Detection of vectors in Scotland

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EUPHRESCO B-115 PhyLib II Project Meeting
27-28 April 2017
University of Bologna, Italy
Outline

1. Euphresco

2. ‘Ca. Liberibacter solanacearum’ & Phytoplasmas

3. PhyLibII & outcomes of PhyLib

4. Why CaLsol is of concern to Scotland

5. Detection of vectors in Scotland
EUropean PHytosanitary RESearch Co ordination

AIM Coordinate national programmes with each other; commission trans-national research; develop common research agendas

GOAL to support phytosanitary policy and operations and improve European phytosanitary science capability
Coordinated by EPPO

68 members 54 countries
Annual research call

Countries support projects of national interest

Project descriptions developed

Funding decisions submitted

Research implementation
‘Candidatus Liberibacter solanacearum’ (CaLsol)  
Phloem-limited, gram-negative bacterium

A: America, New Zealand  
B: North America

C: Finland, Sweden, Norway, Germany
D: France, Spain, Morocco
E: France, Spain, Morocco
Psyllid vectors of CaLsol
Phloem-feeding insects in the Sternorrhyncha

3 known vector species:
- Bactericera cockerelli
- Trioza apicalis
- Bactericera trigonica

2 potential vector species:
- Bactericera tremblayi
- Bactericera nigricornis

B. trigonica/nigricornis/tremblayi can be easily misidentified

Bactericera cockerelli
America, New Zealand and Australia
Phytoplasmas
Cell-wall less bacteria present in plant phloem

Vectored by leafhoppers, planthoppers, and psyllids

~1000 phytoplasma diseases identified on range of host plants

12 strains on potato – most important potato stolbur & potato purple top
PhyLibII

Project coordinators
Jennifer Sjölund & Colin Jeffries
- Main contact for research consortium
- Delivery of project proposal
- Delivery of final project report

Topic coordinator
David Kenyon
- Scottish Government representative
PhyLibII

March 2016 - February 2019

14 countries

Researchers: AGES (AT), ILVO (BE), CFIA (CA), CISTA (CZ), EVPM (EE), ICIA (ES), LUKE (FI), UHE (FI), ANSES (FR), FN3PT (FR), CREA (IT), UNIBO (IT), NVWA (NL), VNKR (RU), NIB (SI), PPCRI (TR), SASA (UK).
The biology and epidemiology of ‘Candidatus Liberibacter solanacearum’ and potato phytoplasmas and their contribution to risk management in potato and other crops

Epidemiology and diagnosis of potato Phytoplasmas and ‘Candidatus Liberibacter solanacearum’ and their contribution to risk management
Outcomes CaLSol:

Real-time PCR Kit for CaLSol detection.

Haplotype E detected in Spain. Haplotype D and E in Morocco.

No natural hosts found infected in Finland or Spain.

Potato tubers with ZC symptoms in Spain tested positive for Haplotype E.

No resistance found in 6 potato varieties. Two tomato cultivars latently infected for 4 years.
Outcomes CaLsol:

CaLsol positive *B. trigonica*, *B. tremblayi* and *B. nigricornis*.

*B. trigonica* reaches phloem on carrot, celery and potato, but not on tomato. Ingested only from carrot and celery.

*T. apicalis* in Finland - similar feeding habits for carrot and potato.

*B. trigonica* unable to complete life cycle on potato and tomato.
Outcomes CaLsol:

CaLsol positive carrot, parsnip seed (Spain), carrot (France).

Confirmed by EM in phloem sieve tubes of carrot seed coat.

Transmission to carrot seedlings found by Spain but not found by France.

PMA revealed 95% bacteria dead.

Use of CaLsol free carrot seed reduced disease in Spain.

Potato microplants may be latently infected with CaLsol. Uneven spread of infection problematic for quarantine testing.
Outcomes Phytoplasmas:


Varieties differed in their susceptibility to stolbur infection (Hungary).

Germination of tubers was severely reduced when tubers were infected with ‘*Ca. P solani*’ (SASA)

Infected tubers gave rise to hair-like sprouts and weak plants. Phytoplasma not successfully detected in tubers (Genlogs) or microplants (SASA).

First report of ‘*Ca. P asteris*’ in commercial carrot (UK), also in carrot (The Netherlands).
PhyLib 2012-2014

Outcomes Phytoplasmas:


Potato stolbur detected in potato, eggplant and pepper (Turkey).

Proficiency test for phytoplasma detection.

‘Ca. P trifolii’ detection best on microplants, 10w glasshouse plants and progeny tubers. Improved quarantine test procedures at SASA.

Microarray in tube format (1d process) more sensitive than real-time PCR for some species.
Why CaLsol is of concern to Scotland

Global reputation - High Health Quality

14,800 ha – Ware

12,700 ha – Seed (75% of UK seed)

UK one of the top exporters of seed

Scottish potato sector - £287 million

CaLsol Europe in carrots → carrots grown in same regions as potato - East Coast of Scotland
Current status of CaLsol in the UK

**Bacterium:** Free

Not found in growing plants

Found in seed sold locally

Scotland: Imported (non-EU) tested for CaLsol since 2010

2012-13: growers submitted symptomatic carrots for testing

5 samples = no positives, but ‘Ca. Phytoplasma asteris’ found

**Vector:** Present

Carrot psyllid *Trioza apicalis*

Vector causing issues with carrot in Scandinavia and Germany

Not present in high numbers

Not the main pest on carrots in UK

*Carrot aphid - Cavariella aegopodii*
Detection of vectors in Scotland

Using **suction traps** to assess psyllid diversity in UK

Psyllid ID by **DNA sequencing & classical taxonomy**

Design of species-specific **real-time PCR assays**

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Alex Greenslade, Kirsty Hassall, James Bell (Rothamsted Research)

David Ouvrard (Natural History Museum)

Roland Sigvald (Swedish University of Agricultural Sciences)
12.2m vacuum traps
Rothamsted Insect Survey
EXAMINE Network
Psyllid species diversity

Better understanding of psyllid diversity in the UK
June-July & Nov migration peaks
New species discovered for UK and Sweden

Detected the vector *Trioza apicalis* in Sweden and the UK

The suction trap network shows promise as a monitoring system for vector surveillance
Issues with online DNA databases

1. Incorrect species ID
2. Voucher specimens not available
   Exception: BOLD
3. Target species absent
   NCBI: *B. trigonica/tremblayi/nigricornis*
4. Target region absent
   NCBI: ITS2 *T. apicalis*
Non-destructive DNA extraction protocol

Protocols available on POnTE website - www.ponteproject.eu

COI 84.4% pairwise difference

ITS2 85.4% pairwise difference
COI 84.4% pairwise difference

ITS2 85.4% pairwise difference
**COI** 84.4% pairwise difference

**ITS2** 85.4% pairwise difference

Ideal regions for primers/probes
COI (Cytochrome oxidase subunit 1)

Coding region → mutations at third base of codon (degeneracy)

Ribosomal spacers

Less conserved → many insertion/deletions

High copy number → amplified from small quantities of DNA

Internal Transcribed Spacers (ITS) 1 & 2

Intergenic spacer (IGS)

18S, 5.8S, 28S ribosomal genes

External transcribed spacers (ETS)
### Psyllid DNA database

Cross-check morphological ID & Cross-check online DB

Design assays to sequences reliably identified using classical taxonomy and DNA sequencing

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<th>NCR</th>
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Design assays to sequences reliably identified using classical taxonomy and DNA sequencing.
Voucher specimen

Classical taxonomy

Reliable ID

Molecular Methods

Non-destructive DNA extraction

Molecular Assay

DNA Sequencing
Real-time PCR TaqMan assay for *B. cockerelli*

**ITS2:**

*B. cockerelli*

Sensitive to a 1/10,000 dilution

Tested on 52 non-target (incl. 14 species from USA)

*T. apicalis & B. nigricornis*

Initial tests show species specificity

**ITS1:**

*B. trigonica*

Awaiting testing
B. cockerelli assay on bulk samples

Mix A
1:10 - *B. cockerelli* + 9 psyllid spp.

Mix B
1:50 - *B. cockerelli* + 49 psyllid spp.

Mix C
1:100 - *B. cockerelli* + 49 psyllid spp.

Mix D
1:100 - *B. cockerelli* + unknown arthropods from UK suction trap
Psyllid ID Workshop, SASA, 23-24 March 2017
Outcomes and future work

An updated species list of psyllids in Britain and their migration patterns is being prepared for publication.

*B. cockerelli* assay manuscript in preparation. Sequences will be made available online after publication.

Suction trap in Salamanca to collect psyllids for POnTE 2020.

Seed transmission studies (presentation tomorrow).
Thank you
Assunta Bertaccini & University of Bologna

Enjoy the meeting


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