EUPHRESCO & PhyLibII

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 **PH**ytosanitary **RES**earch **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

'Candidatus Liberibacter solanacearum' (CaLsol)

Phloem-limited, gram-negative bacterium

A1
- **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*** } A1
- ***Trioza apicalis***
- ***Bactericera trigonica***

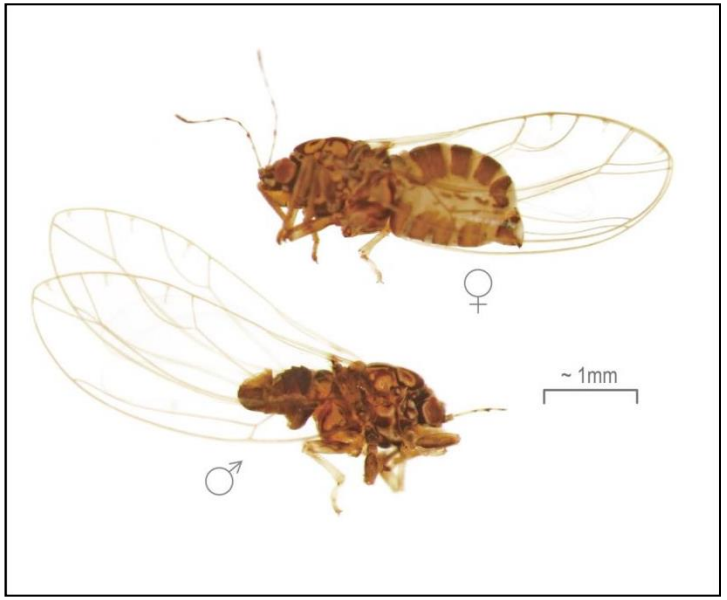
2 potential vector species:

- *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**

Potato stolbur
Maria Kölber
Fitolabs - Hungary

Potato witches broom
SASA - Scotland

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).

PhyLibII

The biology and epidemiology of **'*Candidatus* Liberibacter solanacearum'** and **potato phytoplasmas** and their contribution to risk management in potato and other crops

PhyLib

Epidemiology and diagnosis of potato **Phytoplasmas** and **'*Candidatus* Liberibacter solanacearum'** and their contribution to risk management

PhyLib 2012-2014

Researchers: ILVO (BE), CFIA (CA), IVIA (ES), ICIA (ES), LUKE (FI), UHE (FI), NVWA (NL), PPCRI (TR), SASA (UK), FERA (UK).

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.

PhyLib 2012-2014

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.

PhyLib 2012-2014

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.

PhyLib 2012-2014

Outcomes Phytoplasmas:

Potato stolbur transmitted by Cixiids *Hyalesthes obsoletus* (2012) and *Reptalus quinquecostatus* (2013).

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:

'*Ca.* P solani' associated with stolbur-like symptoms in Belgium (2012). Not found on grapevine, potatoes and strawberry and raspberry (2013).

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)

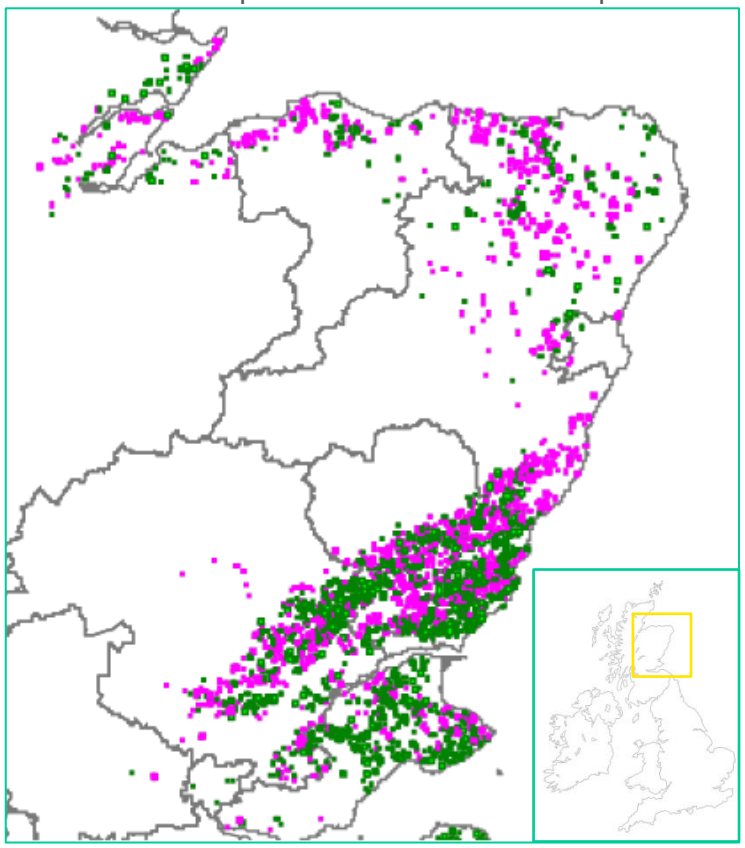
UK one of the top exporters of seed

Scottish potato sector - £287 million

CaLsol Europe in carrots → carrots grown in same regions as potato - East Coast of Scotland

Potato Crop locations in East Scotland

Green – Ware potato Pink – Seed potato



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**

Jennifer Sjölund, Mairi Carnegie, Yvonne Arnsdorf, Fiona Highet, Colin Jeffries, David Kenyon (SASA)

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

David Ouvrard (Natural History Museum)

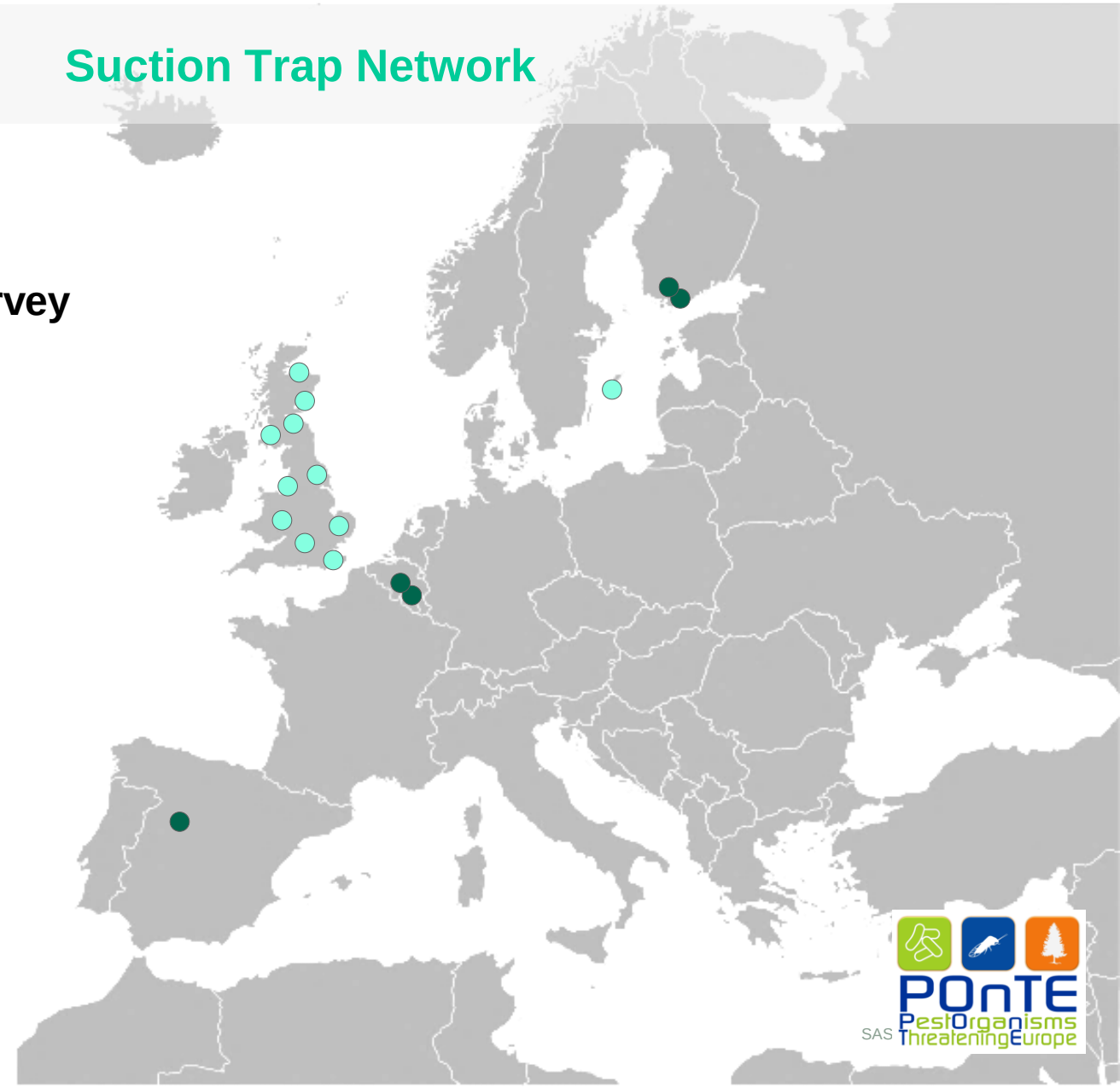
Roland Sigvald (Swedish University of Agricultural Sciences)

Suction Trap Network

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

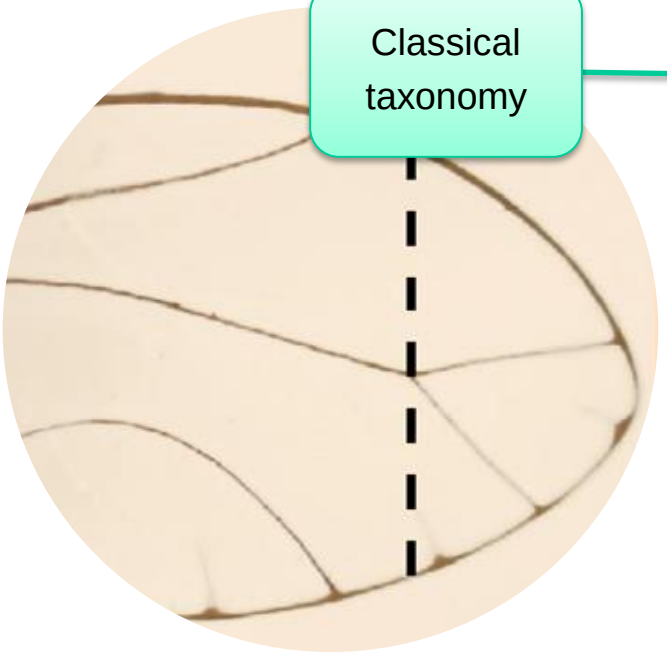
Voucher specimen

Non-destructive DNA extraction

Classical taxonomy

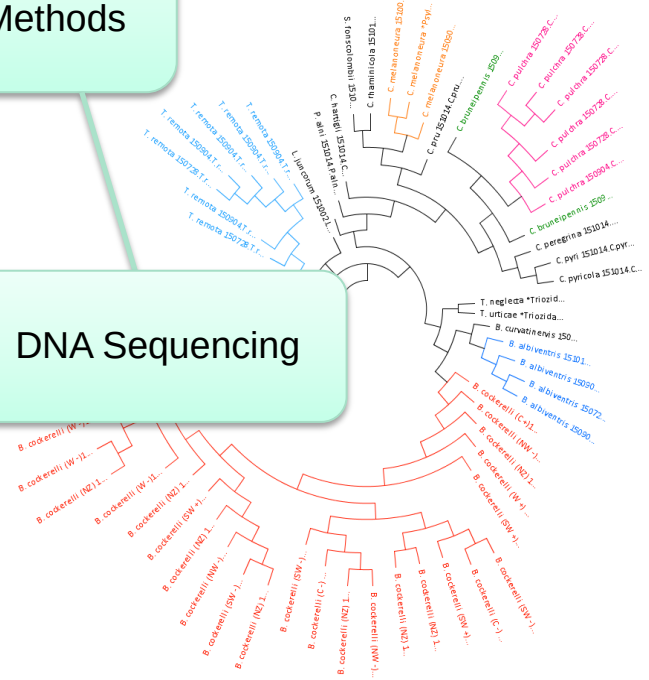
Reliable ID

Molecular Methods



Molecular Assay

DNA Sequencing



Issues with online DNA databases

1. Incorrect species ID
2. Voucher specimens not available

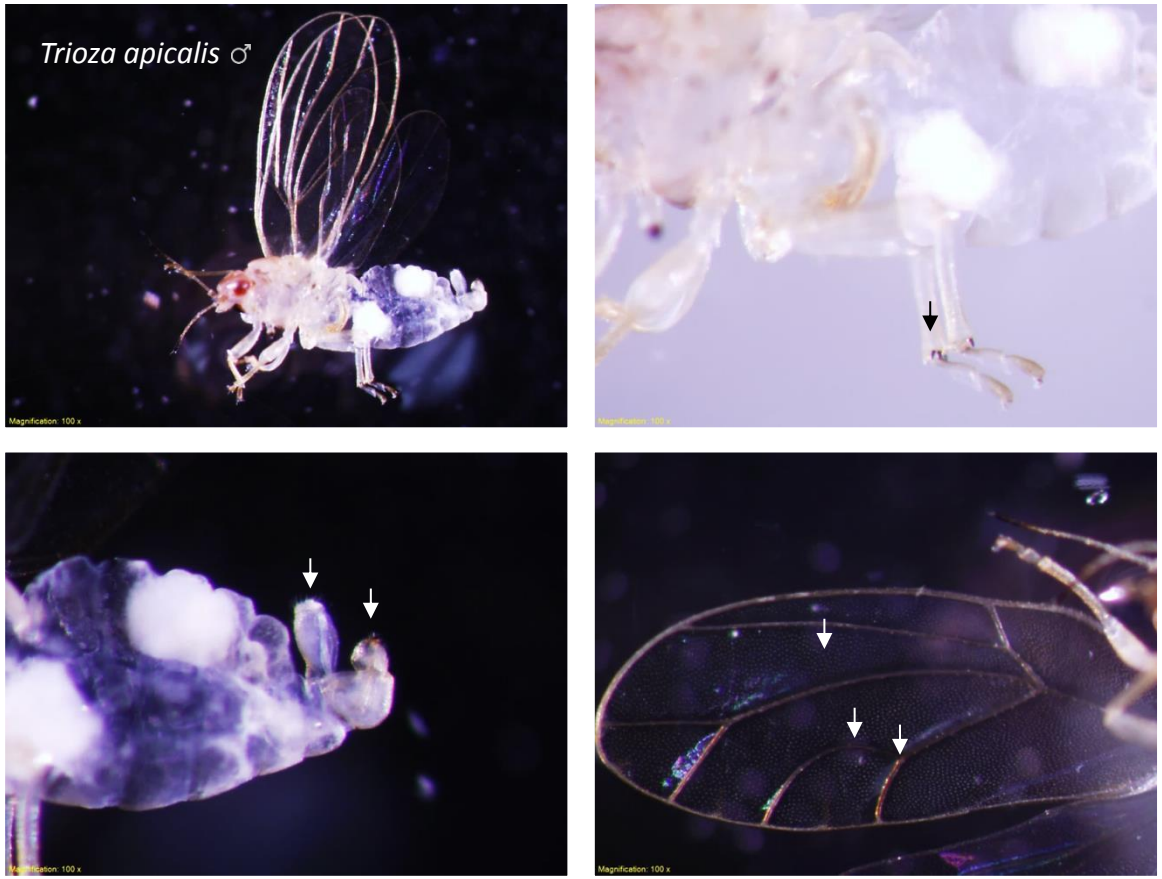
   Exception: BOLD
3. Target species absent

   NCBI: *B. trigonica/tremblayi/nigricornis*
4. Target region absent

   NCBI: ITS2 *T. apicalis*

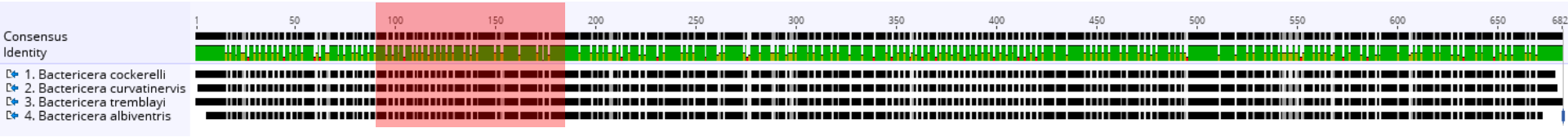
Non-destructive DNA extraction protocol

Protocols available on **POnTE** website - www.ponteproject.eu

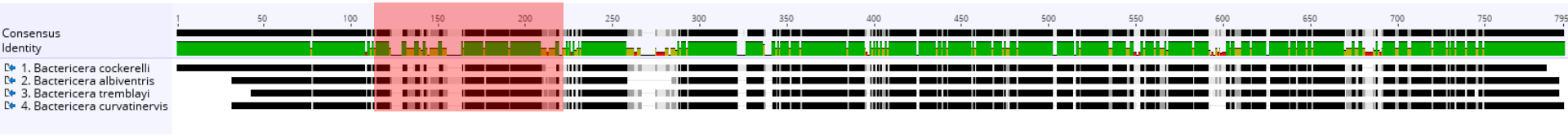


Modified from: Percy (2003) *Evolution* **57**, 2540-2556

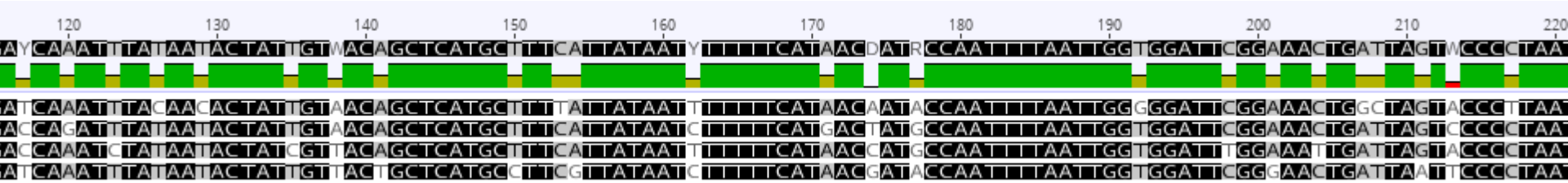
COI 84.4% pairwise difference



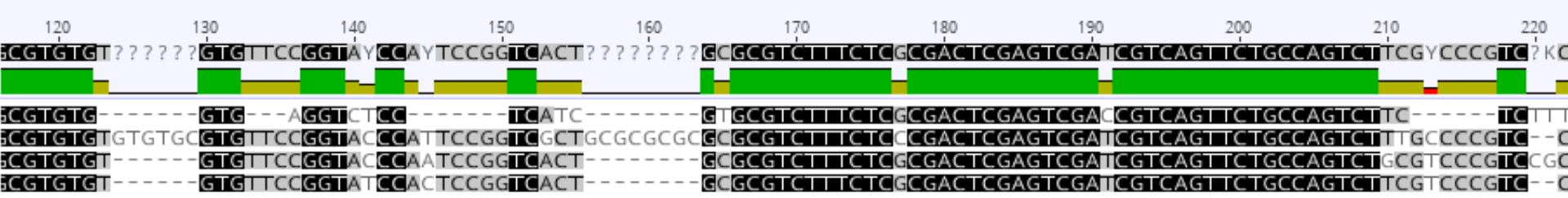
ITS2 85.4% pairwise difference



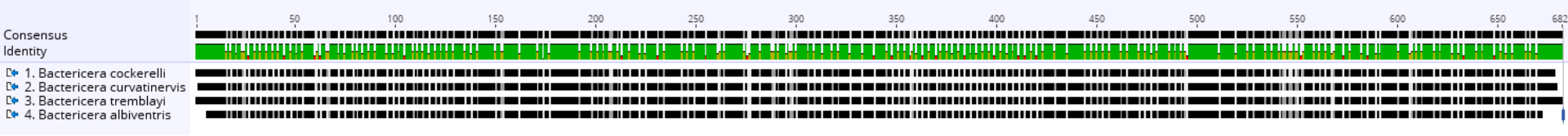
COI 84.4% pairwise difference



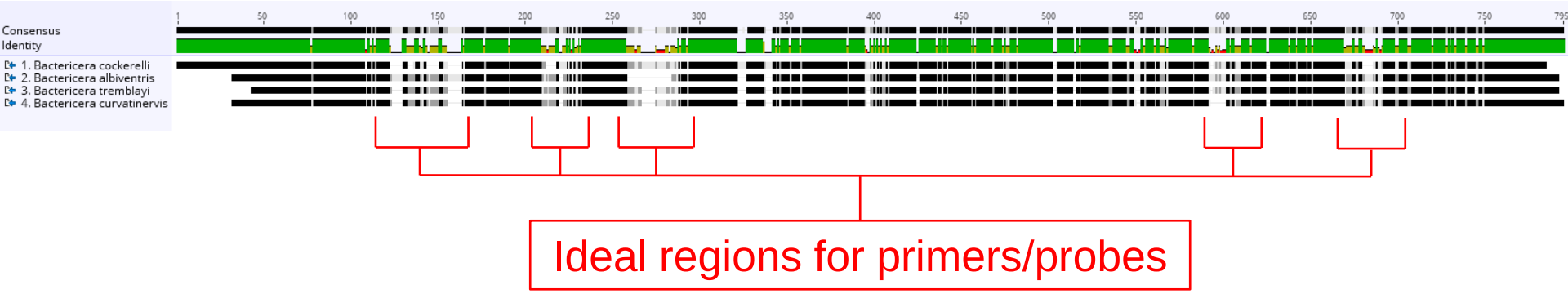
ITS2 85.4% pairwise difference



COI 84.4% pairwise difference



ITS2 85.4% pairwise difference



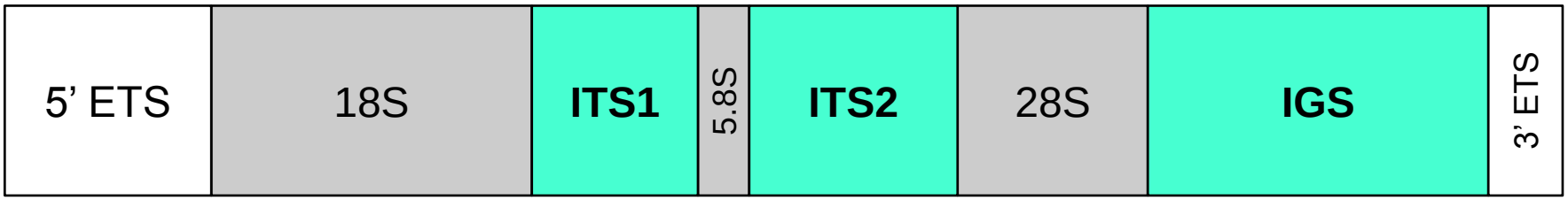
COI (Cytochrome oxidase subunit 1)

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

	Index no.	Sample ID (duplicates formatted in bold)	Cloned Regions	Sequence only	Genus	Species	ITS2 Practitioner (Surname, Institute)	PCR date	Primers	Seq. folder name	Seq. date	Uploaded?	Seq. Length	5' -> 3' sequence	Partial Seq. Length	Partial sequence (R) 3' -> 5'	Closest NCBI species match	NCBI Accession number	NCBI Query coverage (%)	NCBI Identity (%)	Does spec exist on NC
239	238	160120.M13.2			Bactericera	tremblayi	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	682	GGCGTTTCGGGTCACG			Bactericera cockerelli	GQ249860	100	84	No
240	239	160120.M15.3			Bactericera	tremblayi	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	682	GGCGTTTCGGGTCACG			Bactericera cockerelli	GQ249860	100	84	No
241	240	160120.M14.2			Bactericera	tremblayi	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	721	GCGTTTCGGGTCACGT			Bactericera cockerelli	GQ249860	100	84	No
242	241	160120.M14.3			Bactericera	tremblayi	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	723	CGTTTCGGGTCACGTA			Bactericera cockerelli	AY971900	100	85	No
243	242	160120.M15.1			Bactericera	tremblayi	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	679	GTTTCGGGTCACGTAC			Bactericera cockerelli	GQ249860	100	84	No
244	243	160120.M15.2			Bactericera	tremblayi	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	678	GTTTCGGGTCACGTAC			Bactericera cockerelli	GQ249860	100	84	No
245	244	160120.M21.1			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	704	GGCGTTTCGGGTCACG			Bactericera cockerelli	GQ249860	100	85	No
246	245	160120.M21.2			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	676	CCGAGGGTCGGTATCG			Bactericera cockerelli	GQ249860	100	85	No
247	246	160120.M21.3			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	679	GGCGTTTCGGGTCACG			Bactericera cockerelli	GQ249860	100	84	No
248	247	160120.M22.1			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	653	AAACCCTGGCGTTTCG			Bactericera cockerelli	GQ249860	91	85	No
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250	249	160120.M22.3			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	653	GGTCGGTATCGACTCG			Bactericera cockerelli	GQ249860	100	84	No
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258	257	160120.M26.2			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	700	GGCGTTTCGGGTCACG			Bactericera cockerelli	GQ249860	100	85	No
259	258	160120.M26.3			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	701	GTTTCGGGTCACGTAC			Bactericera cockerelli	GQ249860	100	85	No
260	259	160120.M27.1			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	700	GGCGTTTCGGGTCACG			Bactericera cockerelli	GQ249860	100	85	No
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262	261	160120.M27.3			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	677	CGTTTCGGGTCACGTA			Bactericera cockerelli	GQ249860	100	84	No
263	262	160120.M28.1			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	676	GTTTCGGGTCACGTAC			Bactericera cockerelli	GQ249860	100	84	No
264	263	160120.M28.2			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	721	GGCGTTTCGGGTCACG			Bactericera cockerelli	AY971900	100	85	No
265	264	160120.M29.1			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	687	GGCGTTTCGGGTCACG			Bactericera cockerelli	GQ249860	100	84	No
266	265	160120.M29.2			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	723	GGCGTTTCGGGTCACG			Bactericera cockerelli	AY971900	100	85	No
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268	267	160120.M211.2			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	675	TTTCGGGTCACGTACAT			Bactericera cockerelli	GQ249860	100	84	No
269	268	160120.M211.3			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	692	GTTTCGGGTCACGTAC			Bactericera cockerelli	GQ249860	100	85	No
270	269	160209.M25.1			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	325	GTGTGTGTGTTCCGGT			Bactericera cockerelli	GQ249860	100	84	No
271	270	160209.M25.2			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	708	GGCGTTTCGGGTCACG			Bactericera cockerelli	GQ249860	100	85	No
272	271	160209.M28.3			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	694	GTTTCGGGTCACGTAC			Bactericera cockerelli	GQ249860	100	84	No
273	272	160209.M14.1			Bactericera	tremblayi	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	682	GGCGTTTCGGGTCACG			Bactericera cockerelli	GQ249860	100	84	No
274	273	160209.M11.3			Bactericera	nigricornis	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	678	TCGGGTCACGTACATC			Bactericera cockerelli	AY971897	100	85	No
275	274	160209.M29.3			Bactericera	trigonica	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	704	GGCGTTTCGGGTCACG			Bactericera cockerelli	GQ249860	100	84	No
276	275	160209.M13.3			Bactericera	tremblayi	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	689	GCGTTTCGGGTCACGT			Bactericera cockerelli	GQ249860	100	85	No
277	276	160209.M25.3			Bactericera	nigricornis	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	659	GGGTCGGTATCGACTC			Bactericera cockerelli	AY971897	100	85	No
278	277	160209.C.mel.9			Cacopsylla	melanoneura	J. Sjölund, SASA	19/02/2016	CAS5p8sFcm-F +	MJS_SEQ_160223_A +	23/02/2016	Yes	682	CCTGGCGTTTCGGGTC			Cacopsylla pruni	KF305148	81	84	No, not ITS

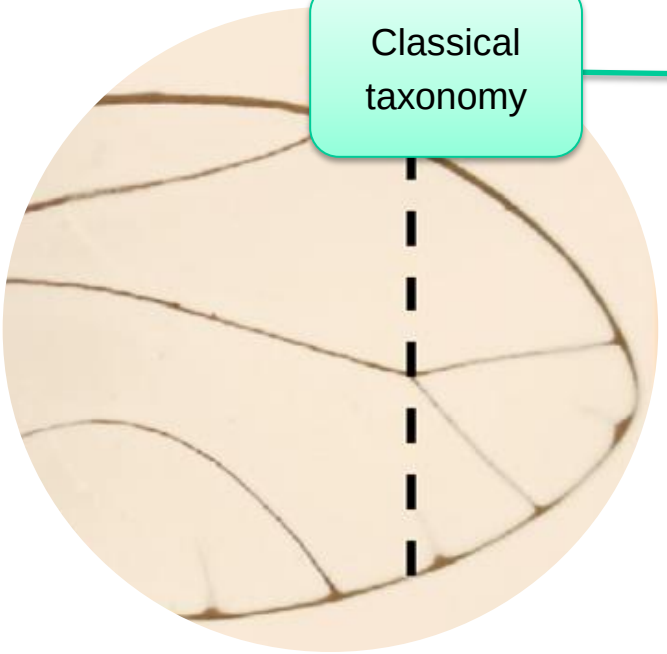
Voucher specimen

Non-destructive DNA extraction

Classical taxonomy

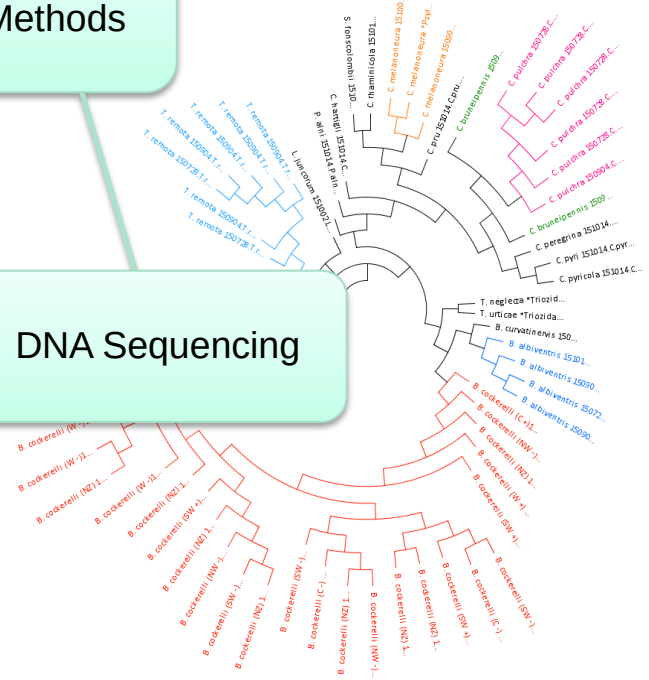
Reliable ID

Molecular Methods



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)

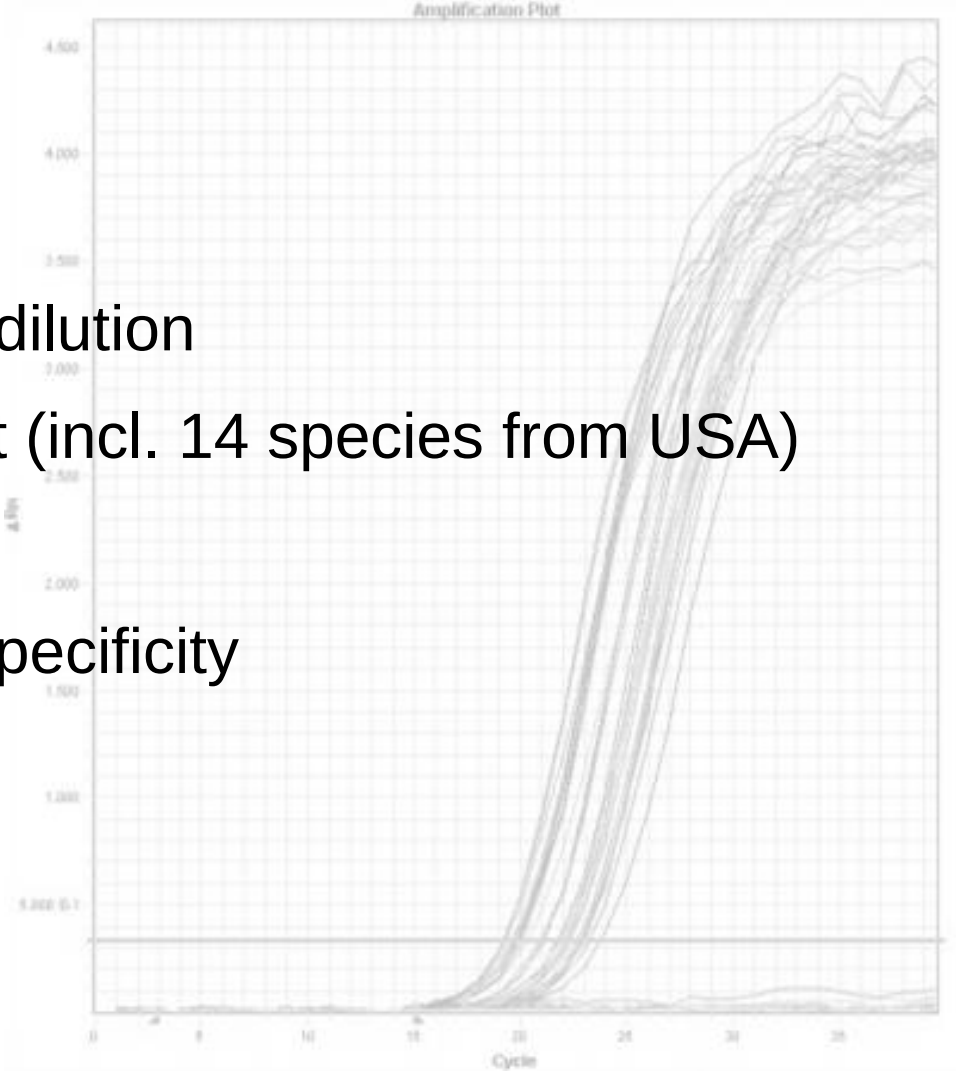
T. apicalis & B. nigricornis

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

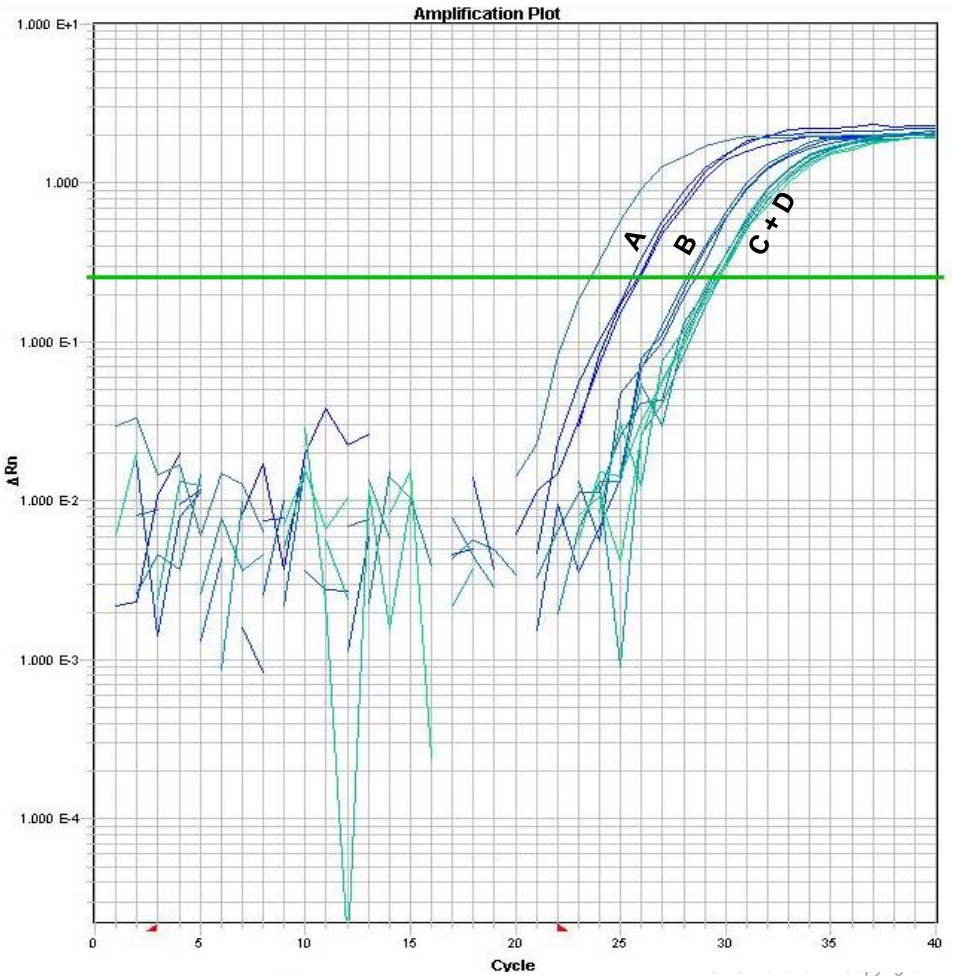
1:50 - *B. cockerelli* + 49 psyllid spp.

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

J. Munyaneza, K. Swisher, D. Percy, S. Bulman, J. Robinson, A. Nissinen, A. Fereres, A. Antonilez-Delgado, J. Palomo Gómez, M. Clark, K. Pearson, R. Cairns.

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