

Salicaceae, a potential plant network for the spread of *Xylella fastidiosa* in temperate regions

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SCREENING OF SEVERAL BELGIAN POTENTIAL HOST PLANTS BY MECHANICAL INOCULATION

Which plants ?

- First screening : *Prunus domestica* cv. Opal, *Quercus petraea*, *Salix alba* with four *X. fastidiosa* strains (LMG9063, LMG17159, LMG15099 and KLN59.3)
- Second screening : *Salix alba*, *Salix caprea*, *Populus tremula*, *Populus canescens* with strain KLN59.3 Kan^r GFP⁺ (Newman *et al.*, 2003)

Inoculation

- Controlled biosafety conditions (fig. 1)
- Pinprick inoculation method (Hill & Purcell, 1995; Almeida *et al.*, 2001) (fig. 1)
- Conditions: 3×10^6 UFC inoculated per branch, 2 branches, at 22° C and 28° C
- 10 inoculated plants + 3 mock-inoculated + 3 not inoculated

Post-inoculation monitoring

- PCR (Minsavage *et al.*, 1994) on leaves petioles at 1, 3, 6, 9 months
- qPCR (Harper *et al.*, 2010) on stem and roots 9 months after inoculation
- Confocal microscopy (fig.2)
- Symptom monitoring
- Re-isolation



Fig.1 Inoculation in biosafety glasshouses

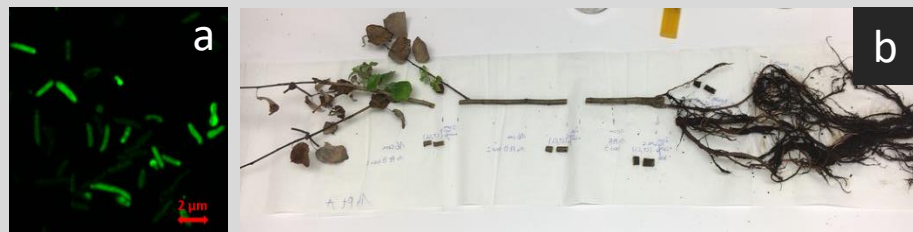


Fig.2 Destructive plant monitoring to observe *Xf*-GFP-tagged strain KLN59.3 from Newman *et al.*, 2003 by confocal microscopy (a) in pieces of the plant stem (b)

MECHANICAL INOCULATIONS REVEAL THE POTENTIAL OF THE SALICACEAE AS XYLELLA HOSTS...

- **PCR** (Minsavage *et al.*, 1994) on petioles x months post-inoculation, example for *Populus tremula* on fig.3 and fig.4
- 9-10 months post-inoculation, stem destruction and observation in **confocal microscopy** of transversal sections (TS) and longitudinal sections (LS) (slide 4)
- 9-10 months post-inoculation, stem destruction and **qPCR** on stem pieces of 15-20 cm (under progress)
- *Populus tremula* are **symptomatic** and show clearly their susceptibility (fig.5)
- *Salix alba* and *Populus canescens* are **more complex to diagnose** visually → Difficulties to identify clear differences between control group and *Xf* inoculated plants because of chlorosis and necrosis, probably due to **greenhouse growing conditions**

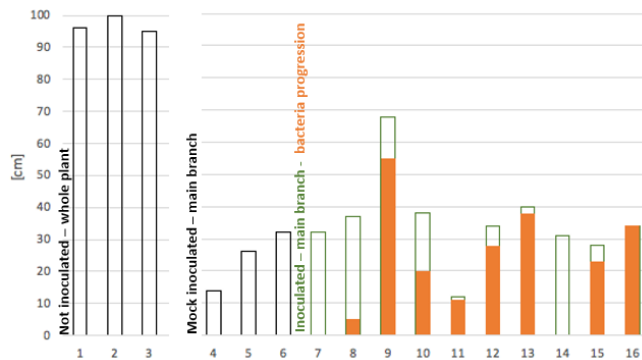


Fig.3 Progression of *Xf* in *P. tremula* 3 months after inoculation in the 22° C-greenhouse

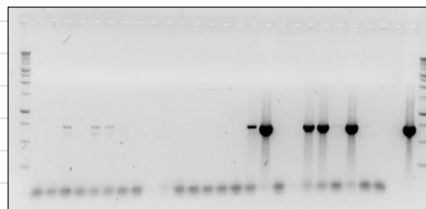


Fig.4 Electrophoresis gel of *Xf* detection with PCR Minsavage *et al.*, 1994

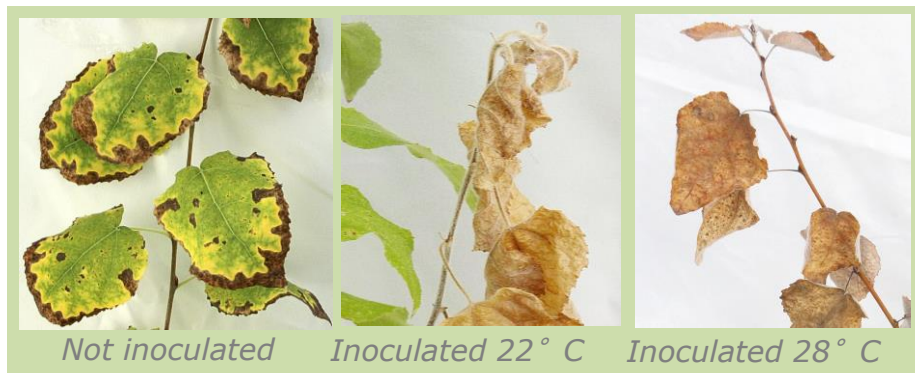
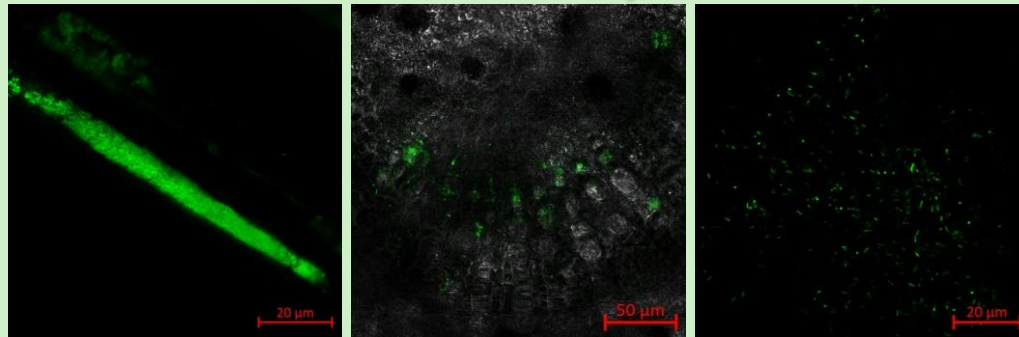


Fig.5 *Populus tremula* symptoms

CONFOCAL MICROSCOPY FOR A LOOK INSIDE

	<i>Populus tremula</i> (fig.6)		<i>Salix alba</i> (fig.7)		<i>Populus canescens</i>	
	22°C	28°C	22°C	28°C	22°C	28°C
Inoculation point (IP) detection	3/3	4/4	3/3	3/3	1/3	2/3
Apex detection	3/3	3/4	0/3	0/3	0/3	0/3
Root detection	1/1	3/3	0/1	1/1	NA	NA
Maximum spread from the IP	29 cm above the IP (stem)/ 53 cm below the IP (roots)	65 cm above the IP (stem)/ 70 cm below the IP (roots)	93 cm above the IP (stem)/ 15 cm below the IP (stem)	82 cm above the IP (stem)/ 23 cm below the IP (roots)	no spread	no spread

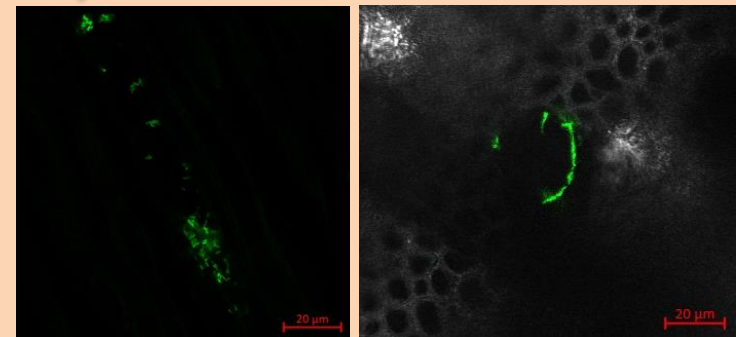


LS in the stem

TS in the stem

TS in the roots

Fig.6 Highlighting of Xf-GFP-tagged strain KLN59.3 in *P. tremula*



LS in the stem

TS in the stem

Fig.7 Highlighting of Xf-GFP-tagged strain KLN59.3 in *S. alba*

WHAT IS THE RISK POSED BY SALICACEAE ?

Salicaceae can form a closed-mesh network of dispersion of *X. fastidiosa*

- Characteristic of riparian areas
- Riparian areas and Salicaceae, respectively niche and hosts of several potential insect vectors (fig.8)
- Along roads, waterways and economic plantations (barrier to wind) (fig.9)



Fig.8 *Aphrophora salicina* and *Philaenus spumarius*

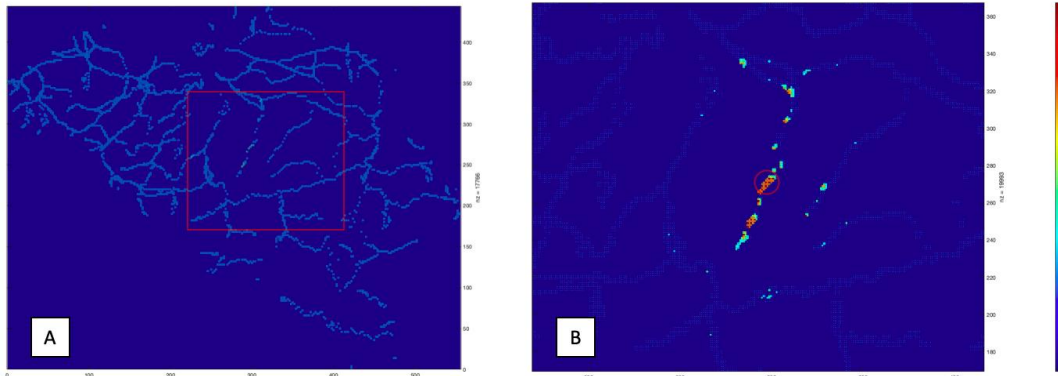


Fig.10 Simulation of the infection of *X. fastidiosa* in Belgium according to the model of White et al. (2016) six years after an infection outbreak in the center of the country, following hydrographic network (Gabriel Carestia in 2019)



Fig.9 Google view of riparian areas with Salicaceae