**Slide 1**

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**Agro-Bio Tech**

**Next Generation Applications**
**of HTS Technologies**
**for plant viruses**
Sebastien Massart
Liege University – Gembloux Agro-Bio Tech - Belgium

**Slide 2**

1. The first decade of HTS

▶ The virus discovery party !



**Slide 3**

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**Agro-Bio Tech**

**Attendees @work :**

**What can be the next generation applications**
**of HTS technologies for plant viruses ?**

**Slide 4**

1. The first decade of HTS

▶ The virus discovery party !
▶ Fruit tree viruses :
   › >90 viral species
   › Exponential growth

frontiers
in Microbiology

REVIEW

**Is There a "Biological Desert" With**
**the Discovery of New Plant Viruses?**
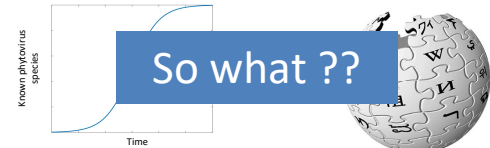**A Retrospective Analysis for New**
**Fruit Tree Viruses**

Wanying Hou[1,2], Shifang Li[1] and Sebastien Massart[1]

**Slide 5**

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**Gembloux**
**Agro-Bio Tech**

**The first decade of HTS**

**Slide 6**

1. The first decade of HTS

▶ The rythm will slow down
▶ Earth wikipedia of phytovirus

Known phytovirus species

So what ??

Time

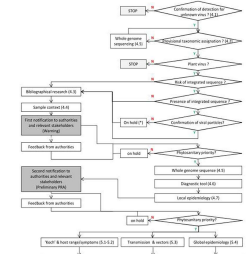▶ But when ?

### Slide 1

Participants @work :

What can be the priorities/further steps once the plant virome has been discovered ?



**www.wooclap.com/SIFAOD**

### Slide 2

2. The next decade with HTS : characterization

▶ Collective characterization framework in 2017 :
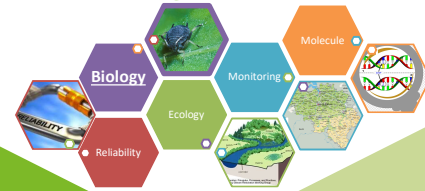


frontiers in Microbiology — PERSPECTIVE

**A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies**

cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

### Slide 3

LIÈGE université
**Gembloux Agro-Bio Tech**



Nuria Fontdevila   Coline Temple

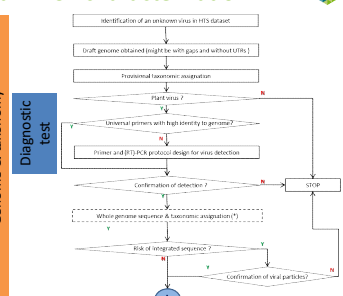**1. Biological characterization**

Frameworks for prioritisation

### Slide 4

2. The next decade with HTS : characterization

▶ Update in 2021:



INEXTVIR

### Slide 5

2. The next decade with HTS : characterization

▶ The hangover of biological characterization

- Impossible to evaluate biological properties of all new viruses
- How to evalute risks … or benefits ?
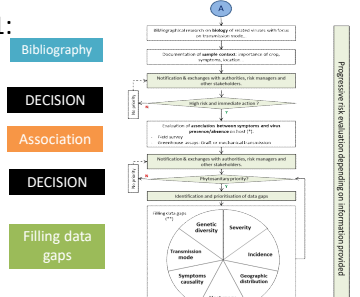
**Plant virus evolution under strong drought conditions results in a transition from parasitism to mutualism**

Rubén González, Anamarija Butković, Francisco J. Escaray, Javier Martínez-Latorre, Izan Melero, Enric Pérez-Parets, Aurelio Gómez-Cadenas, Pedro Carrasco, and Santiago F. Elena

### Slide 6

2. The next decade with HTS : characterization

▶ Update in 2021:



Plant Pathology

LETTER TO THE EDITOR
Reconsidering causal association in plant virology
Adrian Fox

Bibliography

DECISION

Association

DECISION

Filling data gaps

INEXTVIR

**Slide 1:**

LIÈGE université
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**Agro-Bio Tech**

Molecule

Biology

Monitoring

**Ecology**

RELIABILITY

Reliability

François Maclot

**2. Ecology to support biology**
Striking observations

**Slide 2:**

Studying the virome of 3 *Poaceae* communities



Legend for nodes: colour by *Poaceae or virus taxa*

- Barley
- Wheat
- *Apera spica venti*
- *Agrostis capillaris*
- *Bromus hordeaceus*
- *Dactylis glomerata*
- *Lolium perenne*
- *Poa trivialis*
- Virus species

Legend for links: colour by virus lifestyles

— Non persistent viruses (transmission by arthropods, nematodes, seeds or mechanically)
— Persistent viruses and mycoviruses

1. Lolium latent virus
2. White clover mosaic virus
3. Brome mosaic virus
4. Barley yellow dwarf virus GAV-MAV
5. Barley yellow dwarf virus Ker II – Ker III
6. Barley yellow dwarf virus PAS-PAV
7. Cereal yellow dwarf virus
8. Cereal yellow dwarf virus - RPV
9. Cocksfoot streak virus
10. Ryegrass mosaic virus
11. Cocksfoot mild mosaic virus
12. Novel closterovirus
13. Novel bluneirus
14. Novel nymovirus
15. Novel poceevirus
16. Novel repovirus
17. Novel waikavirus
18. Novel machlomovirus
19. Novel umbravirus
20. Novel tymovirus
21. Novel nucleorhabdovirus
22. Novel varicosavirus
23. Oat sterile dwarf virus
24. Hordeum vulgare endornavirus
25. Novel alphaendornavirus
26. Novel amalgavirus
27. Novel alphachrysovirus 2
28. Novel alphapartitivirus
29. Novel betapartitivirus
30. Novel deltapartitivirus 1
31. Novel deltapartitivirus 2
32-38. Novel unclassified partitiviruses
39. Alphacryptovirus
40. Uncultured partitivirus 1
41. Uncultured partitivirus 2
42-58. Novel totiviruses
59. Novel victorivirus
60. Novel unclassified mycoviruses
61. Bromus-associated circular DNA virus 3
62. Bromus-associated circular DNA virus 4

**Slide 3:**

Studying the virome of 3 *Poaceae* communities



Ecosystem simplification, biodiversity loss and plant virus emergence
Marilyn J. Roossinck and Fernando García-Arenal

Disease Risk

**Wheat** or 6-row **barley** fields

**Grazed pastures** with 4 major species

**Grasslands** with high biol. value

Human management

Biodiversity

Current Opinion in Virology

**Impact on the virome ?**

**Slide 4:**

Studying the virome of 3 *Poaceae* communities

Others | Phytoviruses



HF Wheat
HF Barley
HP Lolium perenne
AP Lolium perenne
AO Lolium perenne
LP Lolium perenne
LG Lolium perenne
HG Lolium perenne
HP Poa trivalis
LP Poa trivalis
AF Barley 6-row
AF Barley 2-row
LF Wheat
AG Bromus hordeaceus
LG Bromus hordeaceus
HG Poa trivalis
LG Poa trivalis
AP Poa trivalis
AG Poa trivalis
AG Dactylis glomerata
HG Dactylis glomerata
LG Dactylis glomerata
HG Bromus hordeaceus
AP Agrostis capillaris
AG Agrostis capillaris
AG Apera spica venti
HP Agrostis capillaris
HG Agrostis capillaris

**Slide 5:**

Studying the virome of 3 *Poaceae* communities

- Sampling 2 years in spring
- 50 quadrats
- Botanical identification
- Sampling (4,296 plants)
  - Plant community
  - Targeted species
- VANA sequencing on pool of 50 samples

fnrs   FÉDÉRATION WALLONIE-BRUXELLES



**Slide 6:**

Studying the virome of 3 *Poaceae* communities

BYDV | PoLNVA | PoLV1

*Lolium perenne*

*Poa trivialis*



Viruses highly prevalent while maintaining ecological fitness of the host

Plants infected by many viruses while maintaining ecological fitness

**Slide 1:**

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Molecule

Biology

Monitoring

RELIABILITY

Ecology

Reliability

Wei Rong   Benedicte Lebas   Johan Rollin

**3. Improving reliability of HTS**
Controls & contaminations

**Slide 2:**

Reliability: the proper use of controls

▶ What would be you choice for HTS ?

▶ www.wooclap.com/ SIFAOD

Horizon 2020 European Union Funding for Research & Innovation   Valitest

**Slide 3:**

Reliability: the proper use of controls

HTS is about counting sequences by millions
&
Determining presence of viral sequences

Horizon 2020 European Union Funding for Research & Innovation   Valitest

**Slide 4:**

Reliability: the proper use of controls

Source: www.technologyreview.com

▶ Positive control
▶ ~~Negative control~~
▶ Internal control
▶ Alien control

Biologicals ⟷ sequence datasets

Horizon 2020 European Union Funding for Research & Innovation   Valitest

**Slide 5:**

Reliability: the proper use of controls

Source: www.wikipedia.org

▶ Positive control
▶ Negative control
▶ Internal control

Biologicals

Horizon 2020 European Union Funding for Research & Innovation   Valitest

**Slide 6:**

Reliability: the proper use of controls

▶ Large scope of targets without *a priori*
▶ **+ control**
  - Mix of viruses (diversity)
  - Low concentration
    › Evaluate LOD
    › Minimize contamination risk
  - Check absence of other viruses (negative control)

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**Slide 1**

## Reliability: the proper use of controls

- Large scope of target without *a priori*
- **Internal control**
  - Virus not present in analysed samples
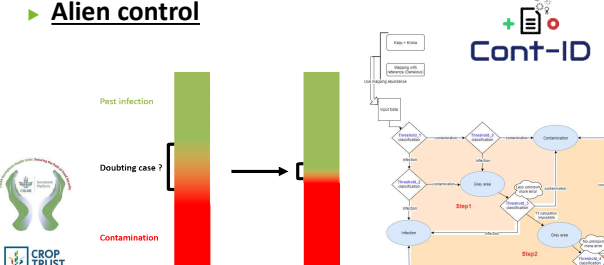  - Spiked at low concentration in all samples

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Screening for plant viruses by next generation sequencing using a modified double strand RNA extraction protocol with an internal amplification control

Prasad Kesanakurti, Mark Belton[1], Hanaa Saeed, Heidi Rast, Ian Boyes, Michael Rott[*]

*Centre for Plant Health, Canadian Food Inspection Agency, 8801 East Saanich Road, North Saanich, BC, V8L 1H3, Canada*

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**Slide 2**

## Reliability: the proper use of controls

- **Alien control**

Cont-ID

Pest infection

Doubting case ?

Contamination

CROP TRUST

**Slide 3**

## Reliability: the proper use of controls

- Large scope of targets without *a priori*
- **Alien control**
  - Virus not present in analysed samples

BYDV

CROP TRUST

Source: www.pinterest.com

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**Slide 4**

## Reliability: the proper use of controls

Biologicals ⟷ sequence datasets

Lucie Tamisier

PCI

Recommendation — https://genomics.peercommunityin.org/

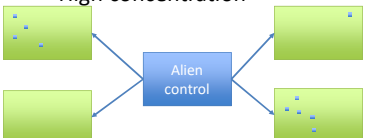**Toward a critical assessment of virus detection in plants**
*Hadi Quesneville based on reviews by Alexander Suh and 1 anonymous reviewer*

A recommendation of:

**Semi-artificial datasets as a resource for validation of bioinformatics pipelines for plant virus detection**
*Lucie Tamisier, Annelies Haegeman, Yoika Foucart, Nicolas Fouillien, Maher Al Rwahnih, Nihal Buzkan, Thierry Candresse, Michela Chiumenti, Kris De Jonghe, Marie Lefebvre, Paolo Margaria, Jean Sébastien Reynard, Kristian Stevens, Denis Kutnjak, Sébastien Massart*
*(2021, Zenodo, 4564718, ver. 4 peer-reviewed and recommended by Peer Community in Genomics)*
**10.5281/zenodo.4564718**

Abstract

*Submitted: 27 November 2020, Recommended: 02 April 2021*

Recommendation

The advent of High Throughput Sequencing (HTS) since the last decade has revealed previously unexpected diversity of viruses as well as their (sometimes) unexpected presence in some healthy individuals. These results demonstrate that genomics offers a powerful tool for studying viruses at the individual level, allowing an in-depth inventory of those that are infecting an organism. Such approaches make it possible to study viromes with an unprecedented level of detail, both qualitative and quantitative, which opens new avenues for analyses of viruses of humans, animals and plants. Consequently, the diagnostic field is using more and more HTS, fueling the need for efficient and reliable bioinformatics tools.

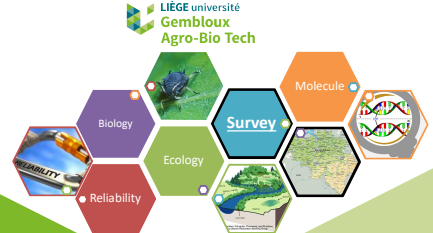federal public service — HEALTH, FOOD CHAIN SAFETY AND ENVIRONMENT — Euphresco

**Slide 5**

## Reliability: the proper use of controls

- Large scope of targets without *a priori*
- **Alien control**
  - Virus not present in analysed in samples
  - High concentration

Alien control

CROP TRUST

Horizon 2020 European Union Funding for Research & Innovation — Valitest

**Slide 6**

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Molecule

Biology

**Survey**

Ecology

RELIABILITY

Reliability

Arnaud Blouin

**4. From a plant to a nation ?**

Scale up of HTS for surveillance

## Slide 1: A primer: analysing 47 field samples in Estonia

**A Survey Using High-Throughput Sequencing Suggests That the Diversity of Cereal and Barley Yellow Dwarf Viruses Is Underestimated** Provisionally accepted The final, formatted version of the article will be published soon. ✉ Notify me

Merike Sõmera[1*], Sebastien Massart[2], Lucie Tamisier[3], Pille Sooväli[3], Kanitha Sathees[4] and Anders Kvarnheden[4, 5]

## Slide 2: Seviplant: analysing the virome at national scale

- ► Purification of viral particules by VANA
- ► Pools of 50 or 100 plants (with internal control)
- ► Alien control
- ► >10,000 plants sequenced so far

## Slide 3: Seviplant: analysing the virome at national scale

ILVO
Wallonie recherche
CRA-W

30 689 km²

## Slide 4: Seviplant: analysing the virome at national scale

| Potato | Tomato | Nightshade | Eggplant | Capsicum | Mix |
|---|---|---|---|---|---|
| 9300 plants | 1187 plants | 488 plants | 82 plants | 145 plants | 317 plants |
| PVY | PhCMoV | PVY | PhCMoV | PhCMoV | PVY |
| PVS | Tomato black ring virus | Solanum nigrum ilavirus 1 | Tomato bushy stunt virus | Cucumber mosaic virus | Tomato chlorosis virus |
| PVA | Strawberry latent ringspot virus | Cucumber mosaic virus | Satellite Tomato bushy stunt virus | Satellite Cucumber mosaic virus | Cucumber mosaic virus |
| PLRV | Alfalfa mosaic virus | Sambucus virus S | Moroccan pepper virus | Tomato mosaic virus | Olive latent virus 1 |
| Tobacco rattle virus | Lettuce ring necrosis virus | Alfalfa mosaic virus | Olive mild mosaic virus | Pepper mild mosaic virus | Solanum nigrum ilavirus 1 |
| Potato virus V | Olive mild mosaic virus | Melon chlorotic spot virus | | PVY | Plantain virus X (AcVX) |
| Alfalfa mosaic virus | Satellite Tobacco necrosis virus | | | | Colombian datura virus |
| Poinsettia latent virus | Pepino mosaic virus | | 22 virus species not reported in Belgium | | Potato yellowing virus |
| novel Tombusviridae | Olive latent virus 1 | | 12 new hosts for viruses | | PhCMoV |
| PhCMoV | Tomato chlorosis virus | | | | PLRV |

## Slide 5: Seviplant: analysing the virome at national scale

- ► Total 17,600 plants
- ► 24 different genera
- ► In *Solanum* alone, 14 species
- ► Intra-species diversity
  - *Solanum lycopersicum*  > 60 cv.
  - *Solanum tuberosum*  > 45 cv.
  - *Capsicum annuum*  > 15 cv.

(pie chart: 6%, 2%, 4%, 4%, 7%, 16%, 8%, 88%, 61%)

## Slide 6

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Molecule
Biology
Monitoring
Ecology
Reliability

Lucie Tamisier

**5. SNPs & consequences ?**
Behind the consensus

## What do we miss with consensus ?

- Sanger time : 1 seq        >Consensus sequence
  ATCAGACTAGACGATAG
  CAGAGGATACATTATGA
- HTS time: Million seqs    GACGATACAGATCAGAG
  CAAGCATATTACAGAAA
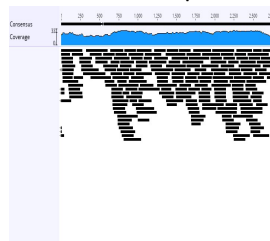- Behind the consensus ?   ATTGCAATTTGCATATTG
- Quasi-species

## Experimental evolution with BYDV

- Luteovirus
- Aphid-transmission
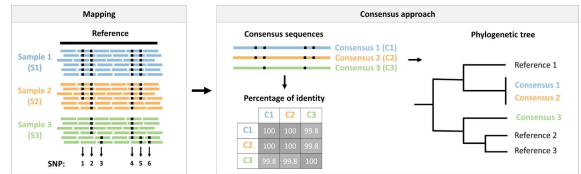- Mixed PAS/PAV population

- Wheat – Oat –Barley (2 & 6 rows)
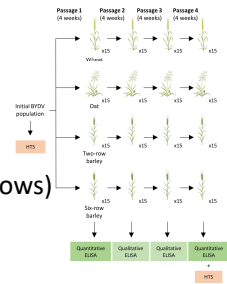


## What do we miss with consensus ?

- Sanger time : 1 seq        >Consensus sequence



- HTS time: Million seqs

- Behind the consensus:
- Quasi-species

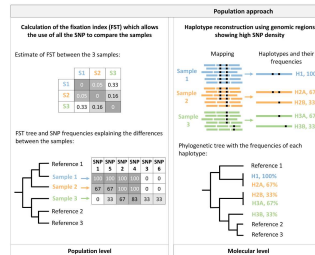## The classical approach: consensus tree



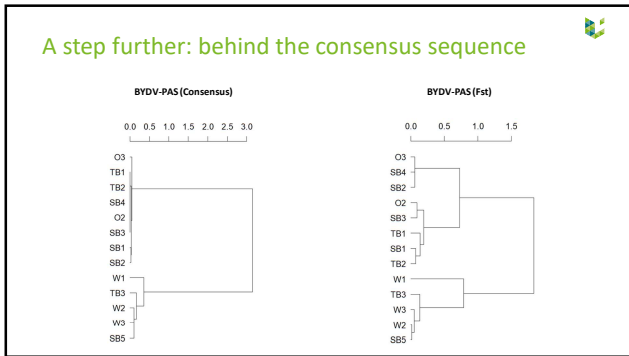## On the importance of SNPs behind consensus genome

**PLOS PATHOGENS**

RESEARCH ARTICLE

Transmission modes affect the population structure of potato virus Y in potato

Washington da Silva[1,2], Denis Kutnjak[3,4], Yi Xu[2,5], Yimin Xu[6,7], James Giovannoni[6,7], Santiago F. Elena[4,8], Stewart Gray[5,7]

## A step further: behind the consensus sequence



Rising thanks to Minion technology

## A step further: behind the consensus sequence

**BYDV-PAS (Consensus)**

0.0  0.5  1.0  1.5  2.0  2.5  3.0

O3
TB1
TB2
SB4
O2
SB3
SB1
SB2
W1
TB3
W2
W3
SB5

**BYDV-PAS (Fst)**

0.0  0.5  1.0  1.5

O3
SB4
SB2
O2
SB3
TB1
SB1
TB2
W1
TB3
W3
W2
SB5

---

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Thanks for the support

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**Conclusion**

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Thanks to the organisers for the invitation

Thank you for your attention
& your participation to the pools !

Sebastien Massart
sebastien.massart@uliege.be

@Be_Phytopath

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## Conclusion

▶ Decade of exciting discoveries
▶ Decade of exciting characterization is coming

Biology

Monitoring

Molecule

Ecology

RELIABILITY

Reliability

& many others…