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EUPHRESCO 2015-F-172:
The application of next-generation sequencing technology for the detection and diagnosis of non-cultural organisms: Viruses and viroids (NGS-detect)

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Euphresco

Network of phytosanitary research funders

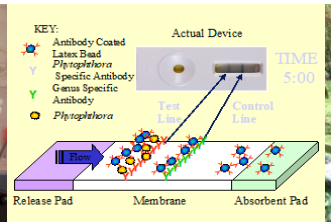
New arrivals of damaging pests….

- Damaging plant pests have increased steadily over the last century
- Global trade, potentially exacerbated by climate change, is likely to increase the risks from pests

- Madeira Declaration (2004):
  - The NPPO services increasingly lack staff, funds and training
  - The whole scientific basis of the phytosanitary field is quickly eroding
  - Indispensable expertise and scientific disciplines will irreversibly disappear
  - NPPO will be unable to do their duty

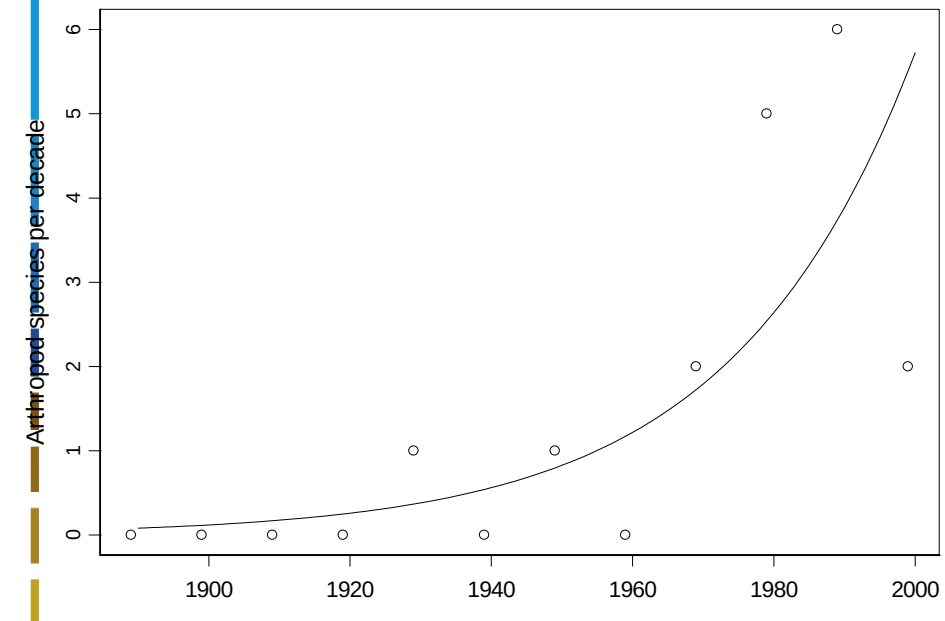




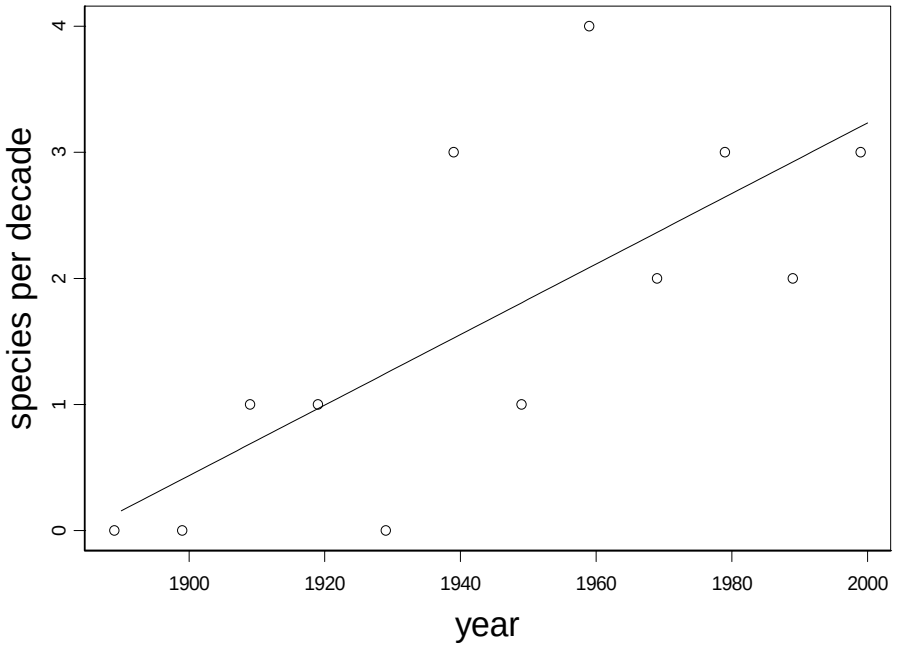


…a risk….

- First recordings of non-native arthropod **pests** in Europe (data from Smith 1997)



- First recordings of non-native plant **diseases** in Europe (including bacteria, fungi and nematodes) (Smith 1997)








… followed by an opportunity

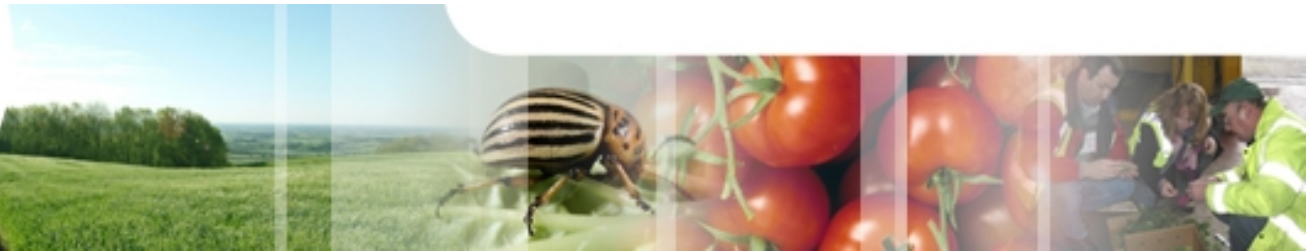
- Need for improved coordination and collaboration between national phytosanitary research programmes and with EU-funded research

- To ensure effective support for plant health policy and its implementation across Europe

Preparing the ground

- EUPHRESCO I (2006-2010)
- From mapping programmes and identifying players to funding calls for proposals
- Two rounds for a total volume of 2.1 Million €
- 8% of the total national budget for phytosanitary research (2008-2009)
- 17 pilot projects funded

Euphresco
Phytosanitary ERA-NET



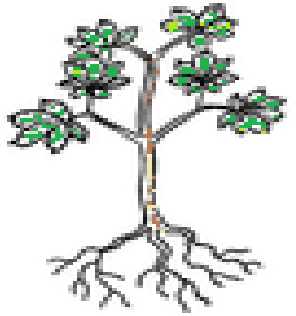
Some projects funded under EUPHRESCO I

- Strategies for *Ambrosia artemisiifolia* **control**
- Development and validation of innovative **diagnostic** tools for the detection of fire blight (*Erwinia amylovora*)
- Evaluating the **risk of spread** of *Scaphoideus titanus* with propagation material
- Decision support systems for **control** of alien invasive macrophytes
- Development of **validated procedures** for whole genome amplification of DNA/RNA for quarantine plant pathogens and pests
- **Ring test** on diagnostic methods for *Pantoea stewartii* spp. *stewartii*, maize bacterial blight
- **Detection and management** of the quarantine nematodes *Meloidogyne chitwoodi* and *Meloidogyne fallax*






Nurturing further growth



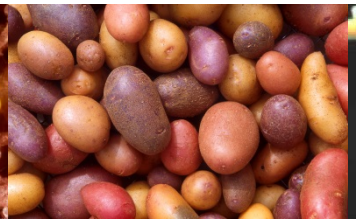
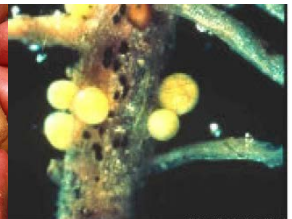
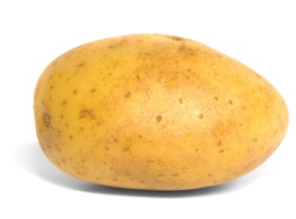
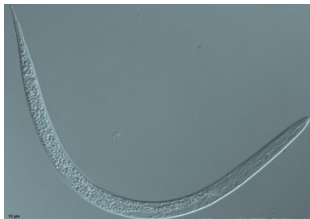
- EUPHRESCO II (2011-2014)
- Strenghtening the collaboration, improving the tools and enlarging the network
- Three annual rounds for a total volume of 6.8 Million €
- 12% of the total national budget for phytosanitary research over 2011-2014
- 30 projects funded

Euphresco
Phytosanitary ERA-NET

Some projects funded under EUPHRESCO II

- Use of novel **molecular methods** to understand population diversity and its implications on disease management through the use of resistant potato varieties (Potato Cyst Nematode)
- Development and validation of innovative **diagnostic** tools for detection and identification of Meloidogyne enterolobii in support of integrated plant protection strategies
- **Epidemiological studies** on reservoir hosts and potential vectors of Grapevine flavescence dorée (GFD) and validation of different diagnostic procedures for GFD
- Bursaphelenchus xylophilus methods for early **detection**
- Plant Health **Fellowship**
- **IPM** strategies against Drosophilidae



Bearing fruit for the long term

- Since April 1, 2014
- 34 member organisations from 25 countries
- *Modus Operandi* agreed
- Coordination hosted within EPPO

Bearing fruit for the long term

- Looking for enlargement within Europe and abroad
- Widening the pool of disciplines
- Developing fast procedures to fund projects to face emergency situations

2015-F-172: The application of Next-Generation Sequencing technology for the detection and diagnosis of non-culturable organisms: viruses and viroids (NGS-detect)

NGS-detect

- 17 partners:
- Germany, France, Belgium, Netherlands, UK, Ireland, Denmark, Greece, Hungary, Italy, Russia, Spain, Canada, Peru, Slovenia and South Africa
- Total budget: €620.000 (non-competitive funding)
- Project duration 07/2016 to 06/2018; kick-off meeting in 10/2016

Objectives

- Development and validation of a common pipeline from sample preparation to data analysis
- Developing reference sequence data for viruses and viroids (e.g. Q-bank) in a plant quarantine setting or certified scheme
- Etiology of uncharacterised diseases

Work-packages

1 Project management and co-ordination

2 Sample preparation and library optimisation for different matrices

3 Comparison of sequencing platforms

4 Bioinformatic pipeline development and analysis

5 Validation of protocols (inter-laboratory comparisons/test performance studies); preparation of EPPO standards

6 Providing reference data for databases (Q-bank)/bioinformatic pipelines (VirTool, VirusDetect)

WP 2 sample preparation

- Different methods for nucleic acid extraction/enrichment (dsRNAs, ribodepleted total RNA, siRNAs, RCA, partial partical purification)
- Matrices have huge influence on success (esp. woody host plants)
- Establishment of own library protocols not in the interest of majority

WP3 Sequencing Platforms

- Many platforms/providers available but:
- If sequence depth deep enough, the effects are negliable for detection of novel viruses
- Recommendation for beginners: fast extraction (e. g. total RNA) and cheapest sequencing platform

WP4 Bioinformatics

- Many software solutions available but not easy to use for none specialists
- Alternatives: VirTool/Virusdetect for detection of known and unknown viruses
- Problems: Curation of databases, hosting of platforms, integration into other software solutions („Galaxy“)

WP5 Validation

- Proficiency test organised by Mike Rott (Canada); 13 participating laboratories
- Two sample sets (fruit trees, grapevine) containing 6 samples each
- Task: Find the viruses!

WP6 Reference data

- Close link to Euphresco project: VirusCollect II (Annelien Roenhorst)
- Aim: Physical collection of reference isolates in reference collections
- Reference sequence data should be made available in Qbank

Interaction and Dissemination

- Two of the main requirements for EPHRESCO projects

→ Interaction between project partners

→ Interaction with other research consortia

→ Public access to project results/raw data (not only by publication but also through policy statements and support of EPPO/EFSA risk assessments

Open questions

- Harmonisation of protocols and pipelines possible?
- Can the NGS process simplified for users such as NPPOs, test laboratories etc.?
- Is EPPO standard on NGS possible at all?
- Consequences of novel findings for import/export regulations, quarantine legislation etc.
- What technology advancements will be available in the future?

NGS-detect Team

Thierry Candresse, Luca Ferretti, Adrian Fox, Yahya Gaafar, Pascal Gentit, Kerstin Herz, Willy Jelkmann, Kris de Jonghe, Denis Kutnjak, Nataša Mehle, Annelien Roenhorst, Mike Rott, Yury Shneyder, Marcel Westenberg, Mogens Nicolaisen, Jean-Phillippe Renvoise, Christina Varveri, George Melilka, Lázló Krizbai, Maria Cullen, Elisa, Konstantin Kornev, Antonio Olmos, Jan Kreuze, Hano Maree, Heiko Ziebell