Sentinel insects to anticipate, detect and monitor Xylella fastidiosa outbreaks

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INTRODUCTION

Monitoring of plant pathogens is usually carried out by analyzing symptomatic plant material. However when plants are frequently asymptomatic, which is the case for *Xylella fastidiosa (Xf)*, this approach is problematic because it limits our ability **to early detect the bacterium** and **to understand and control its spread.** To complement plant survey and facilitate large-scale monitoring, we propose to **generalize the use of insect vectors (*Philaenus spumarius*) as sentinels.**
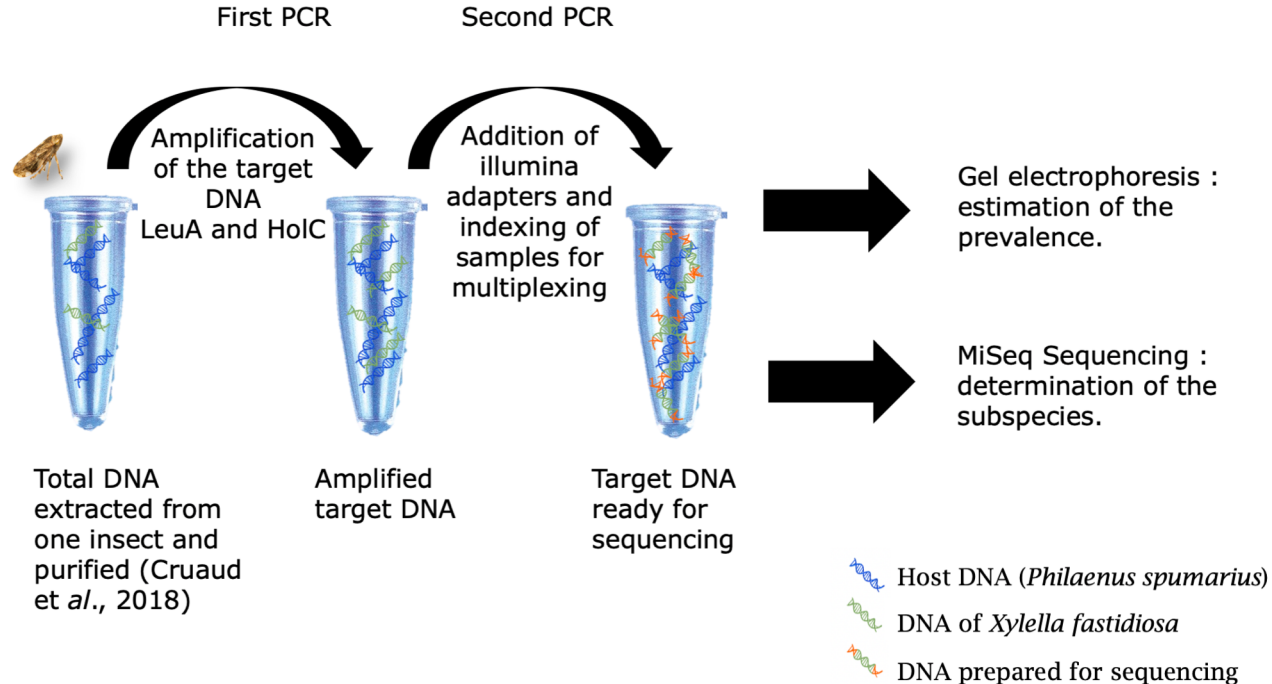
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QUESTIONS

- **How to carry out a large-scale monitoring of *Xylella fastidiosa* ?**
- **What is the prevalence of *Xf* in vector populations in Corsica ?**

MATERIALS AND METHODS

We developed a **high throughput method** based on a two-step PCR approach followed by Illumina sequencing (Cruaud et *al.*, 2017). LeuA and HolC were targeted **to identify the three main subspecies of Xf** (*multiplex, pauca or fastidiosa*). This approach enables larger screening than classic (nested) PCR approaches followed by Sanger sequencing.

First PCR

Second PCR

Amplification of the target DNA LeuA and HolC

Addition of illumina adapters and indexing of samples for multiplexing

Gel electrophoresis : estimation of the prevalence.

MiSeq Sequencing : determination of the subspecies.

Total DNA extracted from one insect and purified (Cruaud et *al*., 2018)

Amplified target DNA

Target DNA ready for sequencing

Host DNA (*Philaenus spumarius*)

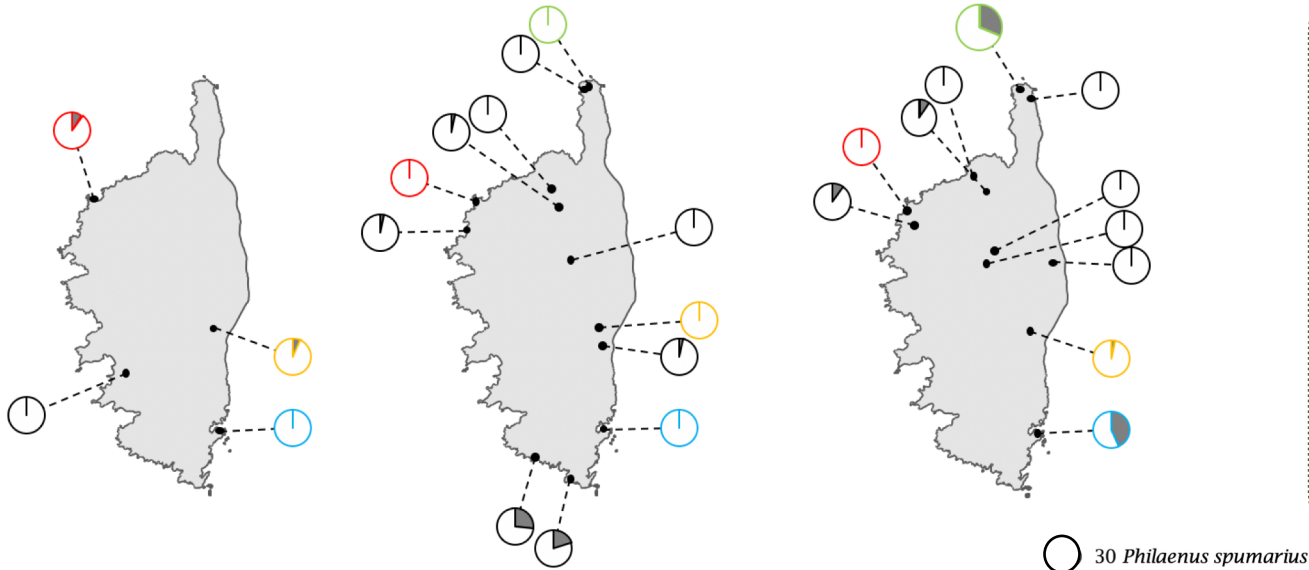DNA of *Xylella fastidiosa*

DNA prepared for sequencing

- We used this approach **to monitor the spatio-temporal prevalence of *Xf*** within populations of *P. spumarius* (27 populations of 30 specimens each).
- DNA was extracted from **single specimens** and **4 replicates of PCR** were performed for each specimen and each gene.

RESULTS AND DISCUSSION

2017   2018   2019

- *Xf* occurs throughout Corsica with a **prevalence in insects of up to 43%** depending on the region and the year of sampling.
- *Xf* is present in ecosystems assumed uncontaminated on the basis of plant monitoring
- **No spatial, temporal or vegetation-related pattern** of *Xf* prevalence in insects seem to emerge from our first results.

◯ 30 *Philaenus spumarius*

Porportion of individuals

▣ Infected

☐ Not infected

Prevalence of Xylella fastidiosa within populations of Philaenus spumarius in Corsica.
Colored pies highlight four sites monitored repeatedly.

Our results confirm that *Xf* introduction in Corsica likely predates 2015, suggest ecological resilience of ecosystems and argue in favor of using insect vectors for *Xf* survey at large scale in Europe.

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ACKNOWLEDGEMENTS