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An update of the situation of *Xylella fastidiosa* in plants and vectors in France

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Validation of *Xylella fastidiosa* detection method for plants and insect (*Philaenus spumarius*)

- Anses – Plant Health Laboratory of Angers: the **national reference laboratory (NRL)** for Ministry of agriculture, in charge of tests in the framework of *Xylella fastidiosa* (Xf) surveillance and provide **validated and efficient protocols** of detection and identification that fit for official surveillance.
- Intra and interlaboratory evaluations are performed according to the following normative references: ISO/IEC 17025 standard, EPPO standards: PM7/98, PM7/76, PM 7/84.



- Simplex Real-Time PCR : Harper *et al.*, 2010 Intralaboratory validation data available on EPPO site and EPPO protocol PM7/24 (4) (2019).
- French official reference method **MA039 V5** (www.anses.fr).
- Other method validated: real-time PCR Ouyang *et al.* (2019).
- Method validation in progress : multiplex PCR Dupas *et al.*, (2020).



- Duplex Real-Time PCR : Harper *et al.*, 2010 / loos *et al.*, 2009 (Internal control).
- Intralaboratory and interlaboratory (Euphresco, POnTE) validation data are available on EPPO site and EPPO protocol PM7/24 V4 (2019).
- French reference method: **MA065 V1** (www.anses.fr).
- Efficient on individuals and groups of ten (*Philaenus spumarius*) (Cunty *et al.*, 2020).

Situation of *Xylella fastidiosa* in France (2015 - June 2021)

- First detection of Xf outdoors in France: in 2015 in Corsica and PACA regions. **NEW: in 2020 in Occitanie.**
- Since 2015, a network of **5 official laboratories** test official samples with the French official detection method, Xf detection on more than 51 800 samples for three regions (Fig. 1; Fig. 2) (Table 1).
- Anses systematically identifies the **subspecies and sequence type (ST)**.
- Plant host range **keeps expanding** as new positive discovered.
- Natural spread of Xf from plant to plant by action of **sucking insects**. *Philaenus spumarius*, known to be the most efficient vector on olive tree in Italy, is also detected as Xf-positive in France.

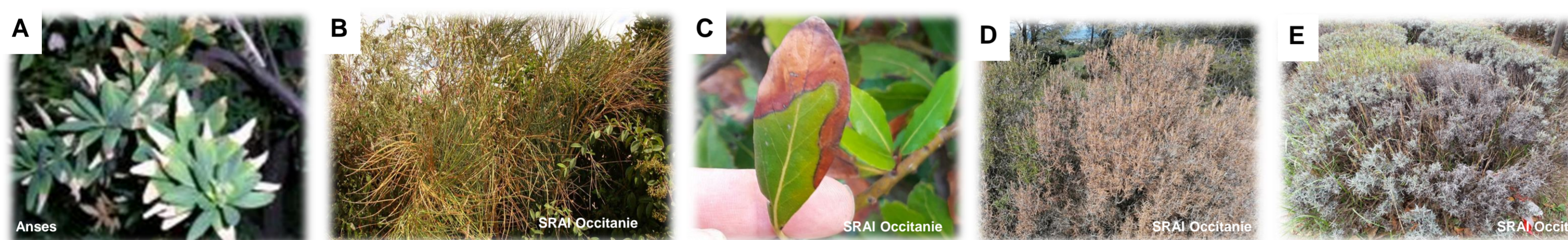


Fig 1: Symptomatic *Polygala myrtifolia* (A); *Spartium junceum* (B); *Laurus nobilis* (C); *Rosmarinus* sp. (D); *Lavandula x intermedia* (E);

Official surveillance 2015 - 2021 (June)	Sampled plant species	Tested samples	POS samples Harper's Cts38	POS samples tested by MLST	subsp multiplex (ST6 and/or ST7)	subsp pauca (ST53)
Corsica	500	19 240	1062 (5,4%)	618	414	0
PACA	659	23 276	424 (1,8%)	403	373	4
Occitanie	210	9 321	101 (1,1%)	100	92	0

Table 1 : Number of samples tested / detected as positive (PCR Harper *et al.*, 2010) and typing by MLST in the framework of official surveillance . Subspecies and/or ST identification rate really increased since 2015.

- **MLST optimized** (Yuan *et al.*, 2010; EPPO, 2019) used for subspecies identification.
- Protocol **efficient** on **most of plant species** and **vector samples** (*P. spumarius*, Cunty *et al.*, 2020).
- Subspecies **multiplex ST6** and **ST7** in Corsica and PACA (sometimes **co-infection ST6/ST7** in plants); subspecies **pauca ST53** in Menton in PACA); subspecies **multiplex ST6** in Occitanie (Fig. 3).
- **Same ST** found in *P. spumarius* and in **plants** for given **infected areas**, in Corsica and PACA.
- Estimation of **bacterial load** per insect applying **Droplet Digital PCR** (ddPCR) (Fig. 4).

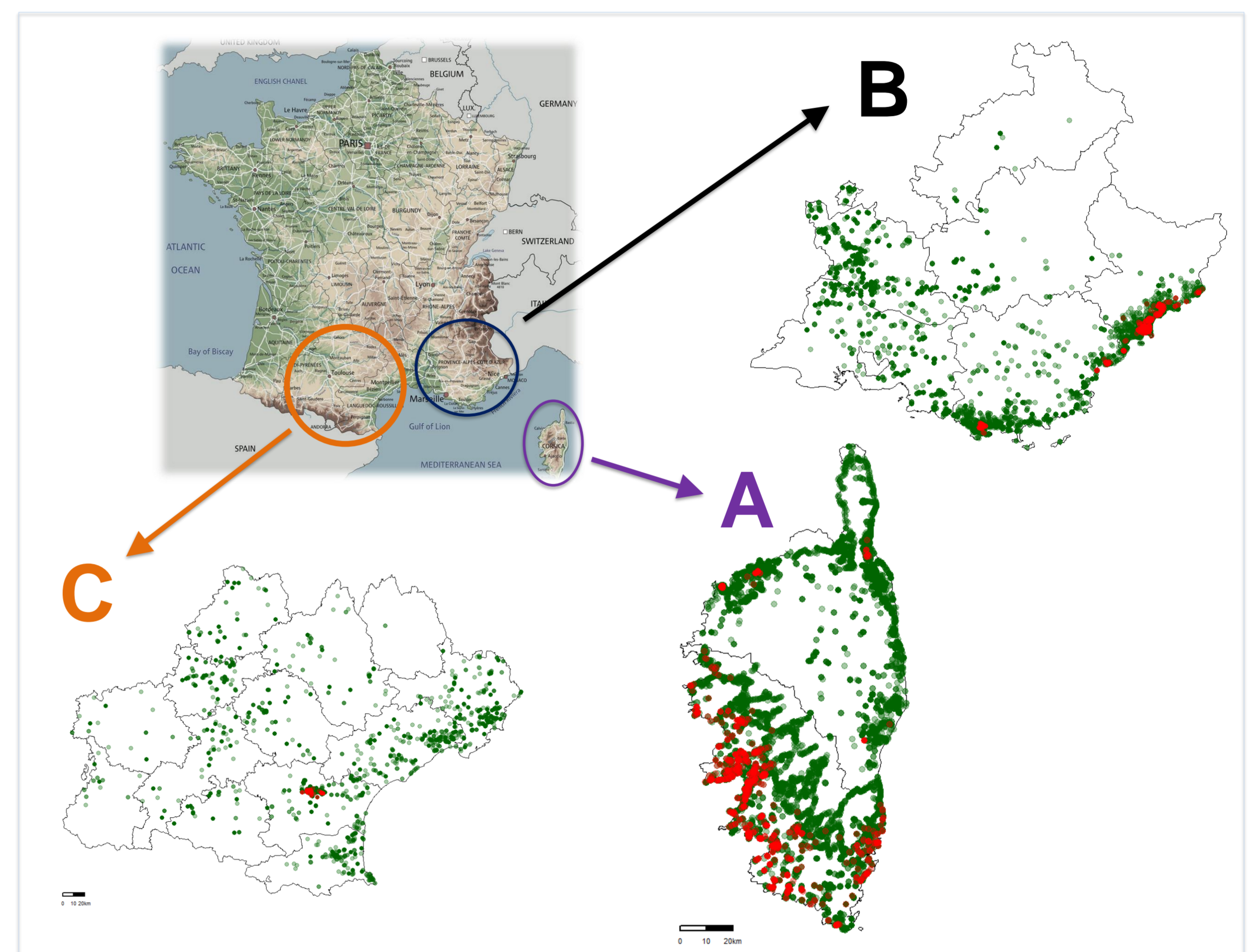


Fig. 2 : Location of positive (in red) and negative (in green) plant samples in Corsica (A), PACA (B) and Occitanie (C).

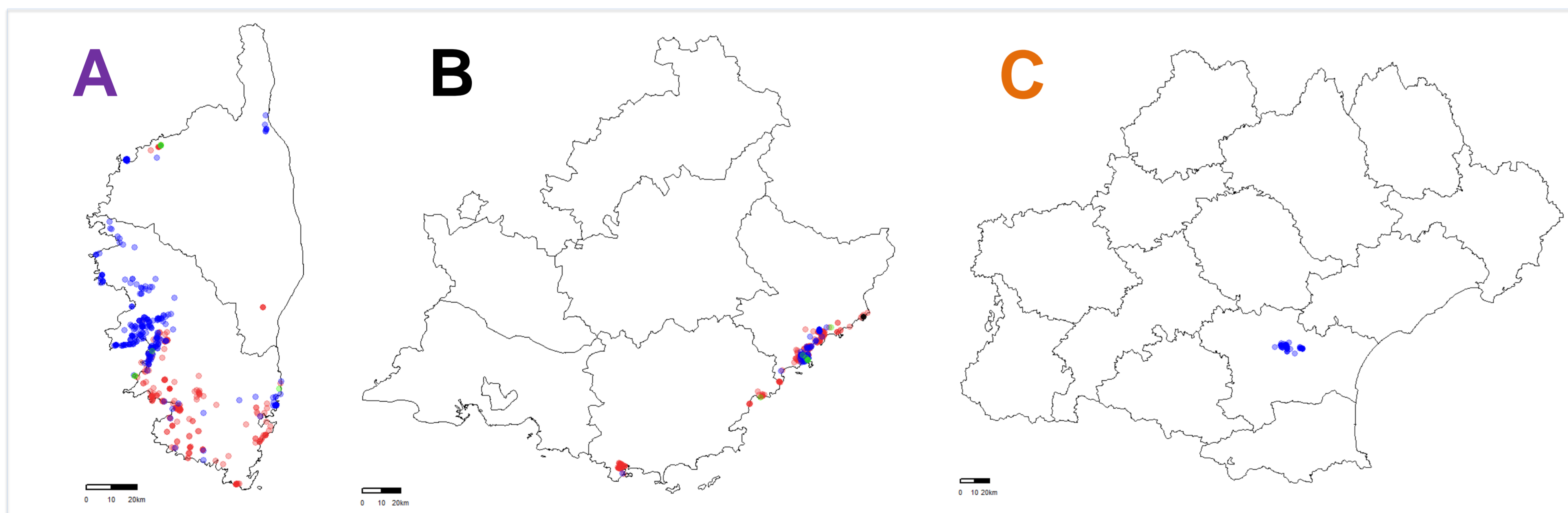


Fig. 3 : Location of ST in Corsica (A), PACA (B) and Occitanie (C). Subsp *multiplex*: ST6 in blue; ST7 in red; coinfection ST6 and ST7 in green; subsp. *pauca* ST53 in black.

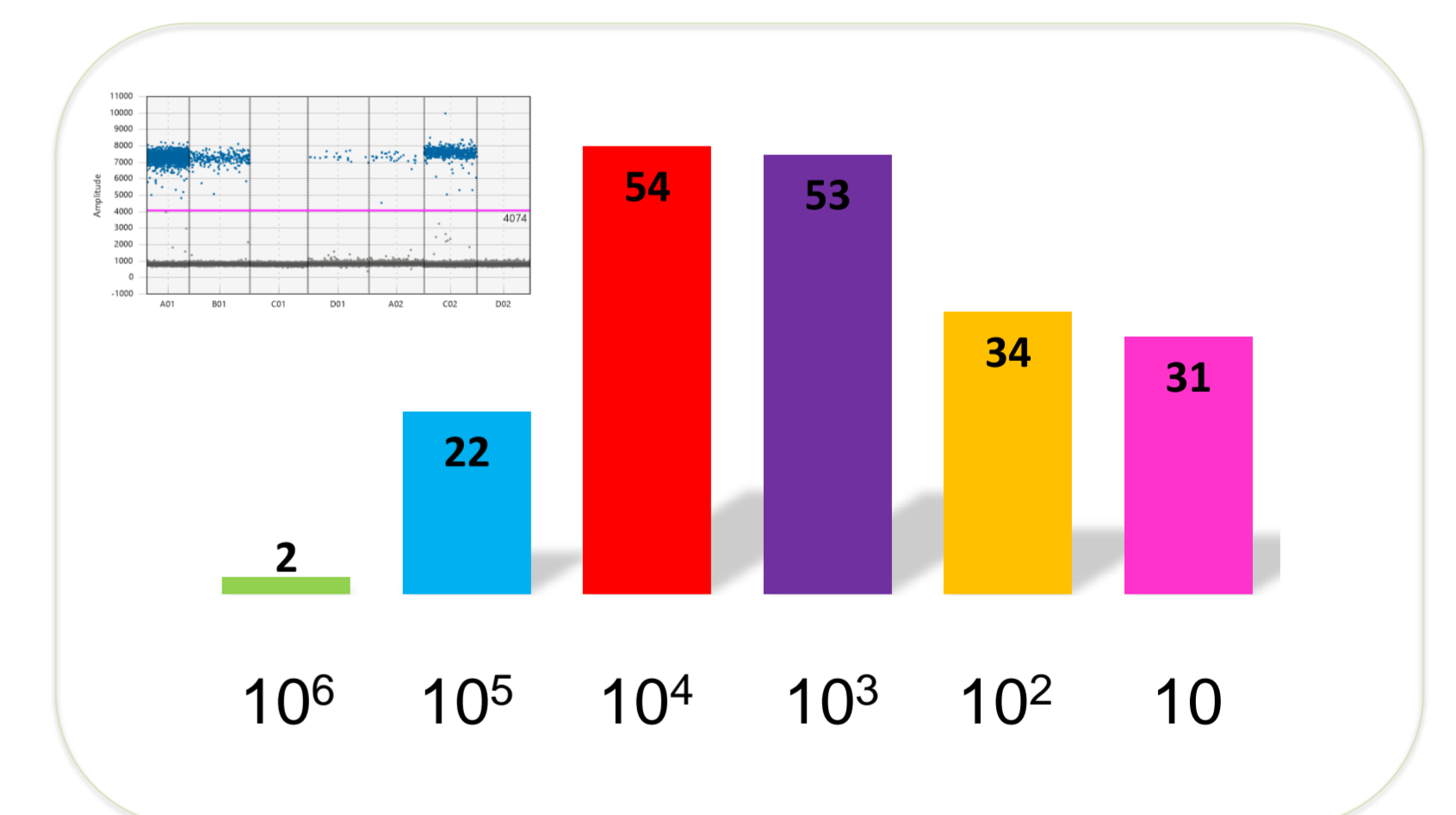


Fig. 4 : Bacterial load (cells/insect head) of 196 Xf-positive *P. spumarius* analysed individually estimated by ddPCR.

Isolation of strains from plants sampled in infected areas (2015 - June 2021)

- Isolation of *X. fastidiosa* strains from **plant samples** on **mPWG medium** (EPPO protocol PM7/24 (4) (2019)) (Fig. 5): more than 60% of success in isolating colonies.
- From 2015 to May 2021, **71 strains** subsp. **multiplex** have been isolated and **typed** from **37 different plant species**:
 - 34 from Corsica; 34 from PACA; 3 from Occitanie.
 - 27 ST6; 42 ST7.
- From **one plant sample** known to be **co-infected by ST6/ST7**, isolation of **colonies of ST6 and ST7** succeed.

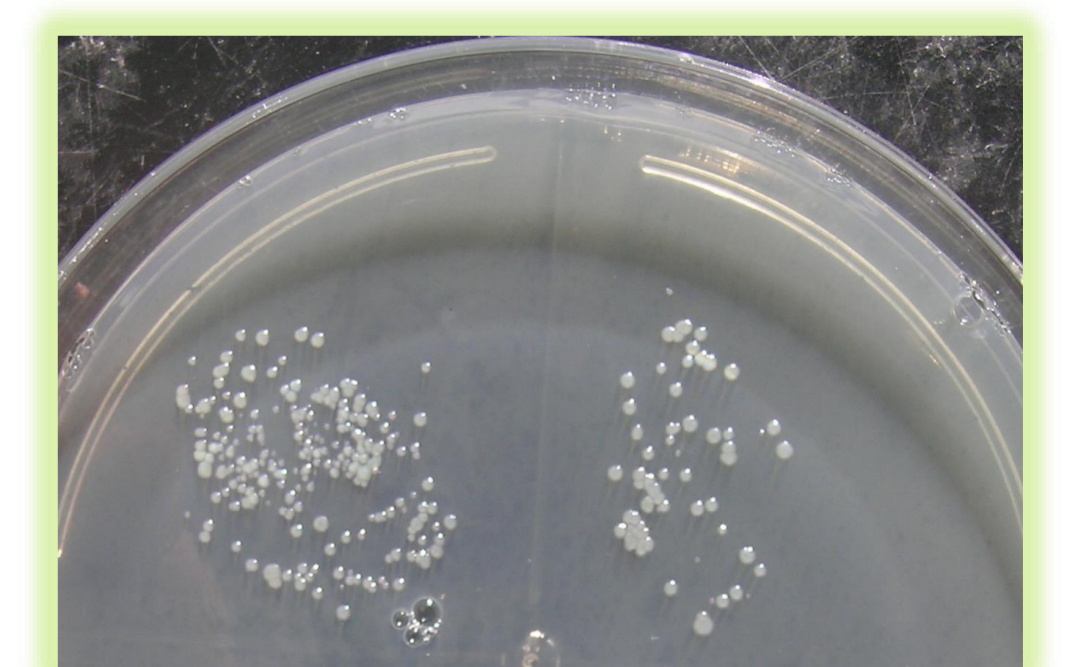


Fig. 5 Colonies of *X fastidiosa* on mPWG

Take-home message

- Anses - Plant Health Laboratory **validated efficient protocols** for the **detection of Xf** in **plants** and in ***Philaenus spumarius*** (individuals or group of two to ten). Since 2015, improvements have been validated and allowed the **detection** of infected area and **identification** of subspecies with a **good efficiency**.
- **Xf** strains detected **outdoors** in France, identified with official methods, are **belonging** to the subspecies **multiplex (ST6 and ST7)**. The subspecies **pauca (ST53)** was **only found in one locality** (Menton in PACA).
- The collection of **strains** isolated by Anses-PLH from Corsica, PACA and Occitanie plants were/are used for **research programs** and **epidemiological studies** (H2020 POnTE; Enora Dupas PhD 2017-2020).