Current investigations on the susceptibility of potential host plants to *Xylella fastidiosa*, to evaluate the risk of introduction, establishment and spread in Belgium

N. Casarin[1*], S. Hasbroucq[1,2], A. Emond[1], J. López-Mercadal[3], M.A. Miranda[3], C. Bragard[1], J-C. Grégoire[2]

*noemi.casarin@uclouvain.be
[1]UCLouvain, Applied Microbiology, Earth&Life Institute (ELI), Louvain-la-Neuve, Belgium
[2]Spatial Epidemiology lab (SpELL), Université libre de Bruxelles, Brussels, Belgium
[3]Zoologia Aplicada i de la Conservació (ZAP), Universitat de les Illes Balears, Palma, Spain

Abstract. The susceptibility to *Xylella fastidiosa* of three plant species relevant for Belgium is investigated in two different ways, by the establishment of a Sentinel plantation in Palma de Mallorca to evaluate the infection under natural conditions, and by mechanical inoculations in controlled conditions. Nothing has been detected in the Sentinel Plantation which is a long term experiment. *Salix alba* and *Quercus petraea* got infected by *X. fastidiosa* (KLN59.3) by mechanical inoculation. Both methods have their advantages and have to be seen as complementary.

Introduction

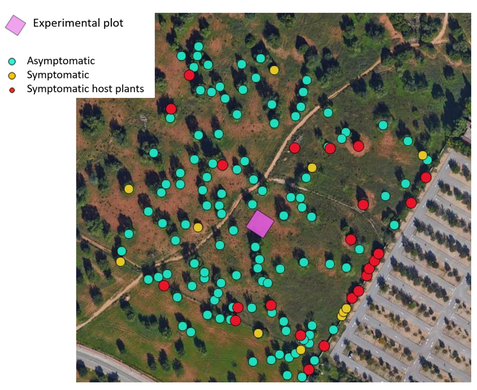
Xylella fastidiosa has been reported in 2018 in a Belgian nursery in West Flanders. Exploring scientifically the threat to the country presented by the bacterium is needed. Based on a first screening of the Xylella host plants, three model tree species were selected : ***Prunus domestica* cv. Opal, *Quercus petraea*, and *Salix alba***. Their susceptibility is simultaneously investigated by two complementary methods.

Materials & Methods

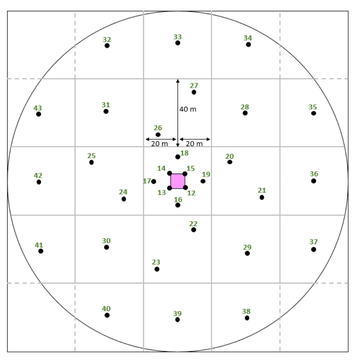
Sentinel Plantation in natural conditions (UIB – Palma de Mallorca)

- **Strains in Mallorca : ST81, ST7, ST6, ST1**
- 81 Belgian plants brought from Belgium to the UIB campus: 27 for each species.
- Detection in insects and plants : PCR of Minsavage *et al.* (1994), qPCR of Harper *et al.* (2010), nested PCR of Cruaud *et al*. (2018).
- Monitoring of the presence of the bacterium in a 100-m area around the plot :

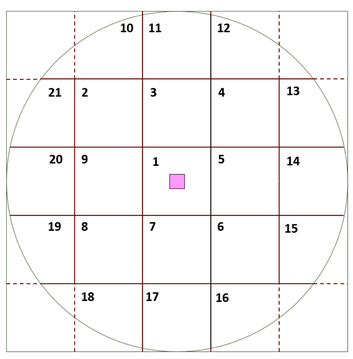
Floristic inventory



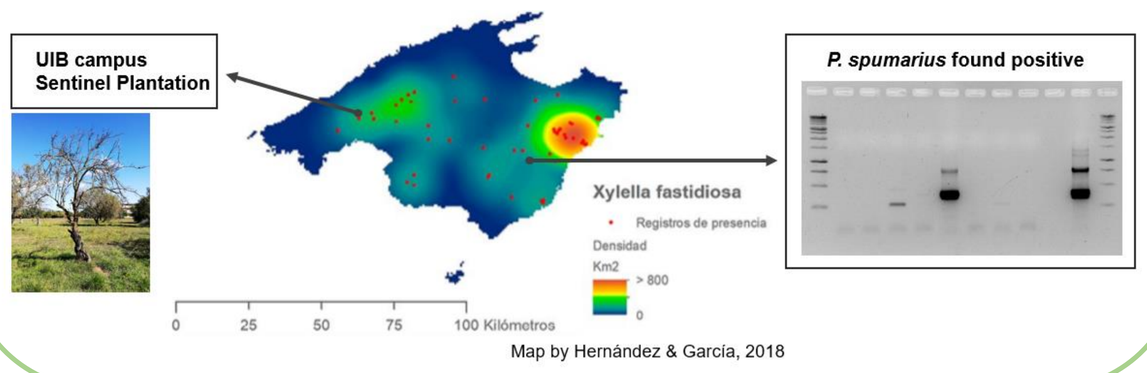
Plantation of 32 « Spy plants » ***Rosmarinus officinalis***



Insect vector sampling 25 blocks



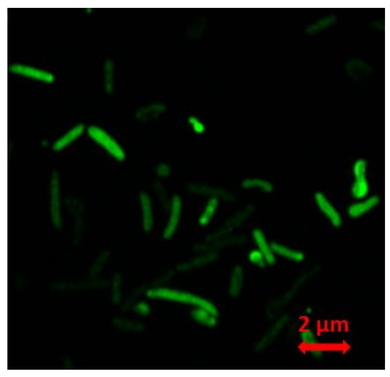
- Presence of insect vectors and symptomatic host plants.
- No bacterial detection in the plot due to detection threshold of the methods or a low infection pressure in that area.
- Detection in other parts of the Islands including the UIB campus area.



Map by Hernández & García, 2018

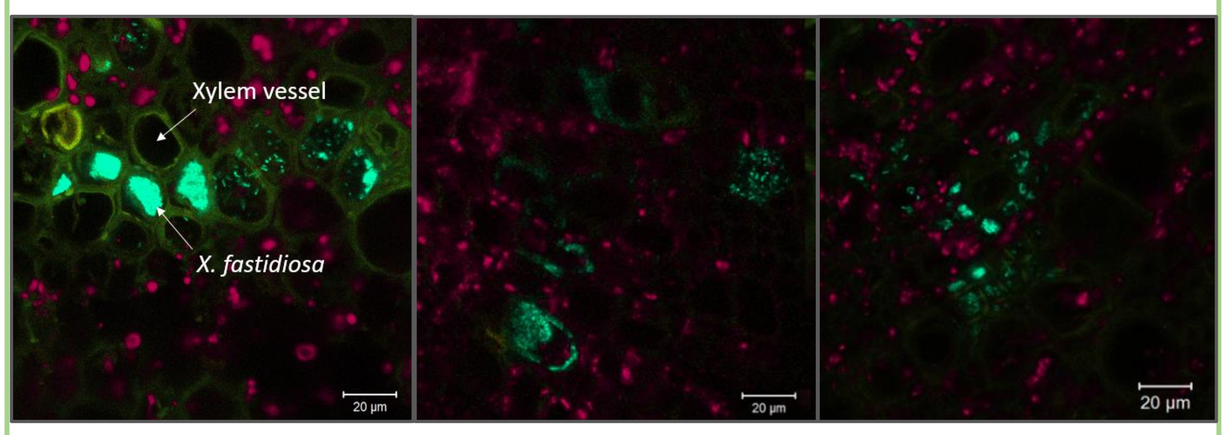
Mechanical inoculation in controlled conditions (G2Q)

- **Strain : KLN59.3 Kan^{r} GFP^{+} → ST1** (Newman *et al*., 2003)
- Control plant : *Catharanthus roseus.*
- Pinprick inoculation method (Hill & Purcell, 1995; Almeida *et al*., 2001).
- Conditions : 10^{8} UFC/ml, at 28°C, in G2Q biosafety glasshouse.
- 3 months after inoculation : detection in stem with confocal microscopy.
- PCR (Minsavage *et al*., 1994) and isolation on BCYE for Koch's Postulates.



Results

	C. roseus	*S. alba*	*Q. petraea*	*P. domestica*
PCR [No. of positive samples]	26/41 (63%)	65/85 (76%)	11/29 (38%)	0/70 (0%)
Average distance [cm]	8,3 ± 3,5	12,1 ± 19	6,3 ± 3,5	0
Isolation [No. of plants]	3/3	1/3	1/3	0/3



- Symptoms observed both on the inoculated plants and on the control plants.

Conclusion

Symptoms are not representative of bacterial detection and presence.

No detection of the bacterium in the plants and insects, probably due to low infection pressure in the region.	Detection, observation, movement and isolation on *S. alba* and *Q. petraea*. *P. domestica* not susceptible to strain KLN59.3 in these conditions.
Natural conditions, taking into account insect vector preferences and transmission efficiency.	Artificial conditions. Further from reality.
Environmental conditions can be very different from country of origin.	Allowing to work with precise controlled conditions.
Hard to implement : administrative and political barriers, follow-up of the plantation all the year → Good collaboration required.	Easy to implement, if quarantine infrastructure available.

References
Minsavage, G.V., Thompson, C.M., Hopkins, D.L., Leite, R.M.V.B.C., Stall, R.E. (1994). *Phytopathology 84*(5), 456–461.
Harper, S.J., Ward, L.I., Clover, G.R.G. (2010). *Phytopathology 100*(12), 1282–1288.
Cruaud, A., Gonzalez, A-A., Godefroid, M., Nidelet, S., Streito, J-C., Thuillier, J-M., Rossi, J-P., Santoni, S., Rasplus, J-Y. (2018). *Scientific reports*, *8*(1), 15628.
Hernández, O. G., & García, L. V. (2018. *Investigaciones Geográficas (Esp)*, (69), 55-72.
Newman, K.L., Almeida, R.P., Purcell, A.H., Lindow S.E. (2003). *Appl. Environ. Microbiol., 69*(12), 7319-7327.
Hill, B. L., & Purcell, A. H. (1995). *Phytopathology*, *85*(2), 209-212.
Almeida, R. P., Blua, M. J., Lopes, J. R., & Purcell, A. H. (2005). *Annals of the Entomological Society of America*, *98*(6), 775-786.

Acknowledgement
Special thanks to Pr. Steve E. Lindow (UC Berkeley) for the GFP-marked strain.
This project was supported by the SPF Santé Publique Belgium, funding the Xyleris project.
N. Casarin is a fellow of the *Fonds de Formation à la Recherche en Industrie et en Agriculture* (FRIA – FNRS).