Towards an efficient monitoring and sampling procedure for early detection of *X. fastidiosa* in asymptomatic plants

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efsa European Food Safety Authority

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European Commission

PONTE Pest Organisms Threatening Europe

XF ACTORS

Euphresco

OUTLINE

- **Task 4.2: Early detection**
  - **Subtask 1:** Identification of candidate **indicator (spy) plants** for confined conditions (PEQ and greenhouses) that can support early detection of *X. fastidiosa*
  - **Subtask 2:** Development of appropriate **sampling methods** for imported ornamental plant material/plant material in nurseries for early detection of *X. fastidiosa*

SUBTASK 1: INDICATOR PLANTS FOR CONFINED CONDITIONS

- ***Nicotiana tabacum* cv SR1 (Petite Havana)**

- ***Catharanthus roseus***

- ***Coffea arabica***

- ***Nerium oleander***

- ***Polygala myrtifolia***

- ***Prunus avium***

- ***Prunus domestica***

annual

perennial

decidious

ISOLATES USED

Isolate	Samples	Lot size	Sample size	Symptoms	*Harper et al., 2010*	*Minsavage et al., 1994*	Subspecies	*Yuan et al., 2010*
PD 7202	Coffea 2	1 plant	50 leaves/petioles	asymptomatic	27,47	+	*X. f.* ssp. *pauca*	ST-53
PD 7211	Coffea 5	1 plant	50 leaves/petioles	asymptomatic	27,58	+	*X. f.* ssp. *pauca*	ST-73 and ST-53

Isolate	Ultrasonication	Gram reaction	Form of cells	Oxidase reaction	Catalase reaction	IF (Loewe 07319)
PD 7202	30 sec	-	rods	-	+	+
PD 7211	60 sec	-	elongated rods	-	+	+

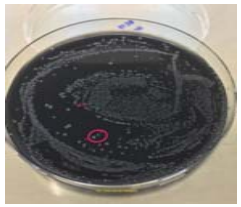
			MLST loci						
Equivalent	Isolate	ST	*leu*A	*pet*C	*mal*F	*cys*G	*hol*C	*nuo*L	*glt*T
CFBP 8495	PD 7202	**53**	7	6	16	24	10	16	14
CFBP 8498	PD 7211	**73**	7	6	8	27	10	16	8

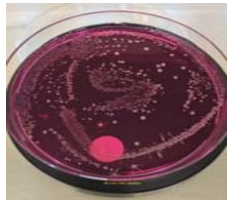
LMG 17159 = CFBP 7970 *Xf* ssp. *fastidiosa* (*Vitis* spp.)

Colonies grown for 2-3,5 wk at 28° C

qPCR (*Harper et al., 2010*):

15.75 - 18.52 (PD 7202)
16.41 - 18.93 (PD 7211)





Bergsma-Vlami et al. 2017, Plant Pathol, 66: 1065–1074

Inoculum drop (10-50 µL)
(EPPO)

14 weeks p.1st i.

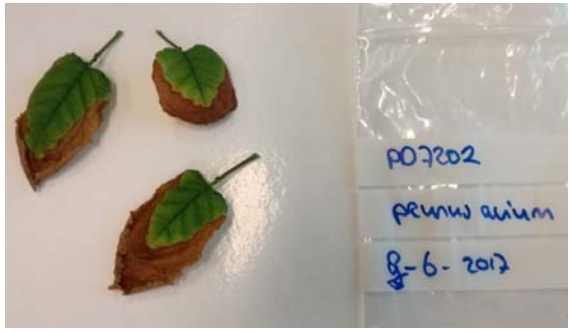
8 weeks p.2ond i.

Two independent experiments (F15 and F12)
F15: 1st inoculation spring 2017
F12: 1st inoculation autumn 2017

28° C during day / 21° C during night / RH 50 %

F15 1st inoculation in spring

SYMPTOMS 10 WEEKS P. 1ST I. (LATE SPRING) ON ALL *PRUNUS AVIUM* PLANTS (PD 7202 AND LMG 17159) BUT NOT ON PLANTS (PD 7211 AND NC)



High Ct values (**37.45** and **38.42)**,
5-10 cm above the inoculation point

21 WEEKS AFTER 1ST / 5 WEEKS AFTER 2OND INOCULATION (F15)

		Plant 1		Plant 2		Plant 3	
		material (g)	Ct value	material (g)	Ct value	material (g)	Ct value
PD 7211	*P. avium*	1.2	NA	1.1	NA	1.3	NA
	P. domestica	0.6	NA	1.3	NA	0.7	NA
	N. oleander	3.6	NA	2.3	NA	3.6	NA
	P. myrtifolia	1.9	NA	1.5	NA	1.3	NA
PD 7202	*P. avium*	1.9	NA	1.8	NA	1.5	NA
	P. domestica	2.0	NA	1.0	**31.37**	1.9	**37.73**
	N. oleander	4.0	**24.45**	3.6	**29.32**	3.2	**33.04**
	P. myrtifolia	3.6	**38.75**	2.7	**27.92**	4.3	**28.21**
NC	*P. avium*	1.0	NA	1.2	NA	0.9	NA
	P. domestica	0.9	NA	0.8	NA	1.1	NA
	N. oleander	3.5	NA	3.3	NA	1.7	NA
	P. myrtifolia	1.7	NA	1.3	NA	1.2	NA

PD 7202, *P. myrtifolia*

PD 7202, *N. oleander*

PD 7202, *P. domestica*

25 WEEKS AFTER 1ST / 9 WEEKS AFTER 2OND INOCULATION (F15)

		Plant 1		Plant 2		Plant 3	
		material (g)	Ct value	material (g)	Ct value	material (g)	Ct value
PD 7211	*P. avium*	0.3	NA	0.3	NA	0.3	NA
	P. domestica	0.1	NA	0.3	NA	0.1	NA
	N. oleander	0.6	NA	0.9	**34.18**	0.9	**35.02**
	P. myrtifolia	0.1	**35.99**	0.15	**36.28**	0.1	**34.99**
PD 7202	*P. avium*	0.4	NA	0.5	NA	0.5	NA
	P. domestica	0.2	NA	0.3	**28.76**	0.25	**36.97**
	N. oleander	0.75	**22.47**	2.0	**35.76**	2.0	**32.56**
	P. myrtifolia	0.1	**35.36**	0.1	**36.69**	0.1	**30.21**
NC	*P. avium*	0.8	NA	1.2	NA	0.8	NA
	P. domestica	0.3	NA	1.1	NA	1.3	NA
	N. oleander	1.3	NA	3.3	NA	0.9	NA
	P. myrtifolia	0.15	NA	0.7	NA	0.9	NA

Conclusions, so far:

- During spring inoculations (F15), isolate PD 7202 (ST 53) can be earlier detected than isolate PD 7211 (ST 73)
- Repeated inoculations required
- *X. fastidiosa* can be earlier detected in ***N. oleander*** **and *P. myrtifolia*** than *P. domestica.*
- Not detected in *P. avium.*

PD 7211, P. myrtifolia

F12 1st inoculation in autumn

6 weeks after 1st inoculation (F12)

		Plant 1	Plant 2	Plant 3
		Ct value	Ct value	Ct value
PD 7211	*P. avium*	NA	NA	NA
	P. domestica	NA	NA	NA
	N. oleander	NA	**35.98**	**36.86**
	P. myrtifolia	**37.02**	NA	**34.54**
PD 7202	*P. avium*	NA	NA	NA
	P. domestica	NA	NA	NA
	N. oleander	**38.05**	NA	NA
	P. myrtifolia	NA	NA	NA
NC	*P. avium*	NA	NA	NA
	P. domestica	NA	NA	NA
	N. oleander	NA	NA	NA
	P. myrtifolia	NA	NA	NA

Conclusions, so far:

- During autumn inoculations (F12), isolate PD 7211 (ST 73) can be earlier detected than isolate PD 7202 (ST 53)
- *X. fastidiosa* can be earlier detected in ***N. oleander* and *P. myrtifolia*** than *P. domestica.*
- Not detected in *P. avium*

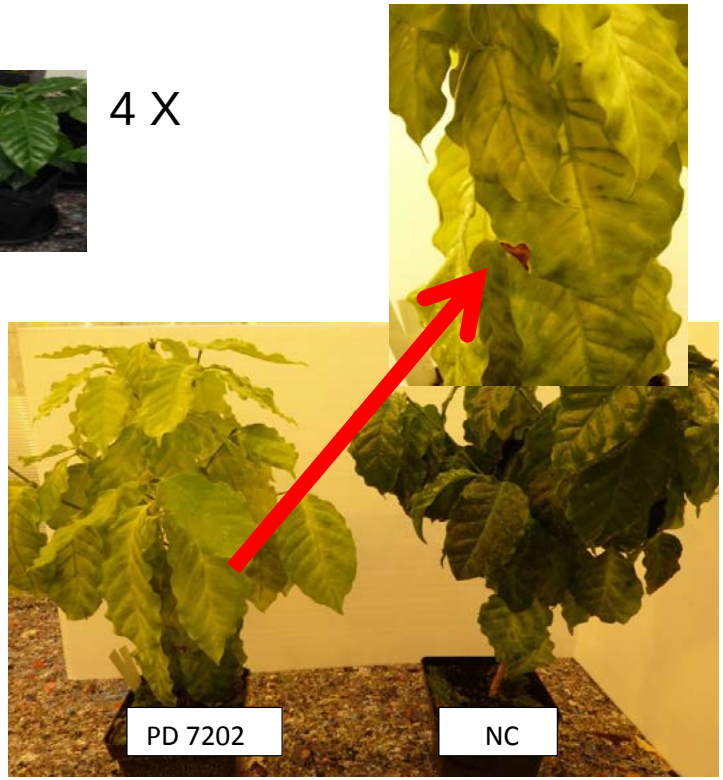
- ***N. oleander* and *P. myrtifolia* both good indicator plants onder confined conditions**

5 wks p.1st i.

12 wks p.2nd i.

40 wks p.3rd i.

4 X



2 x inculated

Inoculum	# inoculations	# plant	leaves (g)	petiole/midrib (g)	Ct
PD7202	2	1	1,43	0,23	N/A
	2	2	1,04	0,16	**24.00**
	2	3	1,4	0,18	**35.22**
	2	4	1,1	0,16	N/A
	3	5	1,27	0,22	N/A
	3	6	1,99	0,29	N/A
	3	7	1,74	0,23	N/A
	4	8	1,27	0,16	**35.36***
	4	9	1,41	0,2	**38.64****
	4	10	1,21	0,17	**32.27**
	4	11	1,08	0,17	**38.12**
PD7211	2	1	1,09	0,15	**34.59**
	2	2	1,22	0,18	N/A
	2	3	1,21	0,16	N/A
	2	4	1,11	0,14	**33.76**
	3	5	1,28	0,17	**36.27**
	3	6	1,01	0,14	N/A
	3	7	1,16	0,16	**34.85**
	4	8	1,69	0,25	N/A
	4	9	1,08	0,19	**34.47**
	4	10	1,37	0,2	N/A
	4	11	1,67	0,25	**32.76**
PC	3	4	1,34	0,16	**34.07**
NC	2	2	1,29	0,2	N/A
	3	5	1,28	0,175	N/A

PD 7202

2x inoculated

4 x inoculated

1 X

PD 7211 NC
11 weeks p.i

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  – **Subtask 2:** Development of appropriate **sampling methods** for imported ornamental plant material/plant material in nurseries for early detection of *X. fastidiosa*

- *Nicotiana tabacum* cv SR1 (Petite Havana)

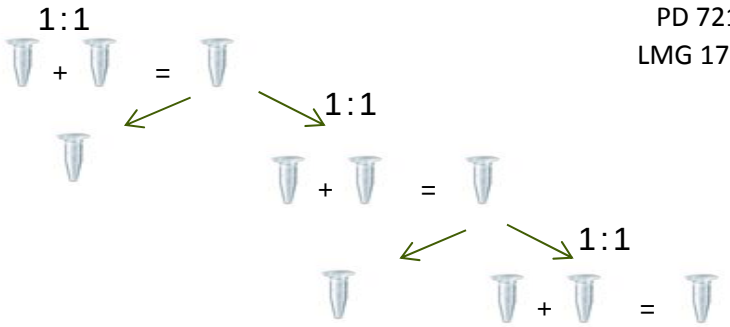
- *Coffea arabica*

- *Nerium oleander*

→ Healthy extracts from petioles/midribs/stems
Predetermined weight/#leaves

↓ Spike in duplicate

Strain	Subspecies	ST	Isolated	Ct values qPCR	# copies *rim*M
PD 7202	*X. f.* ssp. *pauca*	53	*Coffea* spp.	15,93	apprx 10^6
PD 7204	*X. f.* ssp. *pauca*	53	*Coffea* spp.	14,75	apprx 10^6
PD 7211	*X. f.* ssp. *pauca*	73	*Coffea* spp.	16,31	apprx 10^6
LMG 17159	*X. f.* ssp. *fastidiosa*		*Vitis* spp.	15,03	apprx 10^6



→ qPCR (*Harper et al., 2010*) in duplicate

↓

copies *rim*M

Preliminary results on *Nerium oleander*

Nerium oleander

	Ct values qPCR (*Harper et al., 2010*)			
	X. f. ssp. *pauca*			*X.f.* ssp. *fastidiosa*
	ST 53		ST 73	
# leaves	PD 7202	PD 7204	PD 7211	LMG 17159
0,50	16,99	17,17	18,67	16,47
1,00	17,36	17,48	19,51	16,40
1,50	18,07	18,30	20,36	17,48
2,00	18,58	19,15	21,29	18,15
2,50	19,62	19,96	22,73	18,85
3,00	20,32	20,26	23,37	19,70
3,50	20,77	21,25	24,12	20,45
4,00	21,03	22,61	25,10	21,12
4,50	23,40	24,47	27,15	24,28
7,50	28,56	29,77	32,03	29,75
10,50	33,54	34,79	36,92	34,64
13,50	35,96	38,97	38,72	37,67
16,50	37,36	nd	nd	nd
19,50	37,87	38,86	nd	nd
22,50	38,95	nd	nd	38,08
25,50	nd	nd	nd	nd
28,50	38,99	39,01	nd	nd
31,50	nd	nd	nd	nd

2100 mg petiole / midrib
2700 mg petiole / midrib

Ct 34,64- 34,79

100 copies *rim*M

Ct 35,96- 36,92

50 copies *rim*M

Preliminary results on *Coffea arabica*

Coffea arabica

2100 mg petiole / midrib

2700 mg petiole / midrib

Ct 34,22- 34,61

100 copies *rim*M

Ct 36,05- 36,13

50 copies *rim*M

Ct values qPCR (*Harper et al., 2010*)

	X. f. ssp. *pauca*			*X.f.* ssp. *fastidiosa*
	ST 53		ST 73	
# leaves	**PD 7202**	**PD 7204**	**PD 7211**	**LMG 17159**
1,50	16,16	16,88	20,01	16,83
3,00	16,86	17,46	20,30	17,02
4,50	17,58	18,26	21,18	17,50
6,00	18,61	19,29	22,18	18,37
7,50	19,56	20,38	23,04	18,91
9,00	20,97	21,16	23,54	19,77
10,50	21,33	22,04	24,63	20,58
12,00	22,33	23,14	25,73	21,49
13,50	23,29	24,57	26,64	22,61
22,50	29,13	30,24	32,36	28,86
31,50	34,61	36,05	36,13	34,22
40,50	38,03	39,00	nd	38,35
49,50	37,99	nd	nd	37,39
58,50	nd	39,06	nd	37,99
67,50	nd	nd	nd	38,01
76,50	38,89	nd	nd	nd
85,50	nd	nd	nd	nd
94,50	nd	nd	nd	nd

Preliminary results on *Nicotiana tabacum*

Nicotiana tabacum

Ct 32,34- 34,22

500 copies *rim*M

Ct 36,05- 36,13

50 copies *rim*M

Ct values qPCR (*Harper et al., 2010*)

	X. f. ssp. *pauca*			*X.f.* ssp. *fastidiosa*
	ST 53		ST 73	
stem (*mg*)	PD 7202	PD 7204	PD 7211	LMG 17159
100	18,23	20,23	nd	17,68
200	18,75	20,93	17,07	18,04
300	20,10	22,39	18,33	18,93
400	19,89	23,76	18,58	19,96
500	22,05	24,33	19,99	20,91
600	22,62	25,40	20,65	21,50
700	23,59	26,38	20,80	22,60
800	24,42	27,28	21,40	23,47
900	24,15	26,32	28,78	24,34
1500	30,53	32,34	34,22	30,26
2100	35,73	38,94	39,98	36,74
2700	37,92	nd	nd	38,49
3300	nd	nd	nd	nd
3900	nd	nd	nd	nd
4500	38,92	nd	nd	39,00
5100	nd	nd	nd	nd
5700	nd	nd	nd	nd
6200	nd	nd	nd	nd

CONCLUSIONS, SO FAR, ON SAMPLING

- Simulation of successive dilution of the inoculum with a simultaneous increase in the sampling volume

- Based on this approach, 50-100 copies *rim*M can be detected using a sample consisting of:
  - 31,50 – 40,50 leaves (2,1 – 2,7 g petiole/midrib) *Coffea arabica*
  - 10,50 – 13,50 leaves (2,1 - 2,7 g petiole/midrib) *Nerium oleander*
  - 2,1 g stem *Nicotiana tabacum*

  Implications for reliable sampling in order to fulfill the requirement of 99% reliability for a 1% detection (ISPM 31) =more sub samples per sample

ONGOING EXPERIMENTS

1. Plant species

2. N=5-8 replicates per experiment

3. Different subspecies, namely multiplex and evt strain from *Morus* spp.

Reference strain		Host plant	Country
LMG 15098 = CFBP 8084	*X. fastidiosa*	*Morus* spp.	USA
LMG 15553 = CFBP 7969	*X. fastidiosa*	*Vitis rotundifolia*	USA
LMG 15554 = CFBP 8083	*X. fastidiosa* ssp *fastidiosa*	*Vitis vinifera*	USA
CFBP 8069	*X. fastidiosa* ssp *fastidiosa*	*Vitis* spp.	USA
CFBP 8071	*X. fastidiosa* ssp *fastidiosa*	*Prunus dulcis*	USA
CFBP 8070	*X. fastidiosa* ssp *multiplex*	*Prunus domestica*	USA
CFBP 8072	*X. fastidiosa* ssp *pauca*	*Coffea arabica*	Equador
CFBP 8074	*X. fastidiosa* ssp *pauca*	*Coffea arabica*	Equador
CFBP 8351	*X. fastidiosa* ssp *fastidiosa*	*Vitis* spp.	USA

ACKNOWLEDGEMENT

Thank you

Netherlands Food and Consumer Product Safety Authority
Ministry of Economic Affairs

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