Surveys on *Xylella fastidiosa*

Crespera
(Montero-Astua et al., 2008)

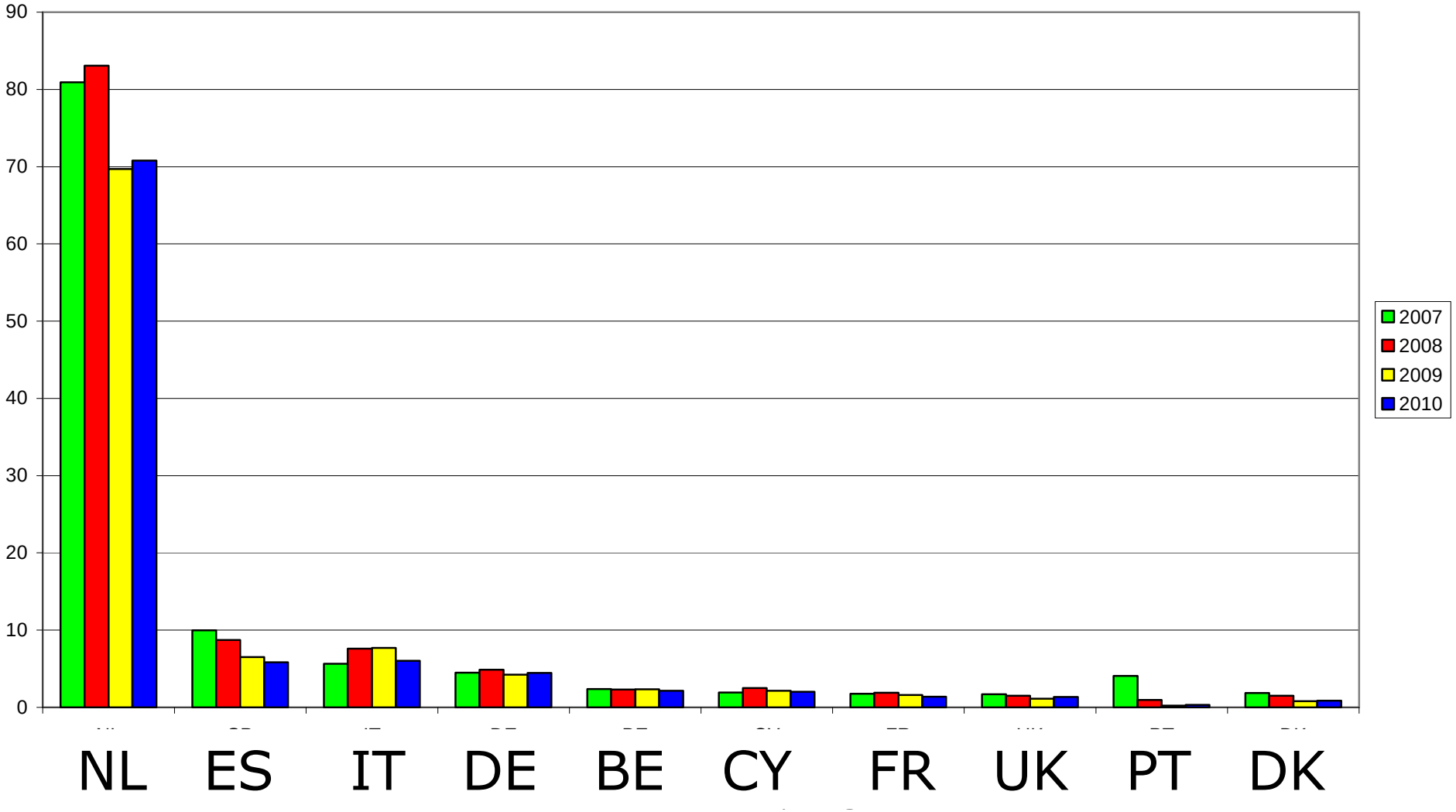
Maria Bergsma-Vlami
Bacteriology
NPPO-NL

Structure presentation

- Detection protocols
- *Xylella fastidiosa* symptoms
- Findings NL 2014, 2015
- Subspecies *X. fastidiosa* in NL

- BUT let's start with something else….

Import volumes of plants for planting material in 10^6 kg 2007 - 2010



Import plants for planting in NL in 2012, incl high risk countries EPPO-PQR 2013

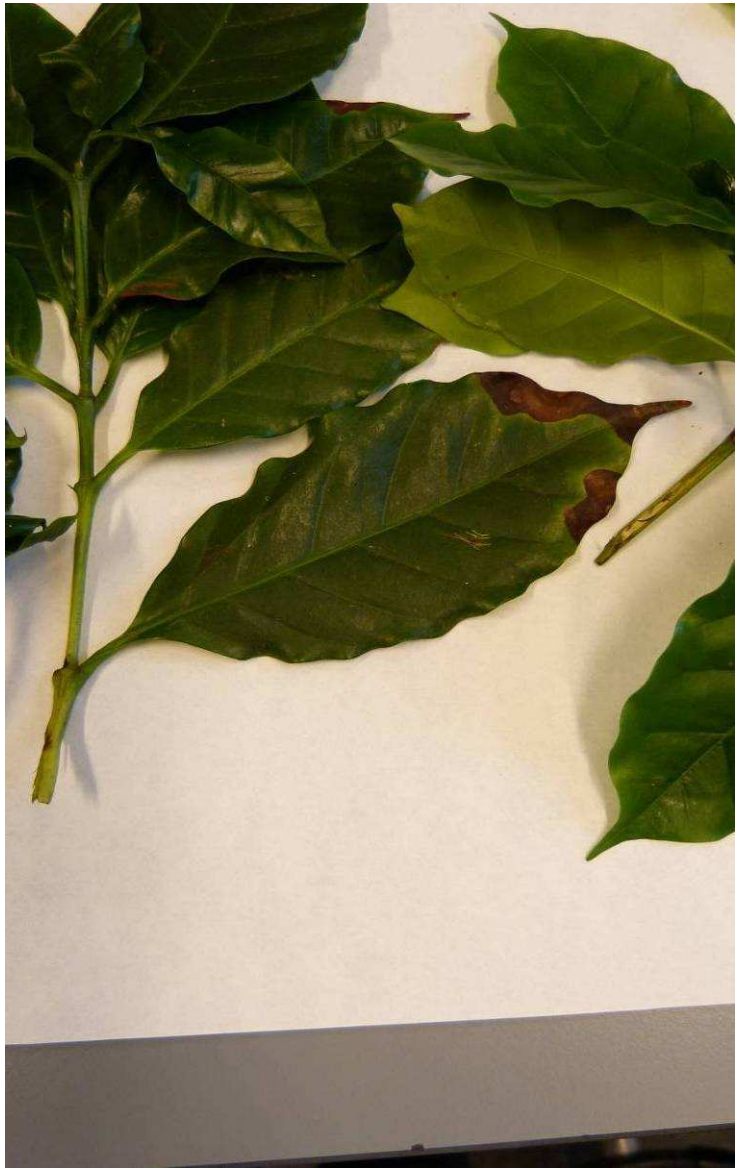
Continent	Country	Number sendings
Afrika	Kenia	13.687
Afrika	Ethiopië	3.558
Afrika	Oeganda	3.258
Afrika	Tanzania	2.465
Afrika	Zuid-Afrika	858
AU+NZ	Nieuw Zeeland	213
Azie	Israël	6.946
Azie	China	3.904
Azie	Maleisië	3.012
Azie	Singapore	2.561
Azie	Indonesië	2.122
Azie	Sri Lanka	1.559
Azie	**Taiwan**	**1.306**
Azie	Thailand	913
Azie	India	675
Azie	Turkije	510
Azie	Vietnam	493
Azie	Japan	299
Azie	Filippijnen	137
CA+USA	**USA**	**1.019**
M-Amerika	**Costa Rica**	**6.107**
M-Amerika	Guatemala	1.889
M-Amerika	Honduras	501
M-Amerika	El Salvador	161
M-Amerika	**Mexico**	**113**
Z-Amerika	Ecuador	817
Z-Amerika	Colombia	729
Z-Amerika	**Brazilie**	**489**
Z-Amerika	Chili	315
TOTAAL		62.594

Import of host plants of *Xylella fastidiosa* (number sendings) from America and Taiwan (**http://www.cnr.berkeley.edu**)

Genus	**2011**	**2012**
Acer	3 (USA)	5 (USA) 1 (Guatemala)
Catharanthus	2 (USA) 3 (Guatemala)	1 (USA) 3 (Guatemala)
Carya	2 (USA)	0
Cercis	4 (USA)	1 (USA)
Citrus, Prunus	Import restriction	
Coffea	19 (Costa Rica) 1 (USA) 1 (Brazilië) 1 (Guatemala)	28 (Costa Rica) 1 (USA) 2 (El Salvador) 1 (Honduras)
Quercus	6 (USA)	3 (USA)
Salvia	50 (Costa Rica) 5 (USA) 4 (Brazilë) 20 (Colombia) 26 (Ecuador) 14 (Guatemala)	20 (Costa Rica) 5 (USA) 1 (Brazilë) 26 (Colombia) 19 (Ecuador) 7 (Guatemala)
Ulmus	2 (USA)	2 (USA)
Vaccinium	0	8 (USA)
Vinca	30 (Costa Rica) 1 (USA) 15 (Guatemala) 1 (Colombia) 1 (Ecuador)	3 (USA) 27 (Guatemala) 5 (Colombia)

Survey 2014 on *Coffea* spp.

no symptom



atypical curling of leaf margins

leaf scorch

leaf scorch

First detection of *Xylella fastidiosa* end 2014

Plants: *Coffea arabica* and *Coffea* sp.
Import: Costa Rica and Honduras.

coffee leaf scorch (CLS) and "crespera"

- **PCR & sequencing**
- **Electron microscopy**
- **IF**

no symptom

leaf scorch

Journal of Plant Pathology (2015), **97** (2), 391-403

DISEASE NOTE

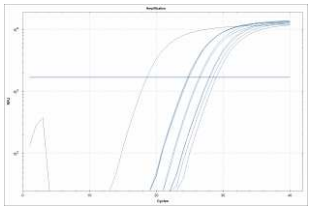
XYLELLA FASTIDIOSA IN *COFFEA ARABICA* ORNAMENTAL PLANTS IMPORTED FROM COSTA RICA AND HONDURAS IN THE NETHERLANDS

M. Bergsma-Vlami, J.L.J. van de Bilt, N.N.A. Tjou-Tam-Sin, B.T.L.H. van de Vossenberg and M. Westenberg

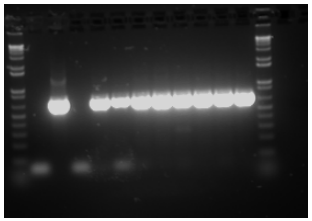
Dutch National Plant Protection Organization (NPPO-NL), P.O. Box. 9102, 6700 HC Wageningen, The Netherlands

Detection protocol

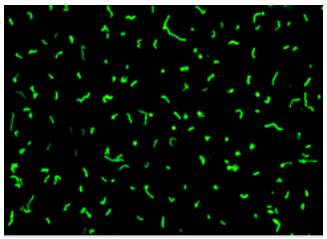
petioles and midribs



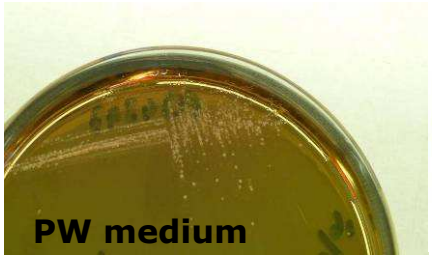
Real-time PCR (Harper *et al.*, 2010)

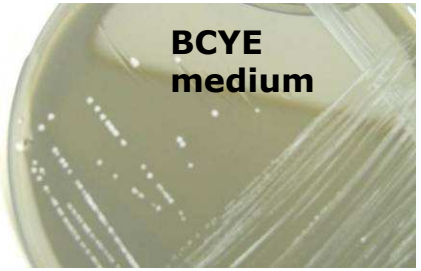


PCR (Minsavage *et al.*, 1994) + sequencing



IF (Loewe 07319)





	Asymptomatic sample (latent)	Symptomatic sample
1	**10 – 50 g petioles and midribs of 100 to 200 leaves (based on lot size), preferably incl stems.**	**1,5 – 3 g petioles and midribs from 3 - 15 leaves, preferably incl stems.**
2	Desinfection: • Soak material; 5' in diluted chlorite (= 0,5% active chlorite). • Rinse material 3x with tap water • Dry in tissue paper • Brief disinfect with 70% alcohol • Dry material in flow cabinet.	Desinfection: • Soak material; 5' in diluted chlorite (= 0,5% active chlorite). • Rinse material 3x with tap water • Dry in tissue paper • Brief disinfect with 70% alcohol • Dry material in flow cabinet.
3	Crush in stomacher bag	Crush in stomacher bag
4	Add 40 ml buffer (PBS 0,01 M)	Add 40 ml buffer (PBS 0,01 M)
5	**Agitate apprx 30' at room temperature**	**Incubate 15' – 60' at room temperature**
6	Pipet 1,5 ml to a tube	Pipet 1,5 ml to a tube
7	Divide the extract in two tubes (one is kept in the freezer)	Divide the extract in two tubes (one is kept in the freezer)
8	100 µl use for PCR: (Harper et al, 2010; Minsavage et al, 1994).	100 µl use for PCR: (Harper et al, 2010; Minsavage et al, 1994).
9	Serial dilutions of the extract voor isolation: • Non diluted ("1:1") • 1:10 • 1:100	Serial dilutions of the extract voor isolation: • Non diluted ("1:1") • 1:10 • 1:100
10	Per dilution, 50 µl plate out on BCYE and PW.	Per dilution, 50 µl plate out on BCYE and PW.
14	**Concentrate the remaining extract:** • **Centrifuge 20' bij 10.000 g; at 4°C** • **Resuspend in 1,5 ml PB 0,01 M and repeat 7 and 8.**	**No concentration step**
11	Incubate 21 - 28 days at 28°C. • Petri dishes in a plastic bag to avoid drying out	Incubate 21 - 28 days at 28°C. • Petri dishes in a plastic bag to avoid drying out

Variables Real time PCR

- **DNA extraction: QuickPick Plant DNA Kit (Bio-Nobile) with a KingFisher isolation robot, 53022-1200, including a purification step with PVPP**
- **Thermocycler: CFX96 (Bio-Rad)**
- **Extraction kit: QuickPick Plant DNA Kit, Bio-Nobile, 53022-1200**
- **Taq: Premix Ex Taq (Probe qPCR) (TaKaRa)**

- DNA extraction: Dneasy Plant Mini Kit - Qiagen using the Qiacube isolation robot
- Thermocycler: CFX96 (Bio-Rad)
- Extraction kit: Dneasy Plant Mini Kit, Qiagen, 148012454
- Taq: TaqMan® Fast Universal PCR Master Mix, No amperase® - Applied BioSystems

Tabel 1. Bacterie isolaten; soort en typen Xf.

Isolaat	Codering isolaat	Identiteit
1	PD4397	Xf: from grapevine
2	PD4491	Xf: Ambrosia artenisiifolia
3	PD5269	Xf: from Prunus salicina
4	PD295 ^{T}	Pantoea agglomerans (P.a.)
5	PD1615	Bacillus subtilus (B.s.)
6	PD760	Pseudomonas s. pv. syringae (P.s.s.)
7	PD1006	Pectobacterium c. ssp. carot. (P.c.c.)

^{T}: isolaten met deze aanduding zijn Type strain voor betreffende bacterie.

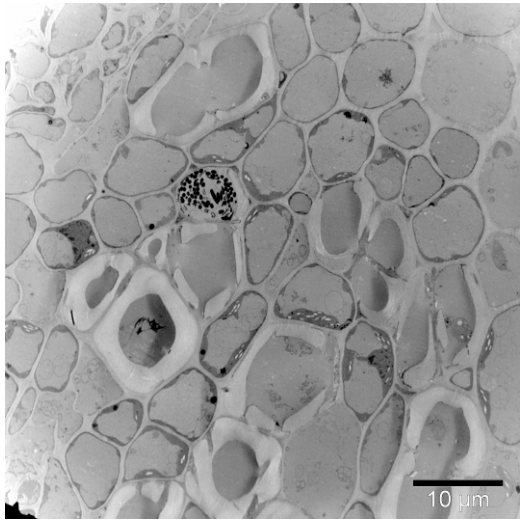
Tabel 2. Toetsmatrix en spikes voor bereiden van experimentele eenheden.

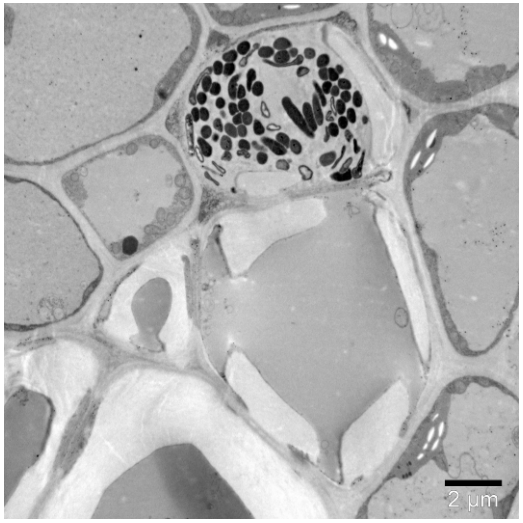
Susp.	Matrix	Bron	Spike	Concentraties spike
1	Extract van bladsteel	Coffea arabica	Xf	$10^3 - 10^5$ en NC
2	Extract van bladsteel	Nerium oleander	Xf	$10^3 - 10^5$ en NC
3	Extract van bladsteel	Olea europaea	Xf	$10^3 - 10^5$ en NC
4	Extract van compleet blad	Olea europaea	Xf	$10^3 - 10^5$ en NC
5	Fysiologische oplossing	PB 0,01 M	Xf	$10^0 - 10^5$ en NC
6	Fysiologische oplossing	PB 0,01 M	P.a.	10^7
7	Fysiologische oplossing	PB 0,01 M	B.s.	10^7
8	Fysiologische oplossing	PB 0,01 M	P.s.s	10^7
9	Fysiologische oplossing	PB 0,01 M	P.c.c.	10^7

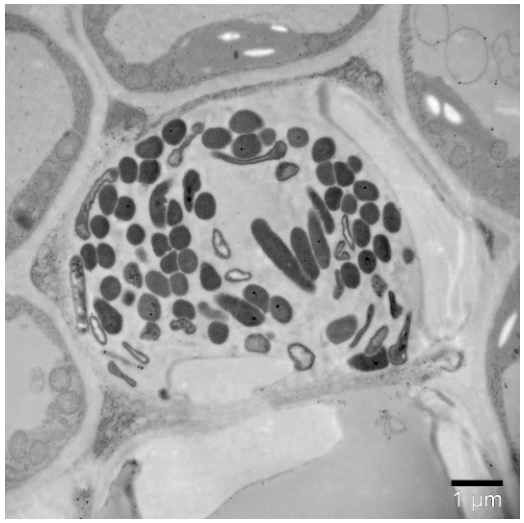
Detection limit Real-Time PCR (Harper *et al.*, 2010): $3,4*10^2$ Coffea, $2,8*10^4$ Olea

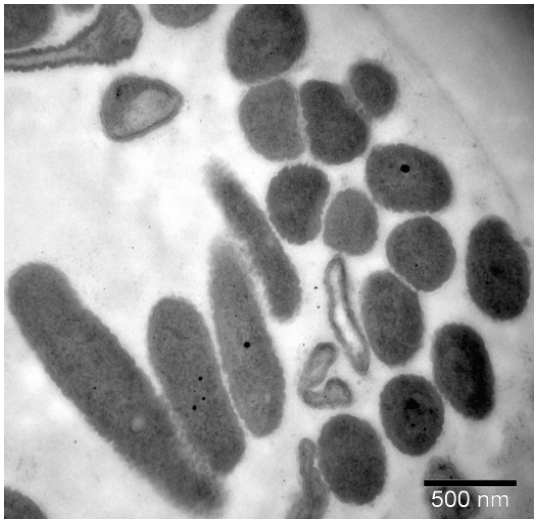
Localization in xylem by Transmission Electron Microscopy (TEM)

- sections with an ultra-microtome (Ultracut S, Reichert),
- analysed with a transmission electron microscope (JEM 1011, JEOL).
- Micrographs made with a digital camera (Veleta, Olympus)

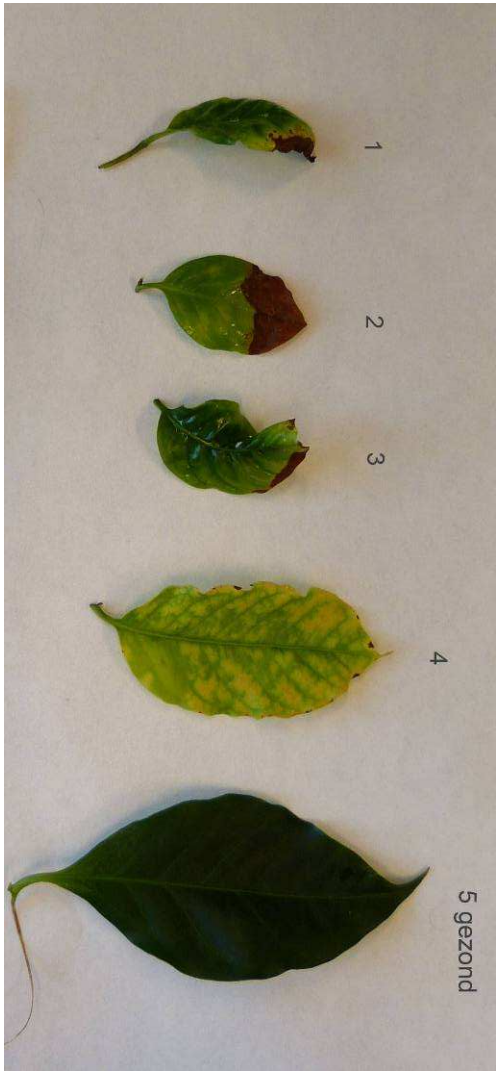








Rod shaped cells in xylem **Thick rippled cell wall**



Leaves 1 to 4 (14.0586)

Electron microscopy images from petiole leaf 2

(Bergsma-Vlami et al., in preparation)

Three genotypes from *Coffea* spp.

- Sequence analysis of the *ca*. 700 bp amplicon (RNA polymerase sigma 70 factor) showed three different sequences with 97-98% identity among each other (GenBank accession **No. KP769842-KP769844).**
- Two of these sequences were related to *C. arabica* plants imported from Costa Rica, whereas the third sequence was only present in plants imported from Honduras.

Thank you