Reconstruction of the plant-vector trophic networks involved in the spread of *Xylella fastidiosa* through hybrid capture.

European conference on Xylella 2019

Jean-Yves Rasplus[1], Anne-Alicia Gonzalez[1], Marguerite Chartois[1], Sabine Nidelet[1], Ileana Quiquerez[2], Xavier Mesmin[1,3], Pauline Farigoule[1], Jean-Marc Thuillier[1], Jean-Claude Streito[1], Domenico Bosco[4], Guillaume Fried[5], Laetitia Hugot[2], François Casabianca[6], Sylvain Santoni[3] and Astrid Cruaud[1]

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INRA SCIENCE & IMPACT

XF ACTORS

European Commission | Horizon 2020 European Union funding for Research & Innovation

Collectivité Territoriale de CORSE
Cullettività Territuriale di CORSICA

Context & questions


Astrid Cruaud


Jean-Yves Rasplus


François Casabianca

Objective: Deciphering interaction networks in which Xf spreads

Questions: Which vector species carry *Xf ?* What proportion of individuals host Xf ? How vector communities and their feeding preferences change in space and time ? Is stochastic assembly an important determinant of trophic network structures ? Can we use plant/vector community composition as a proxy to anticipate colonization route of a strain of Xf ?

Xavier Mesmin

A network of 64 experimental plots

500m² each, multiple biotic and abiotic parameters measured

Marguerite Chartois

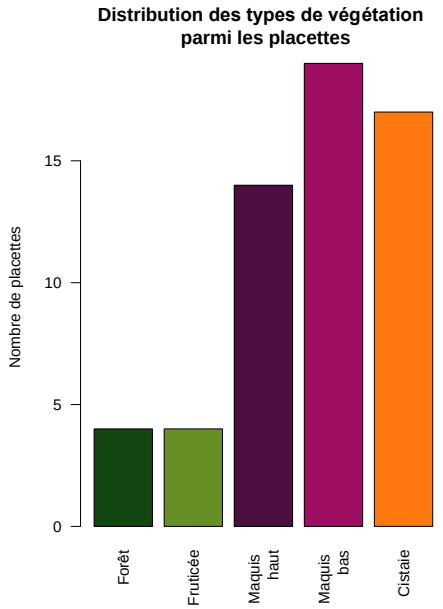
Laetitia Hugo

Ileana Quiquerez

Jean-Marc

Sabrina Borgomano

- Forest
- Shrub vegetation
- High Maquis
- Low Maquis
- *Cistus* vegetation

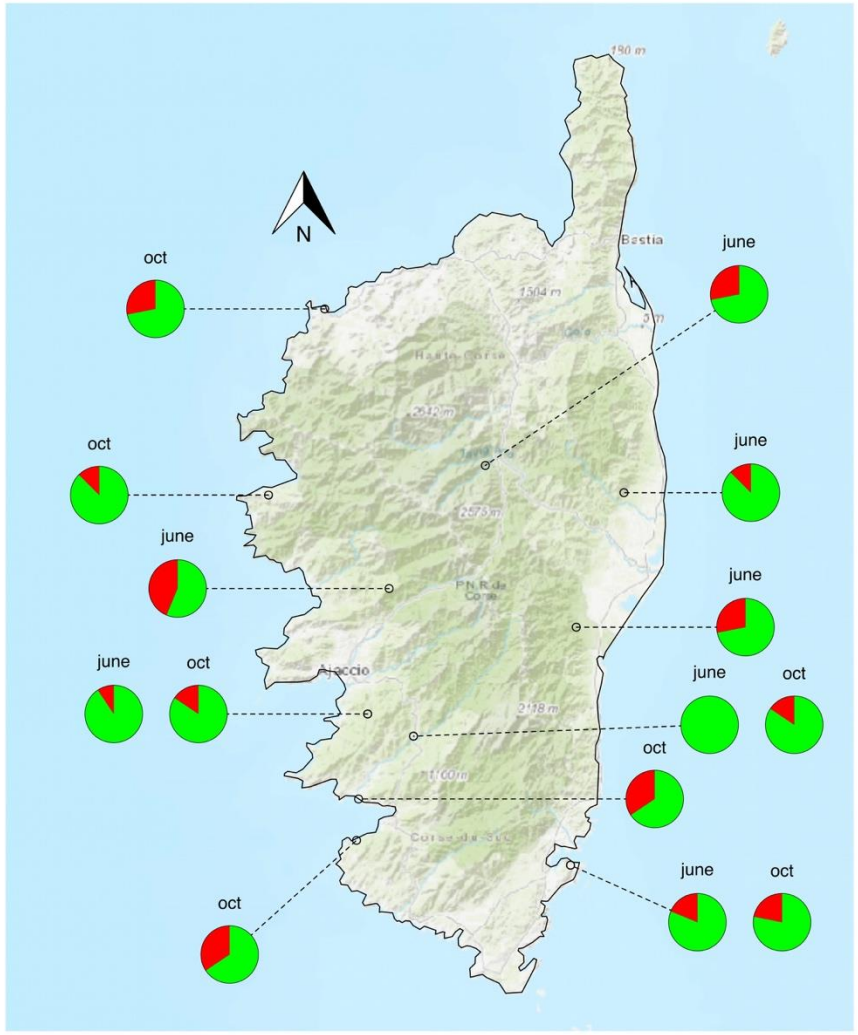


Distribution of vegetation type in the networks

See Poster of Chartois et al (P75)

Need tool to better describe, plant-vector-pathogen interactions

Experiment using PCR to assess prevalence of Xf in vector populations through space and time



11 populations of *Philaenus spumarius* in Corsica, 448 specimens

- *P. spumarius* used to detect and predict the distribution of *Xf* (see Yaseen et al 2015)
- Xf in vectors is widely distributed in Corsica, including natural habitats frequently with no apparent symptoms on plants.
- Compare prevalence in vectors (up to 30%), show importance of Cistus as a food-plant of Ps and as a reservoir of Xf in Corsica.

- No information on feeding plants, on the abundance of the plants in the sites

SCIENTIFIC REPORTS

OPEN

Using insects to detect, monitor and predict the distribution of *Xylella fastidiosa*: a case study in Corsica

Astrid Cruaud[1], Anne-Alicia Gonzalez[1,2], Martin Godefroid[1], Sabine Nidelet[1], Jean-Claude Streito[1], Jean-Marc Thuillier[1], Jean-Pierre Rossi[1], Sylvain Santoni[2] & Jean-Yves Rasplus[1]


Astrid Cruaud


Sylvain Santoni


Jean-Yves Rasplus


Anne-Alicia


Jean-Claude Streito

Hybrid capture: how and what for ?

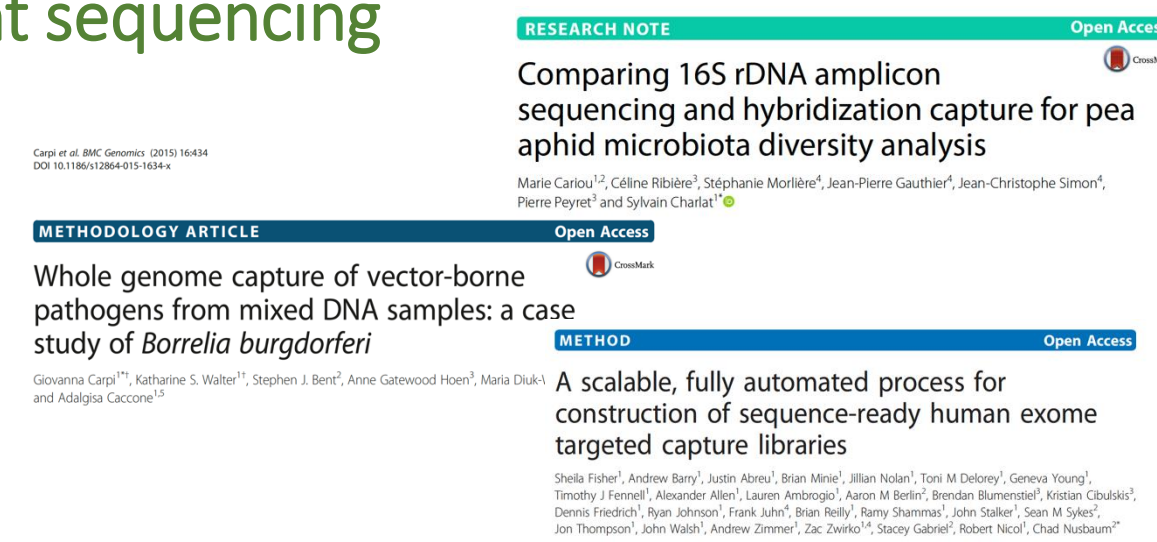
Limitation of PCR, interest of target-enrichment sequencing

NGS approaches have revolutionized ecology of trophic networks
deep sequencing of specimens (metabarcoding) => food, parasitoids, symbionts, and hosted pathogens
PCR = gold standard method for metabarcoding
low cost, rapid processing, automation, sensitivity and specificity

BUT PCR :

- may fail when polymerase are sensitive to inhibitors, targets are too divergent or rare
- Need to be multiplexed for complex diagnostic (MLST),
- Require multiple amplification to describe complex interaction
- May require culture of pathogen (sometimes unsuccessful)

To overcome these limitations:
hybridization capture followed by NGS sequencing
Replace multiplexing for complex diagnostic of human disease / cancers / description of complex bacterial communities
Is highly sensitive


RESEARCH NOTE Open Acces

Comparing 16S rDNA amplicon sequencing and hybridization capture for pea aphid microbiota diversity analysis

Marie Cariou[1,2], Céline Ribière[3], Stéphanie Morlière[4], Jean-Pierre Gauthier[4], Jean-Christophe Simon[4], Pierre Peyret[3] and Sylvain Charlat[1*]

Carpi et al. BMC Genomics (2015) 16:434
DOI 10.1186/s12864-015-1634-x

METHODOLOGY ARTICLE Open Access

Whole genome capture of vector-borne pathogens from mixed DNA samples: a case study of *Borrelia burgdorferi*

Giovanna Carpi[1*†], Katharine S. Walter[1†], Stephen J. Bent[2], Anne Gatewood Hoen[3], Maria Diuk-\ and Adalgisa Caccone[1,5]

METHOD Open Access

A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries

Sheila Fisher[1], Andrew Barry[1], Justin Abreu[1], Brian Minie[1], Jillian Nolan[1], Toni M Delorey[1], Geneva Young[1], Timothy J Fennell[1], Alexander Allen[1], Lauren Ambrogio[1], Aaron M Berlin[2], Brendan Blumenstiel[3], Kristian Cibulskis[3], Dennis Friedrich[1], Ryan Johnson[1], Frank Juhn[4], Brian Reilly[1], Ramy Shammas[1], John Stalker[1], Sean M Sykes[2], Jon Thompson[1], John Walsh[1], Andrew Zimmer[1], Zac Zwirko[1,4], Stacey Gabriel[2], Robert Nicol[1], Chad Nusbaum[2*]

Targeted Retrieval and Analysis of Five Neandertal mtDNA Genomes

Adrian W. Briggs,[1*] Jeffrey M. Good,[1] Richard E. Green,[1] Johannes Krause,[1] Tomislav Maricic,[1] Udo Stenzel,[1] Carles Lalueza-Fox,[2] Pavao Rudan,[3] Dejana Brajković,[4] Željko Kućan,[3] Ivan Gušić,[3] Ralf Schmitz,[5,6] Vladimir B. Doronichev,[7] Liubov V. Golovanova,[7] Marco de la Rasilla,[8] Javier Fortea,[8] Antonio Rosas,[9] Svante Pääbo[1]

frontiers in Microbiology

RESOURCE ARTICLE

WILEY MOLECULAR ECOLOGY RESOURCES

Capture enrichment of aquatic environmental DNA: A first proof of concept

Taylor M. Wilcox[1,2,3] | Katherine E. Zarn[1,2] | Maxine P. Piggott[3] | Michael K. Young[1] | Kevin S. McKelvey[1] | Michael K. Schwartz[1]

Hybrid Capture-Based Next Generation Sequencing and Its Application to Human Infectious Diseases

*Maxime Gaudin and Christelle Desnues**

METHODOLOGY ARTICLE Open Access

Exome-wide DNA capture and next generation sequencing in domestic and wild species

Ted Cosart[1,2,3*], Albano Beja-Pereira[3*], Shanyuan Chen[3], Sarah B Ng[4], Jay Shendure[4] and Gordon Luikart[3,5]

Gene capture : how it works ?

1. Extraction of total DNA

Vector + microbiont

Xylella

Plants

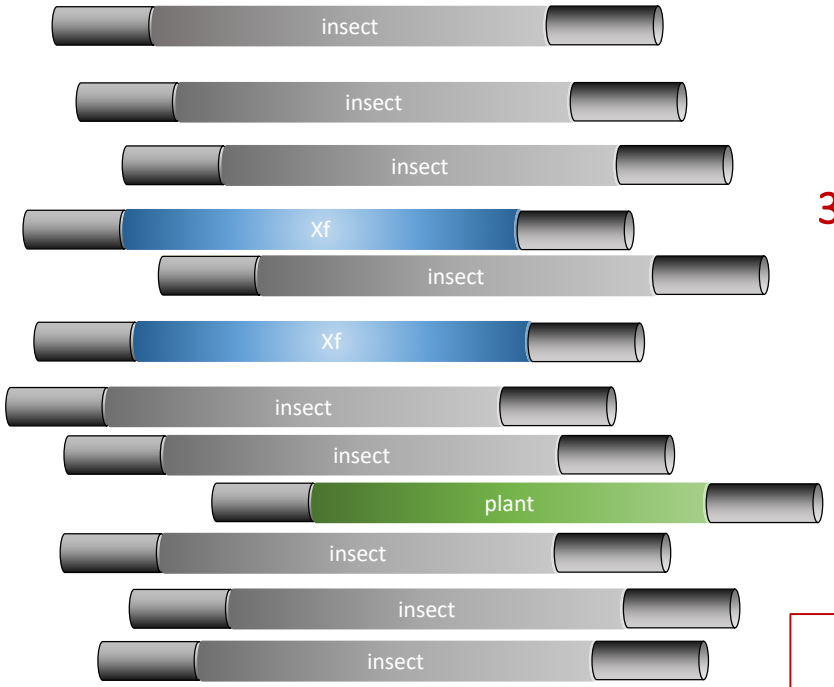
Philaenus

Xylella

Plantes

microbionts

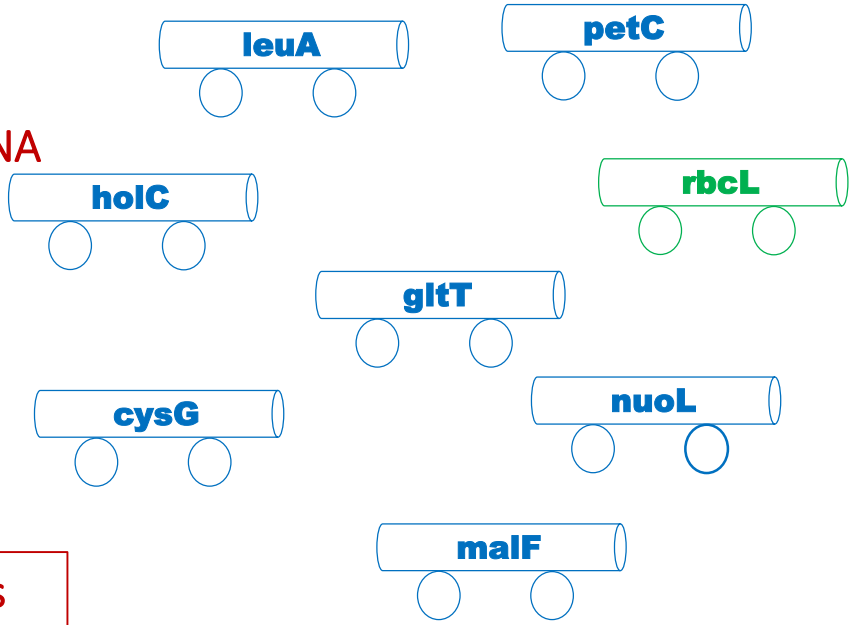
2. Shearing of total DNA and tagging
(1 combination of tags per insect)

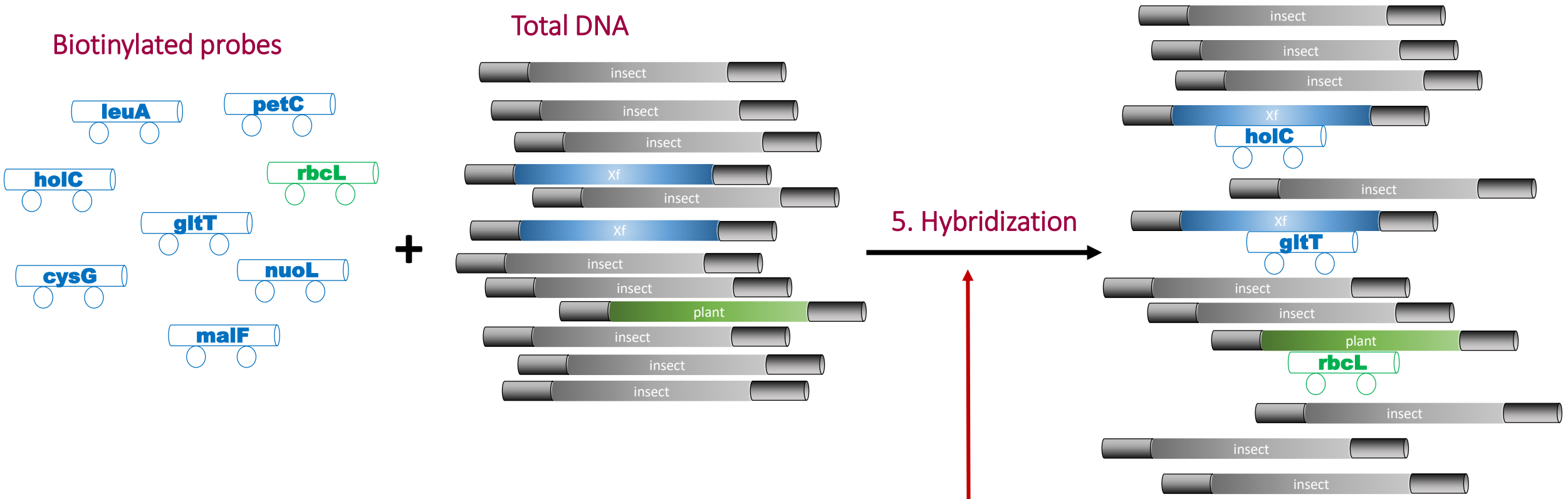


3. Equimolar pooling of total DNA

The number of pooled insects needs to be optimized to avoid competition ensure capture of target DNA (plants & Xf)
1, 2 etc …

4. Addition of probes complementary to :
a) DNA of Xf (7 loci of the MLST scheme)
b) DNA of plants (rbcL – barcoding fragment)



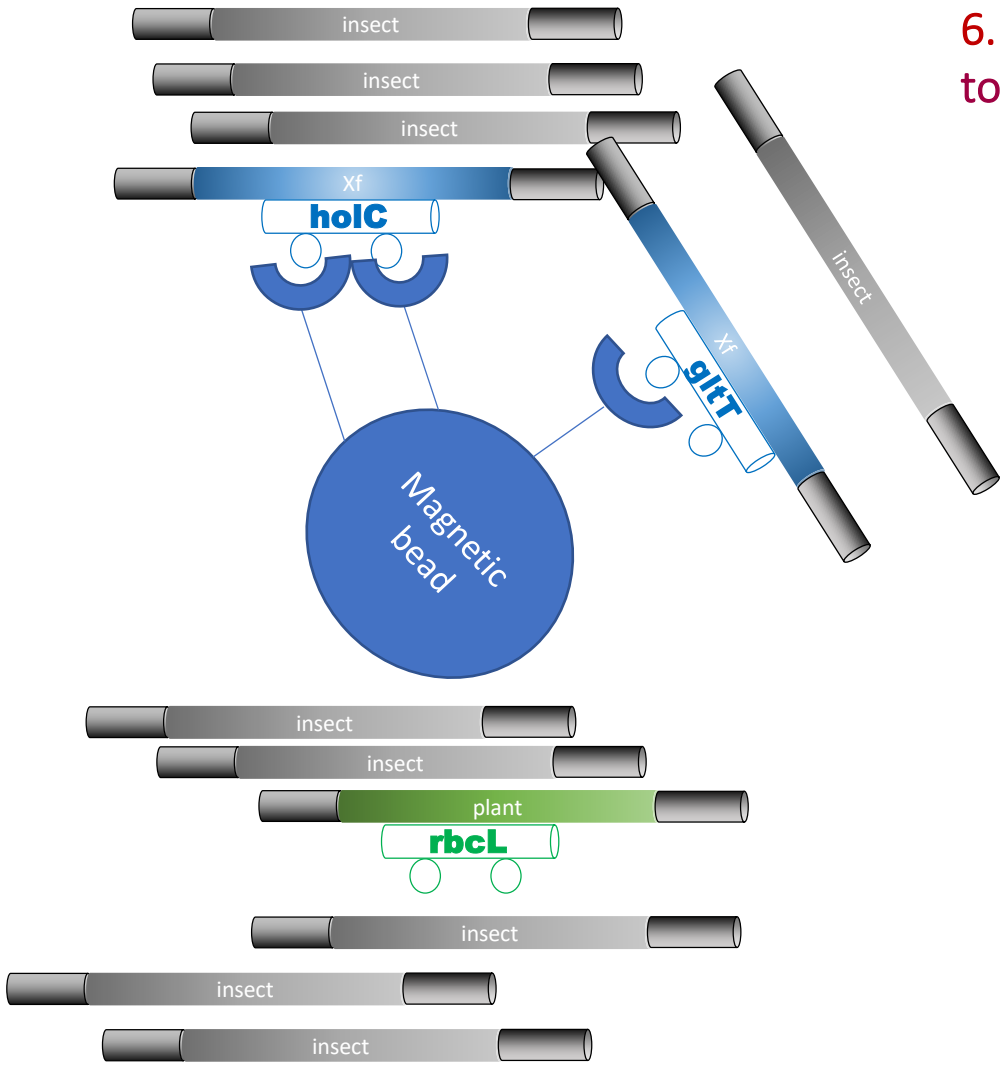


Hybridization time needs to be optimized to:

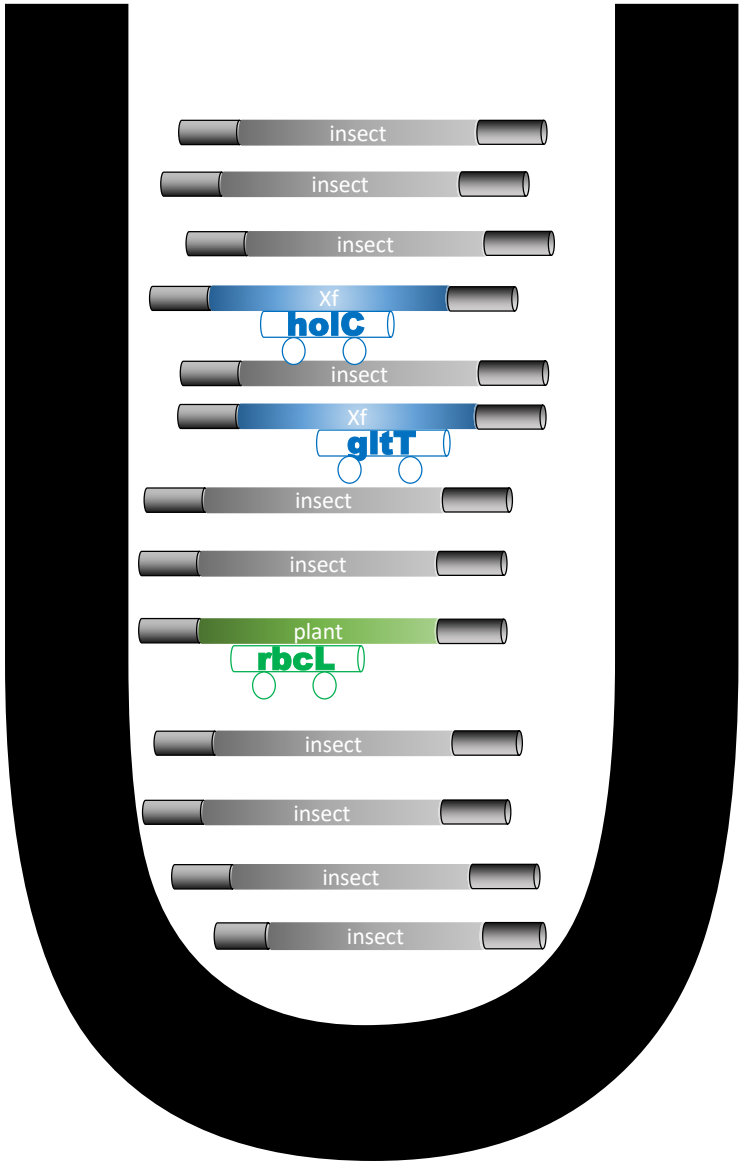
-ensure capture of target DNA (plants & Xf)

-prevent capture of non-target DNA (insect & aerosols)

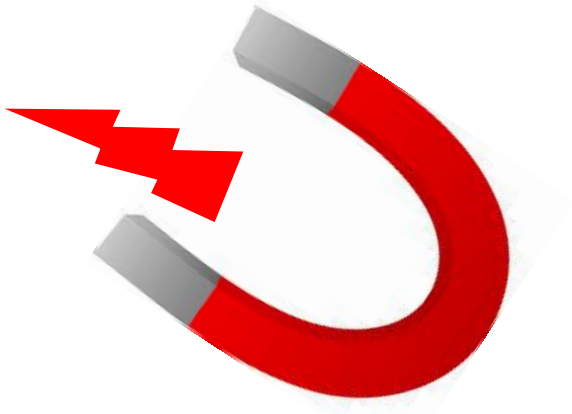
Few hours ? One night ? 48 hours ?



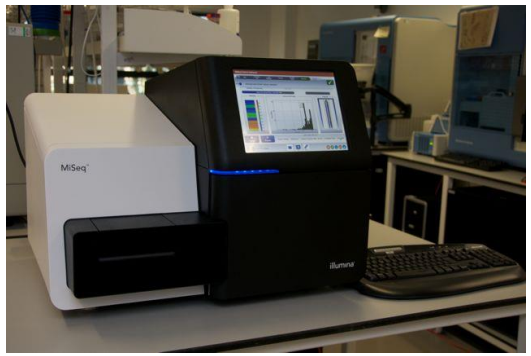
6. Probe-target hybrids (plants & Xf) are bound to streptavidin-coated magnetic beads



8. Most non-target DNA is washed away

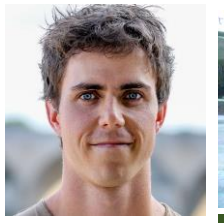


7. Probe-target hybrids are sequestered with a magnet



9. Bead-bound target DNA is amplified, removed from the beads and sequenced with NGS approaches (MiSeq)

Results: tools, pipeline & ingested sap


Xavier Mesmin


Ileana Quiquerez


Marguerite Chartois

Biological material for test

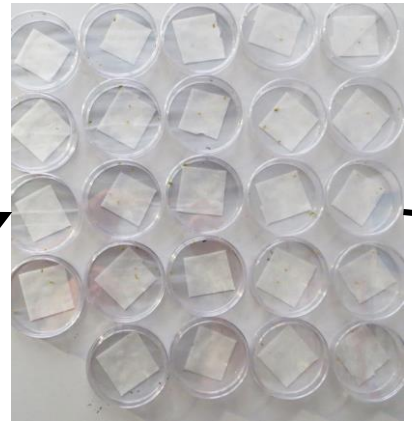
- Sampling (Europe and USA) and rearing on known feeding plants
- Several hundreds of specimens
- Nymphs reared to determine the rate of decay in detectability, typically expressed as the DNA half-life (time during which plants remains are detectable)

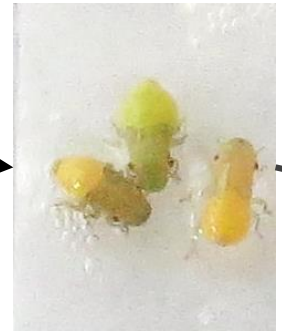


Sampling of nymphs on *Cistus*


30 nymphs in alcohol


120 nymphs in Petri dishes (5/box)


laboratory screening


sampling of 20 nymphs every 2h


alcohol

Probe design for plants

- Starting point rbcL (barcode plants) : 87 360 sequences (NCBI), 443 families, 38052 sp
- Long fragment of rbcL. Reduced set of probes 187 families of European plants
- Sequence cleaning (non coding, outliers, etc.)
- **Filtration to only keep sequences with divergences > 95%** [to reduce bait number]
- Probes of 100bp with 50% overlap → 4,972 probes

Probe design for Xf

- Starting point : all variants of MLST loci available on pubMLST + reference genomes
- Probes of 100bp with 50% overlap → 511 probes

myBaits®

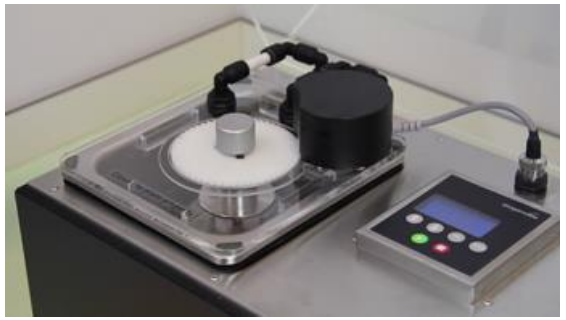
Final mix

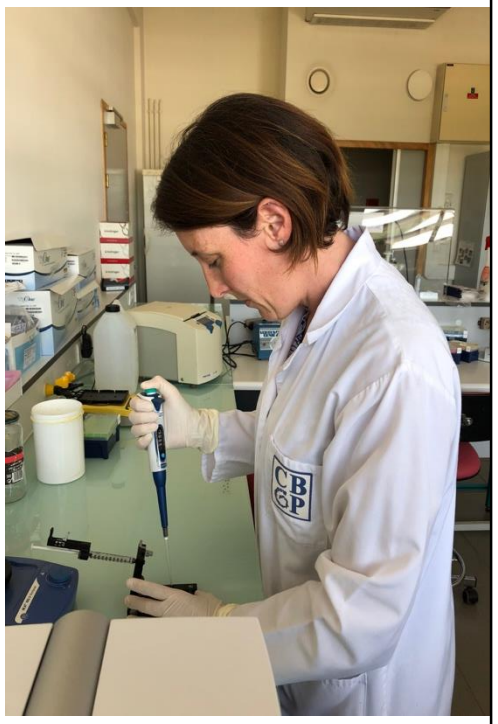
- 4972 plant probes + 10 x 511 probes of Xf MLST [equimolar pool]
- Verified for hybridization on Aphrophoridae genome and synthetized by mybaits

Astrid Cruaud

Construction of two libraries with different protocols

- test of key parameters (hybridization time etc)
- 4 adult individuals previously screened for the presence of Xf
- 32 adults individuals sampled on known plants



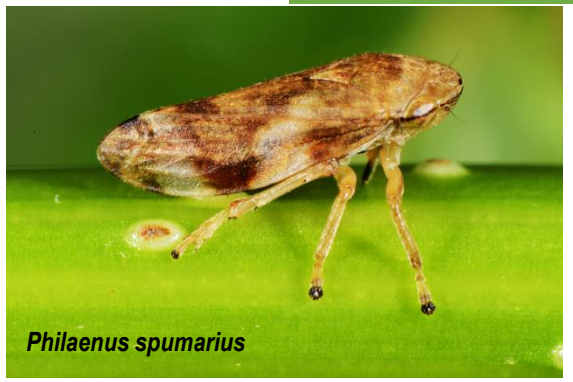


Sabine Nidelet

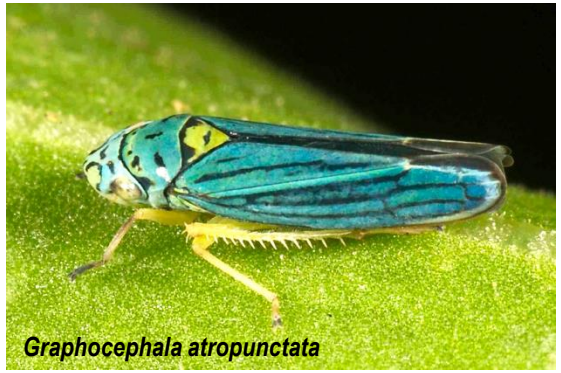
Anna Gonzalez

Sylvain Santoni

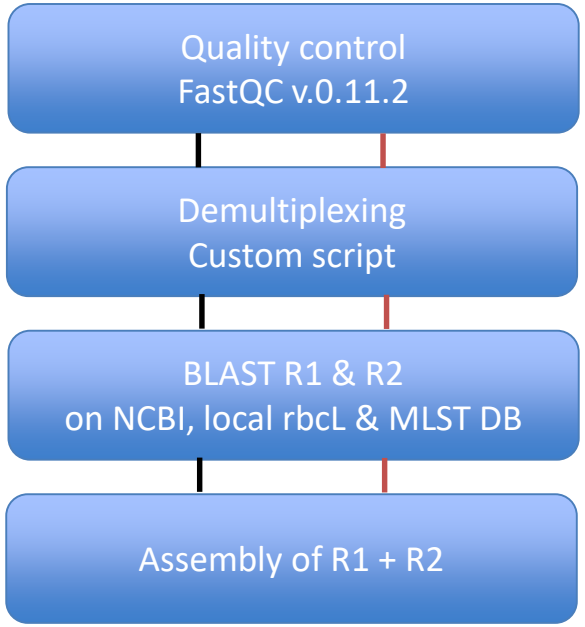
Pauline Farigoule



Philaenus spumarius



Graphocephala atropunctata



Development of a pipeline to process sequences

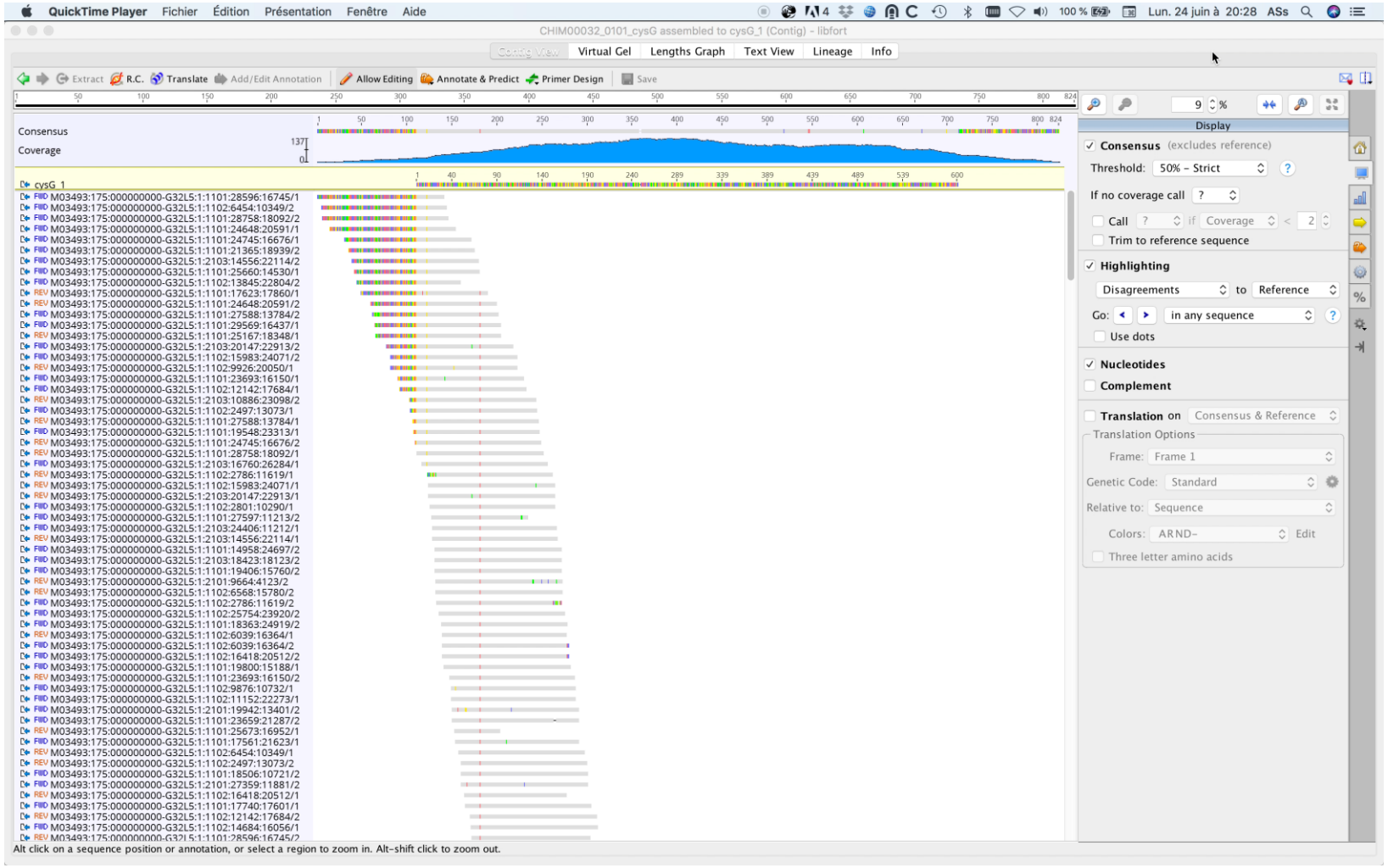
- Reads are sorted by quality
- Low quality nucleotides are trimmed
- Reads are demultiplexed based on combination of barcodes used to tag them
- Forward and reverse reads are blasted on different databases
- Reads are assembled into loci

Astrid Cruaud

Probe validation on cultures of inactivated bacteria (coll. M.A. Jacques)

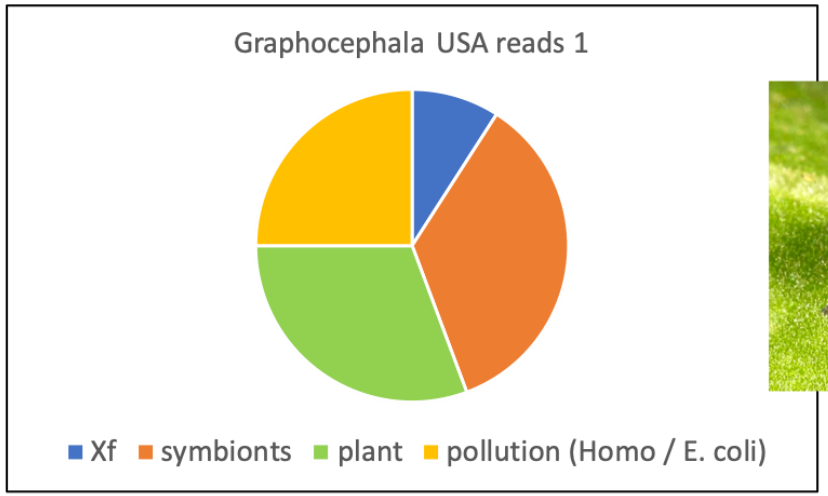
Efficient capture of all loci on all tested subspecies of Xf

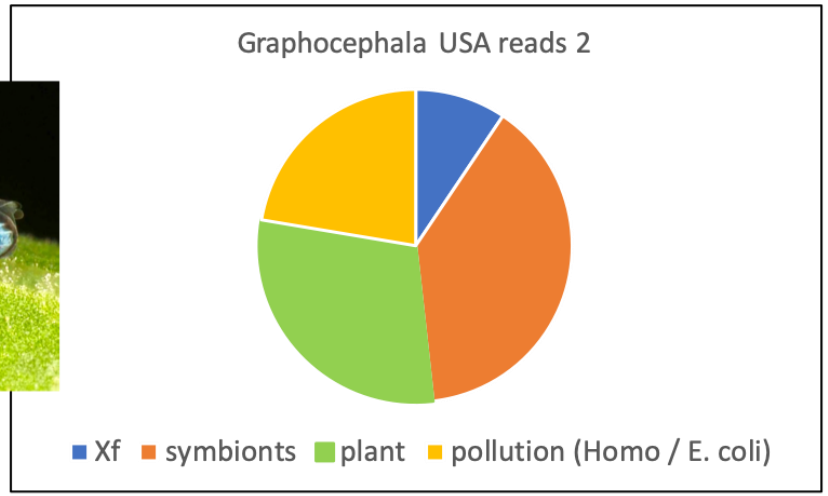
Validation of our probes

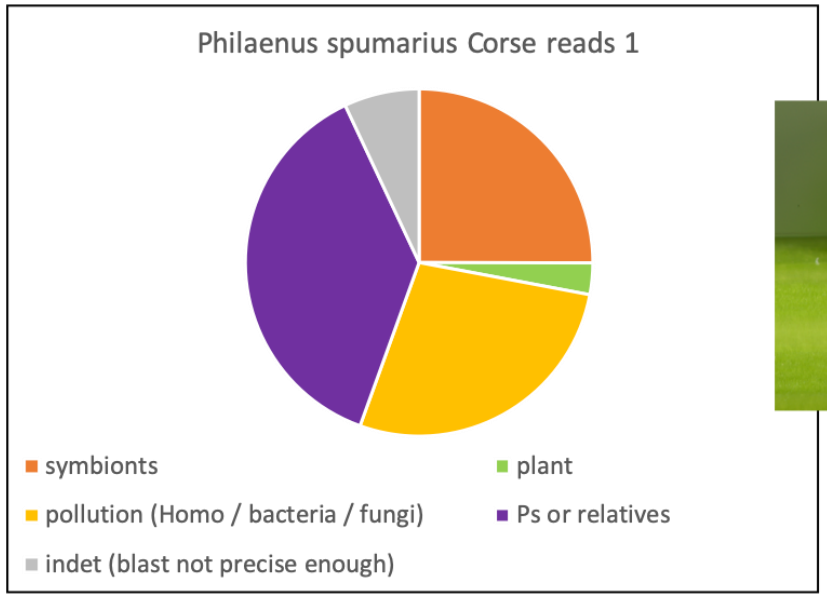


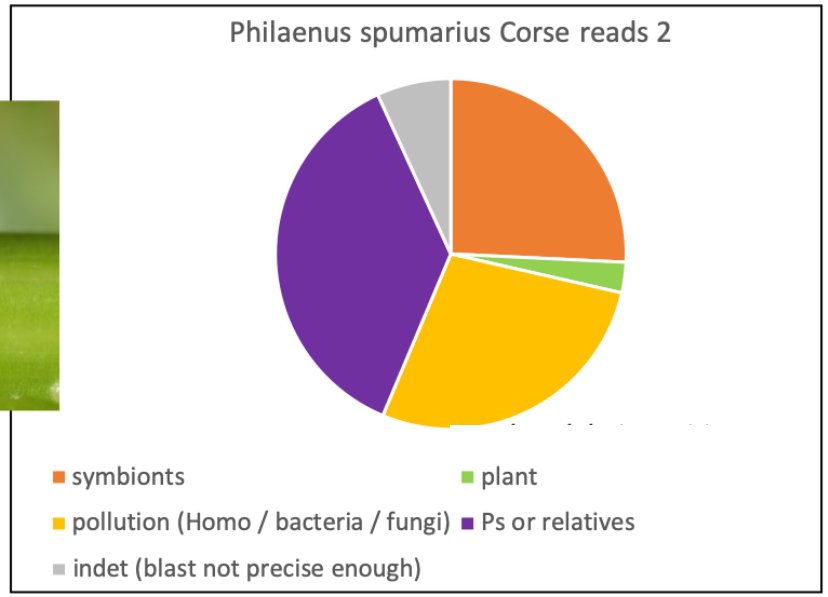
Sequencing depth of cysG locus

BLAST on NCBI of R1 and R2 obtained for two vectors (USA and Corsica) – 60h / 8 ind.



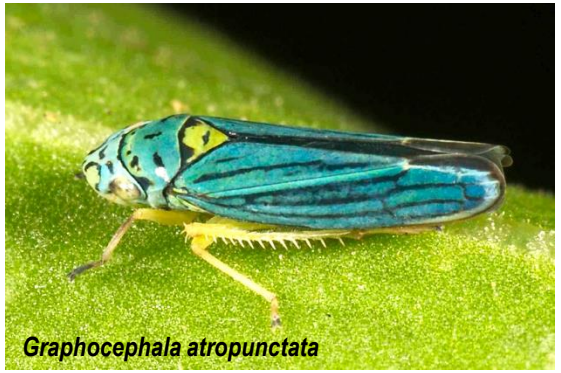




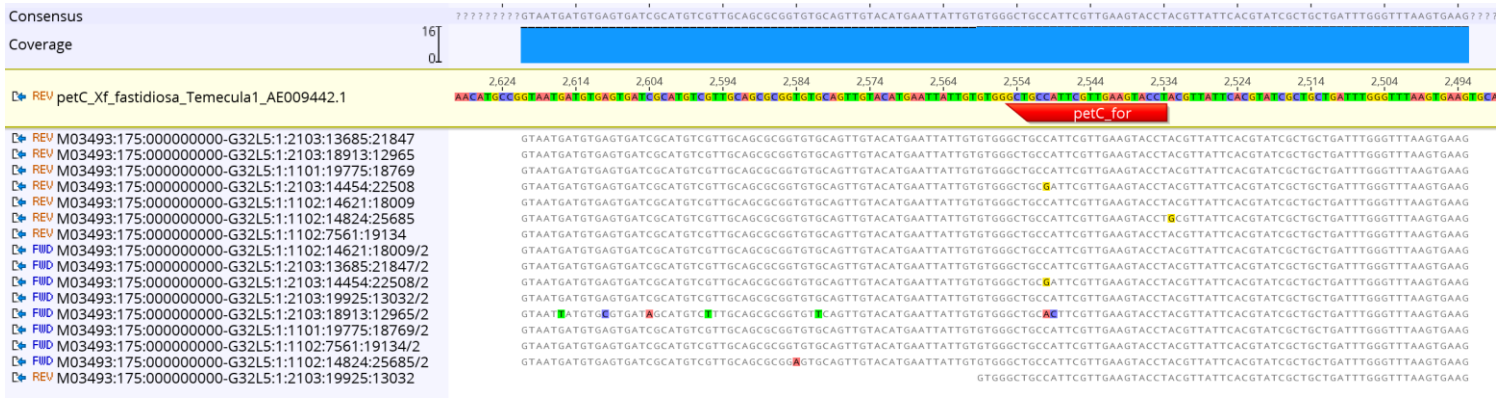


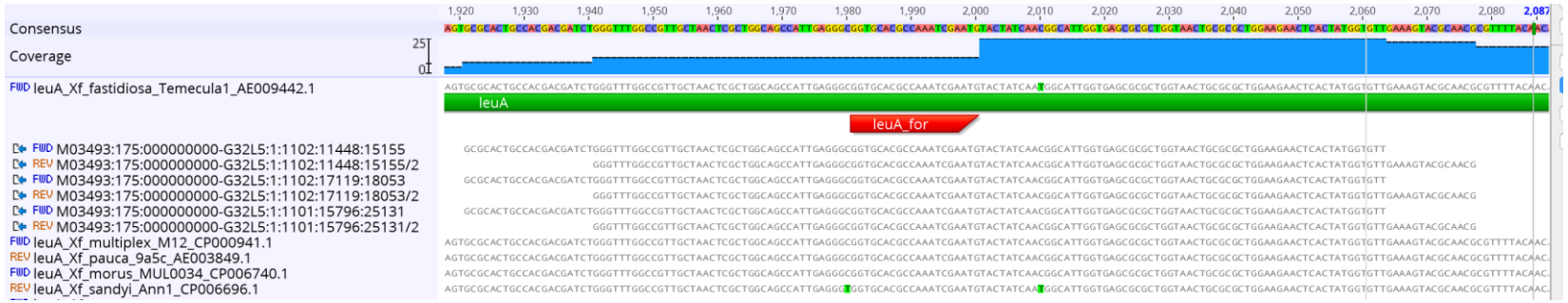
60h may be too much as we have aspecific hybridization (Homo)

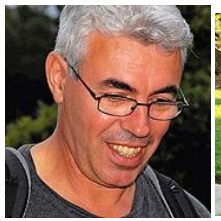
Sequencing of *Xf* MLSTs



Results are limited to leuA and petC => still request optimization
Sequencing depth is problematic in some individuals
Could just be due to competition with inactivated bacteria

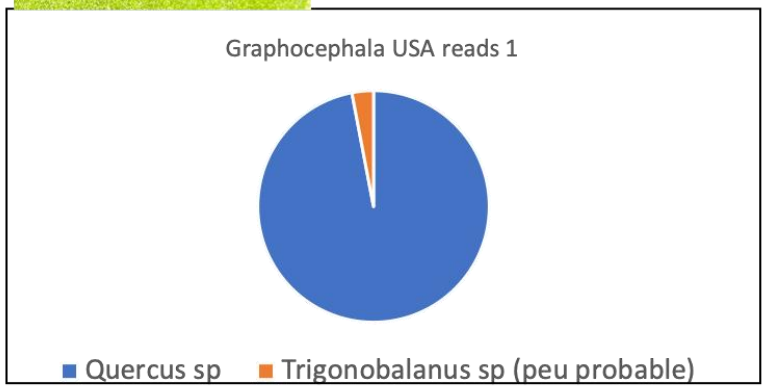


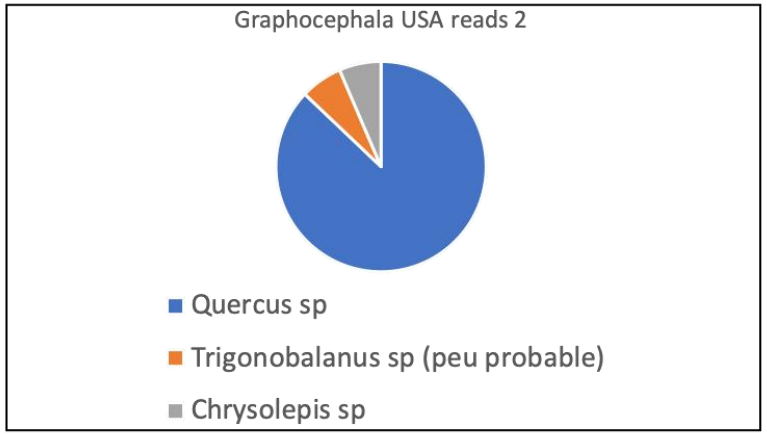


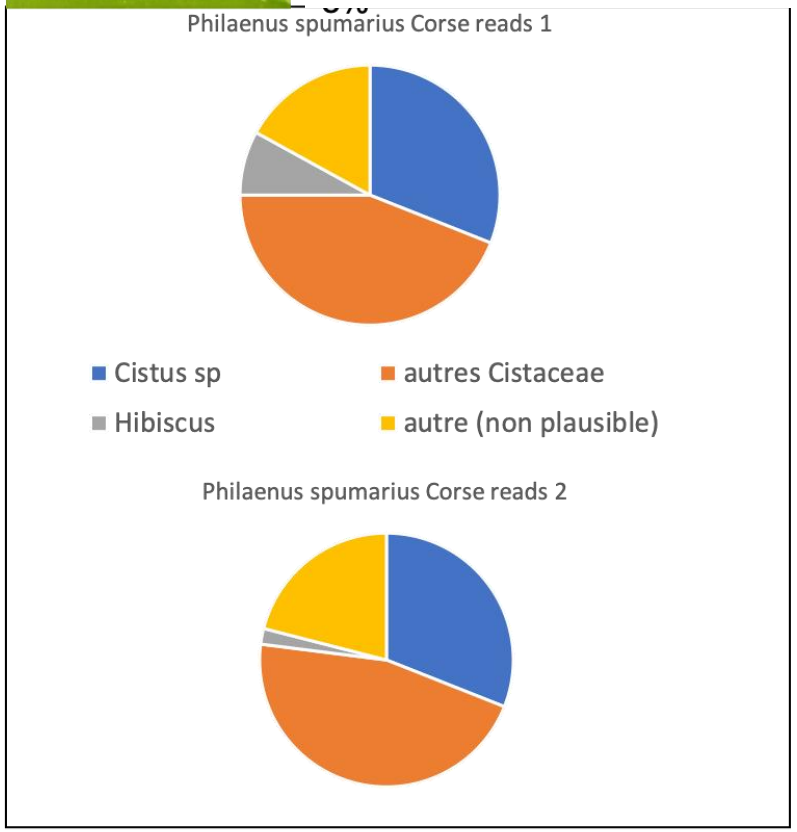

Jean-Yves Rasplus


Astrid Cruaud

Successful identification of ingested sap or plant tissues in piercing mouthparts of Corsican and Californian vectors (sampled in 2012 & kept in EtOH: ***Quercus*** *and* ***Cistus***)







Some American vectors also fed on *Vitis* before dying ☺

Conclusion & perspectives

Preliminary results are encouraging

Optimization still needed for :

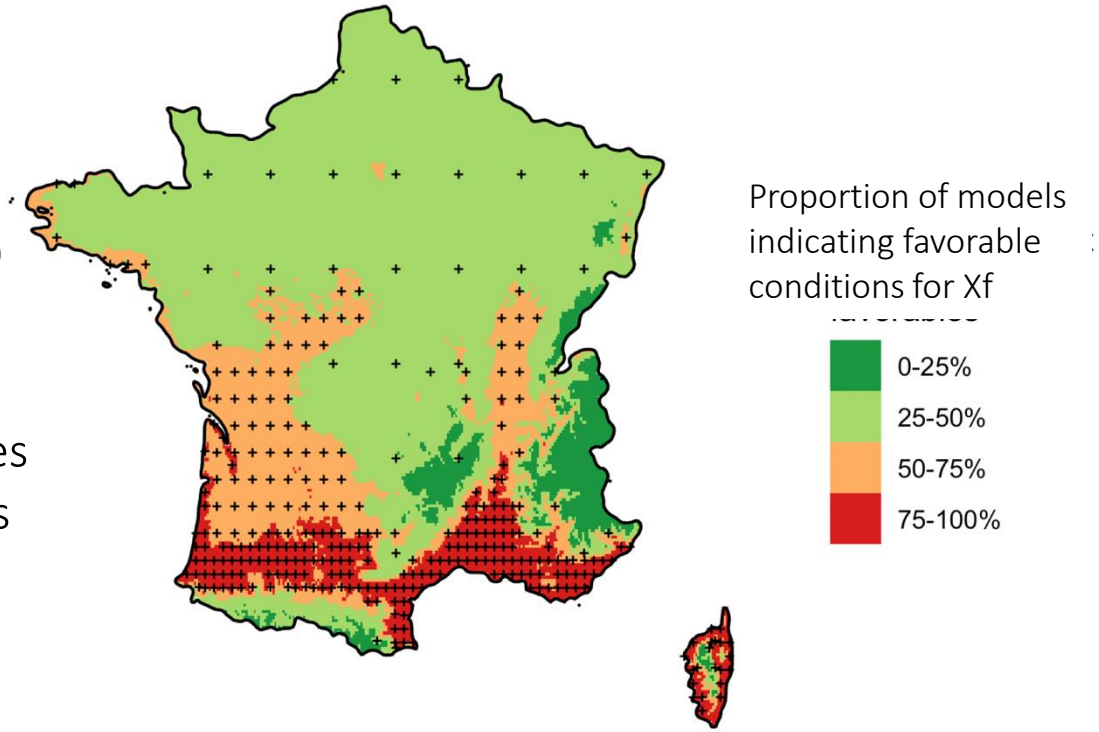
- protocol (hybridization time to avoid aspecific hybridization)
- increasing number of captured vectors in a library (n=32?)

Plant + vector identification :

- Need a complementary locus to rbcL to identify plant species
- Field observations required to corroborate molecular results
- Include COI probes to identify vectors on our database
  (see Jean-Claude Streito presentation)
- Still need to assess plant DNA half-life

Microbiome characterization :

- Include probes to capture obligate and facultative endosymbionts, as well as microbiome (in search for antagonists) => problem of DNA prevalence



SCIENTIFIC REPORTS

OPEN **Xylella fastidiosa: climate suitability of European continent**

Martin Godefroid, Astrid Cruaud, Jean-Claude Streito, Jean-Yves Rasplus & Jean-Pierre Rossi

Extending our network to continental agro-ecosystems

Designing an efficient sampling scheme for Xf based on our SDM analyses

Jean-Pierre Rossi | Martin Godefroid

Thanks for your attention !