total score	Query cover	E value	Ident	Accession
Xylella fastidiosa 9a5c, complete genome	704	100%	0.0	98%	CP006696.1
Xylella fastidiosa MUL0034, complete gen	699	100%	0.0	97%	AE003849.1
Xylella fastidiosa M12, complete genome	693	100%	0.0	97%	CP006740.1
Xylella fastidiosa subsp. multiplex isolate	689	98%	0.0	97%	CP000941.1
Xylella fastidiosa subsp. fastidiosa GB514	673	95%	0.0	97%	JQ694669.1
Xylella fastidiosa M23, complete genome	311	48%	7e-81	95%	CP002165.1
Xylella fastidiosa Temecula1, complete ge	311	48%	7e-81	95%	CP001011.1
Xylella fastidiosa subsp. multiplex isolate	311	48%	7e-81	95%	AE009442.1
	230	36%	2e-56	94%	JQ694670.1

- Further… …drigues *et al.* (2…

Figure 2. (a) Concatameric phylogram based on sequences of seven different loci (*leuA*, *petC*, *lacF*, *cysG*, *holC*, *nuoL*, *gltT*)

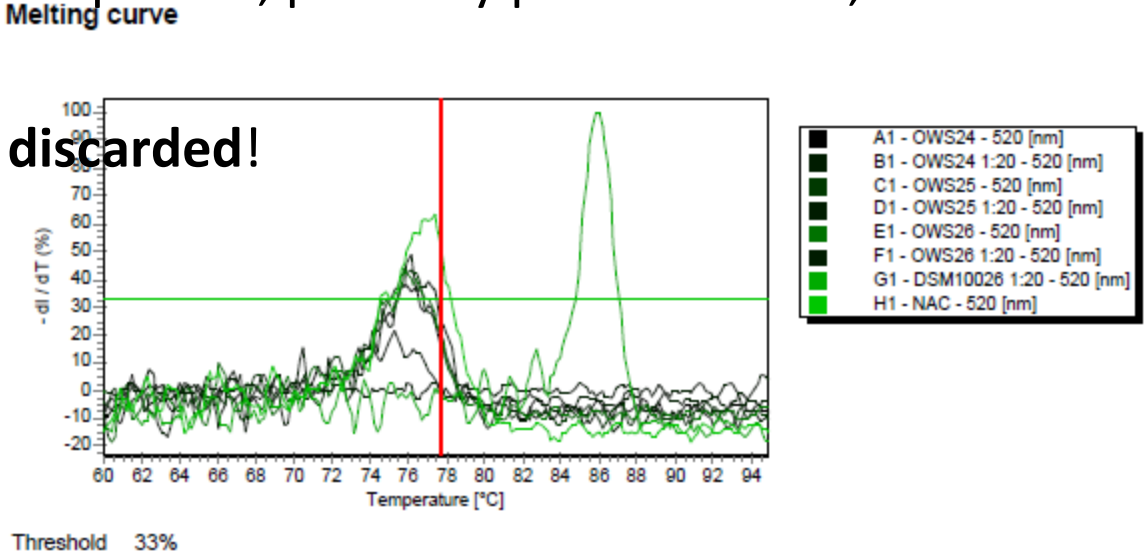
Decision scheme for molecular characterization of *Xf* subspp.

Primer	target	*fastidiosa*	*multiplex*	*sandyi*	*pauca*
RST	sigma-70	x	x	x	x
XF1968	IGS		x	x	
ALM	IGS		x		
XF2542	IGS	x	x		
XYgyr	gyrB	x	x	x	x

Real time PCR tested

- According to Pierce *et al.* 2011. 2 Taqman probes for ssp. *fastidiosa* and *multiplex*
- First test without Taqman probes but with melting curve (EvaGreen) on real *Xf* positive detected samples
- Unspecific amplicons, probably primer dimers, and bad sensitivity with 45 cycles.
- Therefore **discarded**!

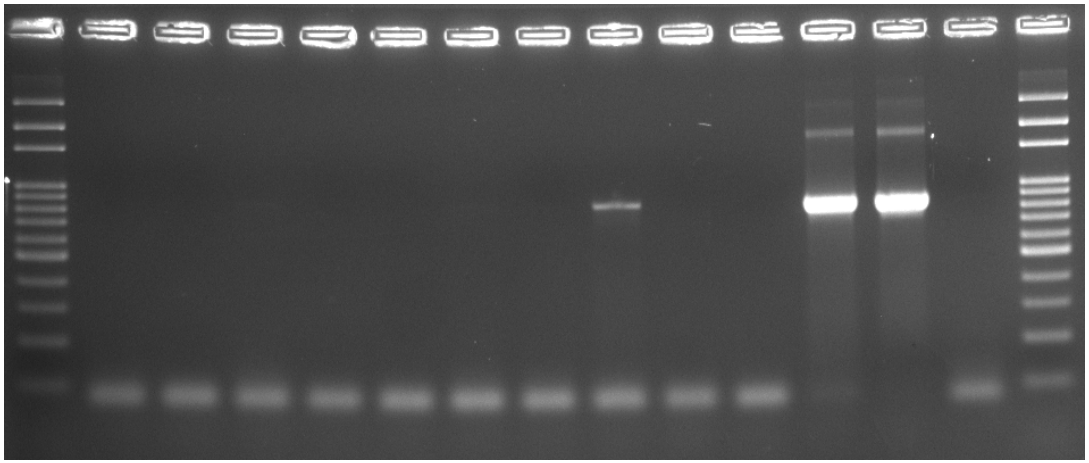
A1	OWS24	1	75.7
B1	OWS24 1:20	1	76.1
C1	OWS25	1	75.8
E1	OWS25	1	75.8
G1	DSM10026 1:20	1	85.9
H1	NAC	1	77.3



Further molecular confirmation
(in testing phase)

- In the frame of the ongoing EUPHRESCO project `DNA Barcoding´ an identification procedure for *Xf* according to M. Maes (unpublished) is being tested. Target is DNA mismatch repair protein coding gene (mutS).
- Can possibly be adapted for molecular confirmation of screening test.

Preliminary tests with positive detected samples:



- Optimization to increase sensitivity for detection needed

Isolation procedure for *Xf* (under optimization)

- When detection positive than isolation attempt on 2-3 media: PW, B-CYE, Bosea medium (DSMZ). At least 3 weeks incubation!
- Problems are overgrowing (probably endophytic) fungi and bacteria
- Sample processing for isolation has to be improved to reduce unwanted and interfering microorganisms!
- Method development on intercepted *Xf* positive tested Coffee plants is underway.
- Pathogenicity test on tobacco for *Xylella fastidiosa* isolates according to Lopez et al. (2000) (to be implemented)

Lopes, S. A., Ribeiro, D. M., Roberto, P. G., França, S. C., and Santos, J. M. 2000. *Nicotiana tabacum* as an experimental host for the study of plant–*Xylella fastidiosa* interactions. Plant Dis. 84:827-830.

AGES

Österreichische Agentur für Gesundheit
und Ernährungssicherheit GmbH

Health. Nutrition. Safety.
Our Concern.