QBOL: Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health

Peter Bonants, EMBL-EBI, 20 oct 2008

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Introduction

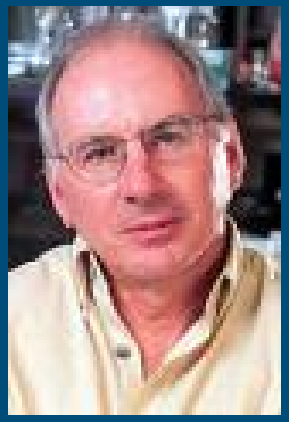
- What is DNA barcoding?
- Examples
- EU Call FP7
- Corsortium / Origin
- Which genes?
- Plant Health: QBOL
- Future

CONSORTIUM FOR THE BARCODE OF LIFE

The **Consortium for the Barcode of Life** (CBOL) is an international initiative devoted to developing DNA barcoding as a global standard for the identification of biological species. DNA barcoding is a new technique that uses a short DNA sequence from a standardized and agreed-upon position in the genome as a molecular diagnostic for species-level identification

CBOL has more than 150 Member Organizations from more than 45 countries including:

- Natural history museums, zoos, herbaria, and botanical gardens;
- University departments of biology and molecular biology;
- Biodiversity and conservation organizations, NGOs;
- Governmental and intergovernmental organizations; and
- Private biotech companies.



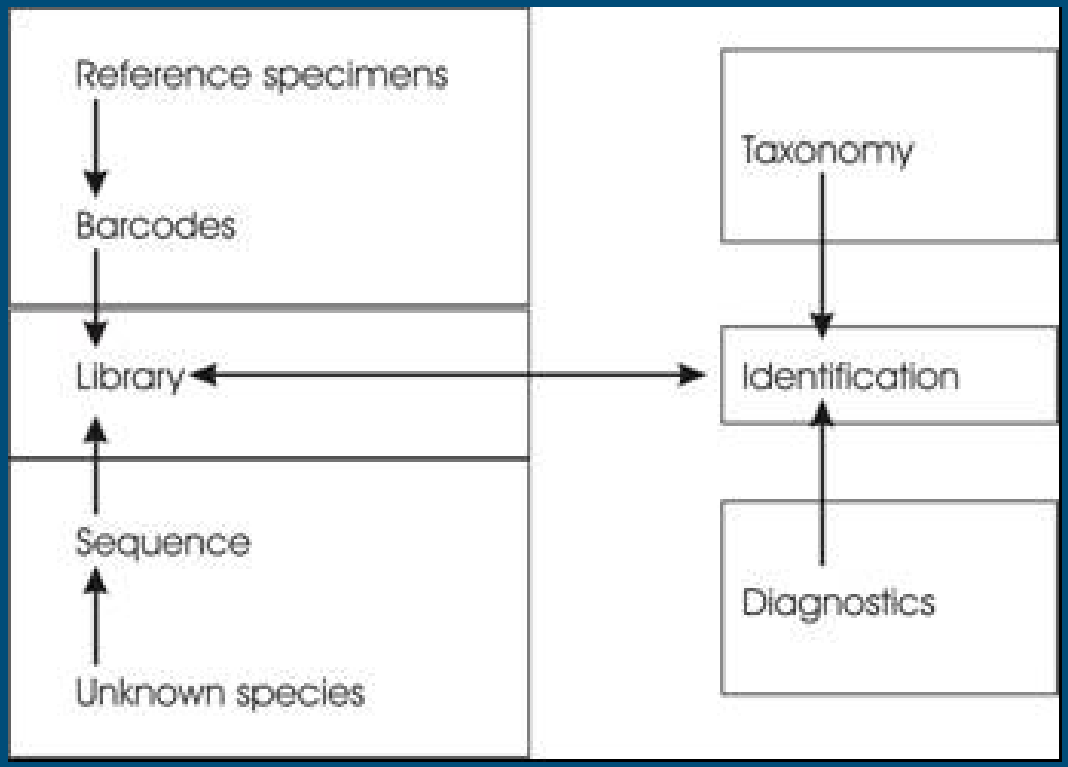
Paul Hebert

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

DNA Barcoding

DNA Barcoding projects have four components:

- *The Specimens*
- *The Laboratory Analysis*
- *The Database*
- *The Data Analysis*



Major Barcoding Campaigns

Polar Bears

Bats

All Birds

Fish

Lepidoptera

Tephritid

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

marine barcode of life
MarBOL Blog
Partners
MarBOL Team
MarBOL Project
Species Checklist | Progress Report | Protocols | Upcoming Meetings
Google™ Custom Search
Search
Meetings
Upcoming Meetings
9th International Meeting - Oct 30, 2008
MEEGID IX, University of California at Irvine, 30 October - 1 November 2008
Communications on genetics, genomics, proteomics, phylogenetics, population biology, mathematical modeling, and bioinformatics are welcome. They can report on the host, the pathogen, or the vector for vector-borne diseases. Papers considering host + pathogen or pathogen + vector (co-evolution) are particularly encouraged. All pathogens are within the scope of MEEGID: viruses, parasitic protozoa, helminths, fungal organisms, and prions. All infectious models can be explored, including those of veterinary or agronomical relevance.
progress
specimens barcoded
26361
species barcoded
4993
View detailed progress reports

FP7 in brief / Specific Programmes

***Cooperation* – Collaborative research**

***Ideas* – Frontier Research**

***People* – Human Potential**

***Capacities* – Research Capacity**

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

FP7 in brief / Cooperation

- 10 themes:
  - Health
  - **Food, Agriculture and Biotechnology**
  - ICT
  - Nanosciences
  - Energy
  - Environment (incl. climate change)
  - Transport
  - Socio-economic sciences and Humanities
  - Security
  - Space

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Text Call FP7-KBBE-2008-2B

KBBE-2008-1-4-01: Development of new diagnostic methods in support of Plant Health policy Call: FP7-KBBE-2008-2B

This Project will build a sustainable diagnostic resource to enable 'DNA-barcode identification' ultimately for all quarantine plant pests or pathogens of statutory importance. Key work will include: obtaining or producing relevant vouchered sequence data for individual pests or pest groups and position them in a correct taxonomic context, developing generic diagnostic tools based on these barcode sequences; linking vouchered sequence information to published biological information; developing strategic approaches and methodologies to enable the establishment of DNA banks and access to digital voucher specimens.

Funding scheme: Small collaborative project.

SEVENTH FRAMEWORK PROGRAMME

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

FP7: Call KBBE-2008-2B

Proposal full title:

Development of a new diagnostic tool using DNA barcoding of Quarantine organisms in support of plant health

Proposal acronym: **QBOL**

Type of funding scheme: **Small Collaborative Project**

SEVENTH FRAMEWORK PROGRAMME

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Targets Quarantine

- Which?
  - Fungi
  - Arthropods
  - Bacteria
  - Nematodes
  - Viruses
  - Phytoplasmas

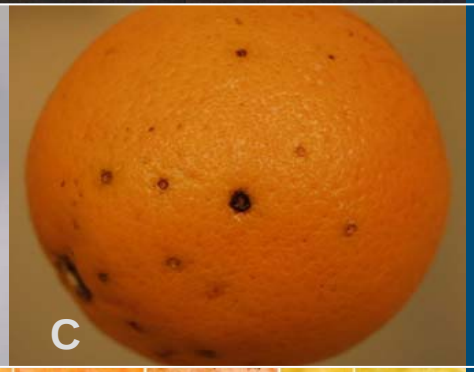
Council Directive 2000/29/EC

EPPO list A1 and A2

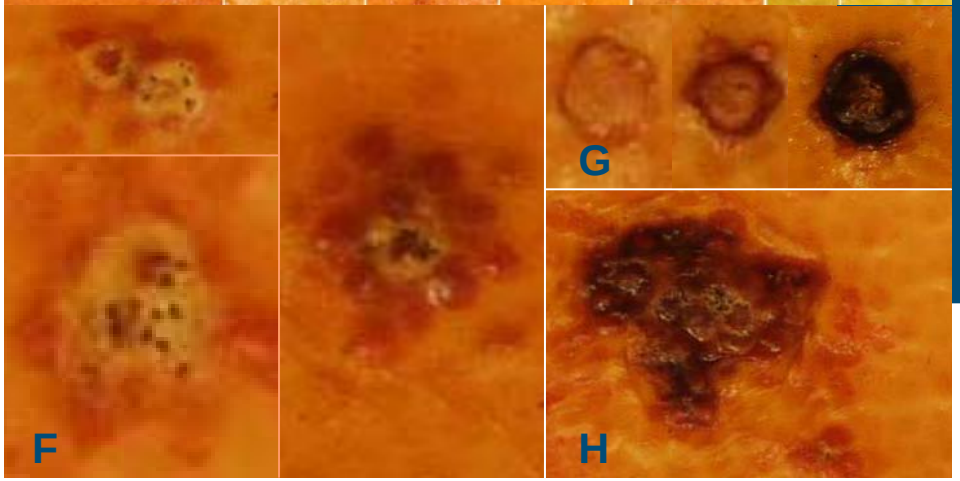
State of the Art

- *Collections and Taxonomic Experience*
- *Quarantine organisms on EU Directive and EPPO list*
- *Sequencing of Q-organisms*
- *Identification methods*
- *DNA Barcoding projects*
- *Databases*

Fungi



Guignardia citricarpa



Phytophthora ramorum

Barcoding Q Arthropods

OBJECTIVES

1. generate 5-10 barcode sequences (Cox1 and ITS) for about 100 species of Q arthropods and 50 closely related species
2. establish a prioritized list of all EU Q arthropods
3. collect all Q arthropods from different localities and crops
4. develop DNA extraction procedures for larvae and adults
5. develop and test primers for PCR amplification
6. collect DNA barcode sequence data
7. test the usefulness of this dataset

Anoplophora chinensis

Bactrocera dorsalis

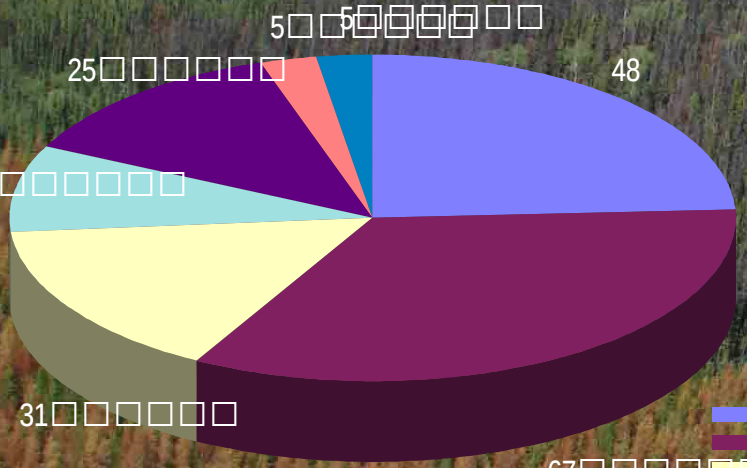
Tetranychus evansi

Scirtothrips spp.

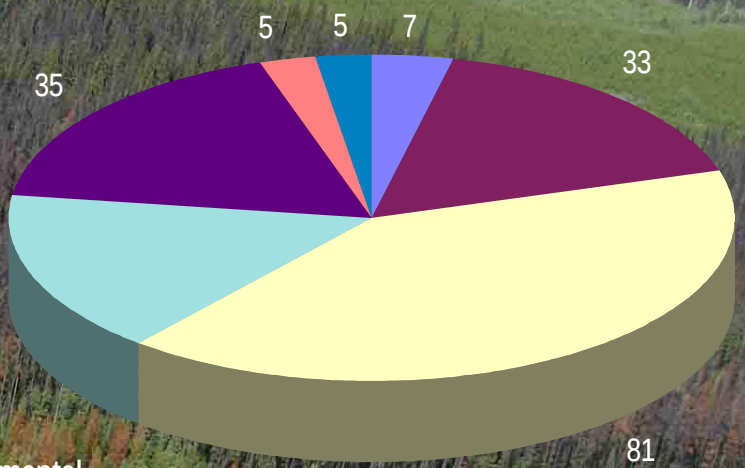
Dendroctonus spp.

Barcoding Q Arthropods

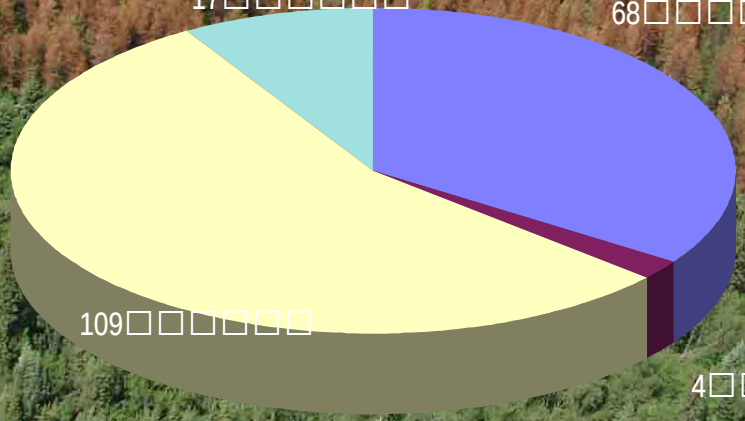
Q pest origin



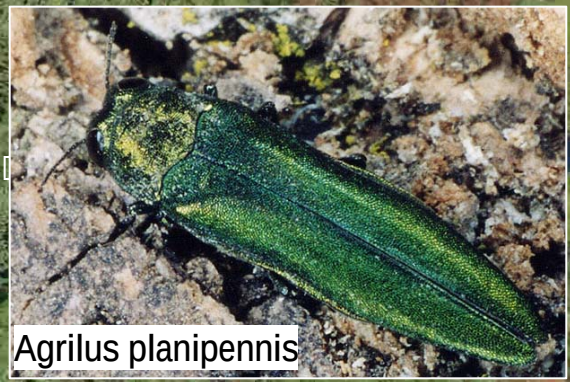
Q arthropod orders



Crop types



Diabrotica virgifera


Agrilus planipennis

Insects

Disciplin Entomology PD-NRL

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2005) **360**, 1813–1823
doi:10.1098/rstb.2005.1713
Published online 15 September 2005

DNA barcodes for biosecurity: invasive species identification

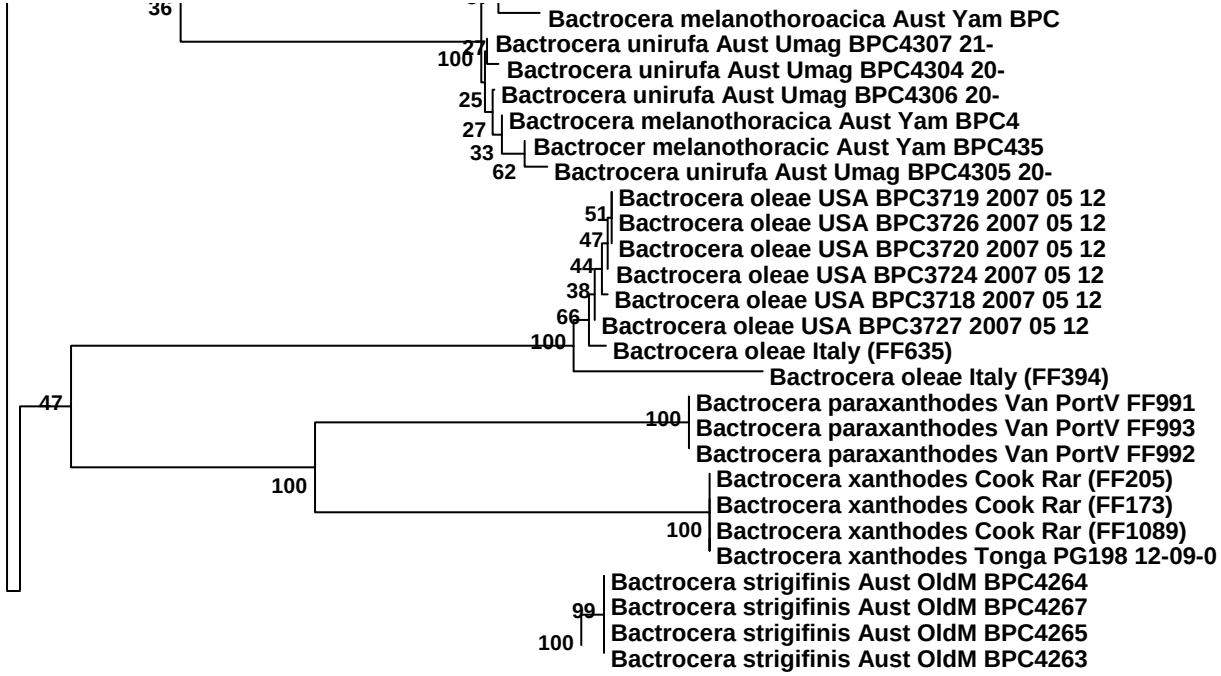
K. F. Armstrong* **and S. L. Ball**

National Centre for Advanced Bio-Protection Technologies, PO Box 84, Lincoln University, Canterbury, New Zealand

Biosecurity encompasses protecting against any risk through 'biological harm', not least being the economic impact from the spread of pest insects. Molecular diagnostic tools provide valuable support for the rapid and accurate identification of morphologically indistinct alien species. However, these tools currently lack standardization. They are not conducive to adaptation by multiple sectors or countries, or to coping with changing pest priorities. The data presented here identifies DNA barcodes as a very promising opportunity to address this. DNA of tussock moth and fruit fly specimens intercepted at the New Zealand border over the last decade were reanalysed using the *cox1* sequence barcode approach. Species identifications were compared with the historical dataset obtained by PCR–RFLP of nuclear rDNA. There was 90 and 96% agreement between the methods for these species, respectively. Improvements included previous tussock moth 'unknowns' being placed to family, genera or species and further resolution within fruit fly species complexes. The analyses highlight several advantages of DNA barcodes, especially their adaptability and predictive value. This approach is a realistic platform on which to build a much more flexible system, with the potential to be adopted globally for the rapid and accurate identification of invasive alien species.

Keywords: mitochondrial DNA; cytochrome oxidase subunit I; COI; molecular diagnostics; quarantine; insects

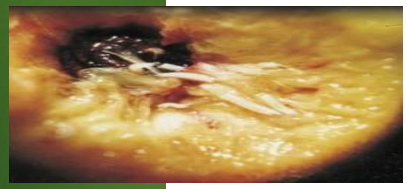
Bio-Protection

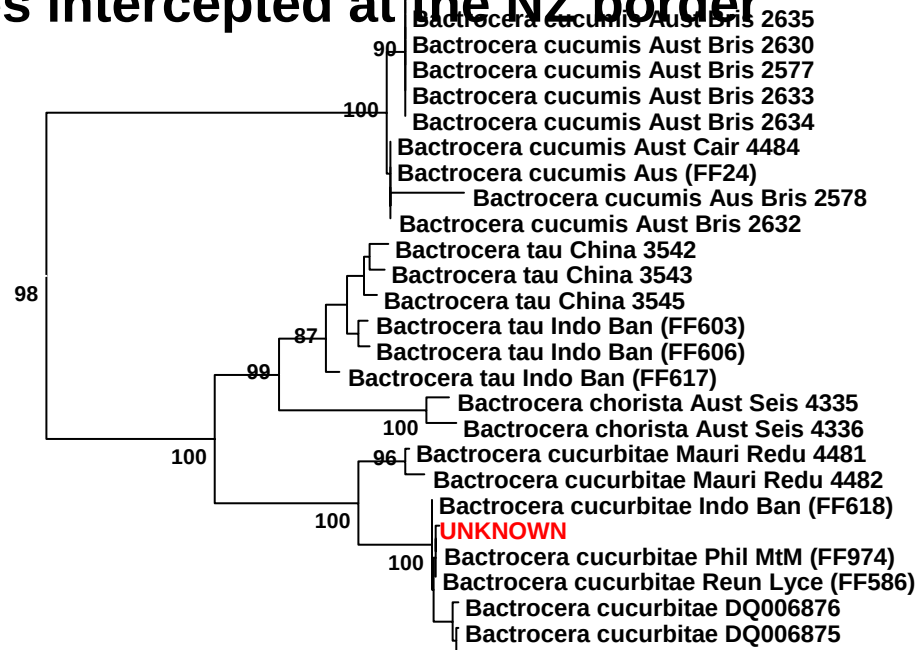


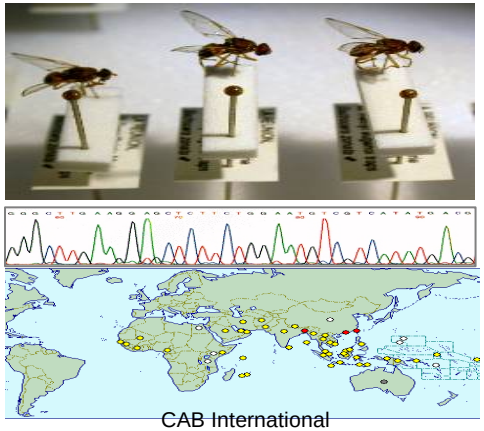
DNA barcodes to identify fruit fly immature life stages intercepted at the NZ border

Karen Armstrong

Lincoln University







CAB International

Combined EU / EPPO list Q and Q-alert bacteria

Annex I	**Part A**	**Section I**
	1 *Xyllela fastidiosa*	
Annex I	**Part A**	**Section II**
	1 *Clavibacter michiganensis* ssp. *sepedonicus*	
	2 *Pseudomonas solanacearum = Ralstonia solanacearum (race 1, race 3)*	
Annex I	**Part B**	
Annex II	**Part A**	**Section I**
	1 *Erwinia stewartii*	
	2 *Xanthomonas campestris* strains pathogenic to Citrus (as *Xanthomonas axonopodis* pv. *citri)*	
	3 *Xanthomonas campestris* pv. *oryzae & oryzicola = Xanthomonas oryzae* pv. *oryzae & oryzicola*	
Annex II	**Part A**	**Section II**
	1 *Clavibacter michiganensis* ssp. *insidiosus*	
	2 *Clavibacter michiganensis* ssp. *michiganensis*	
	3 *Erwinia amylovora*	
	4 *Erwinia chrysanthemi* pv. *dianthicola = Dickeya dianthicola*	
	5 *Pseudomonas caryophylli = Burkholderia caryophylli*	
	6 *Pseudomonas syringae* pv. *persicae*	
	7 *Xanthomonas campestris* pv. *phaseoli*	
	Xanthomonas arboricola pv. *corylina* (EPPO list)	
	8 *Xanthomonas campestris* pv. *pruni = Xanthomonas arboricola* pv. *pruni*	
	9 *Xanthomonas campestris* pv. *vesicatoria = Xanthomonas vesicatoria & Xanthomonas axonopodis* pv. *vesicatoria*	
	10 *Xanthomonas fragariae*	
	11 *Xylophilus ampelinus*	
Annex II	**Part B**	
	1 *Curtobacterium flacumfaciens* pv. *flaccumfaciens*	
Q-alert EPPO		
	Xanthomonas axonopodis pv. *dieffenbachiae*	
	Xanthomoans arboricola pv. *fragariae*	
	Xanthomonas axonopodis pv. *allii*	

Bacteria

Xanthomonas fragariae

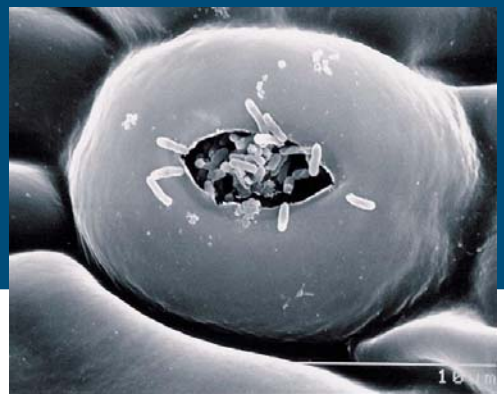
Clavibacter michiganensis subsp. sepedonicus

Ralstonia solanacearum

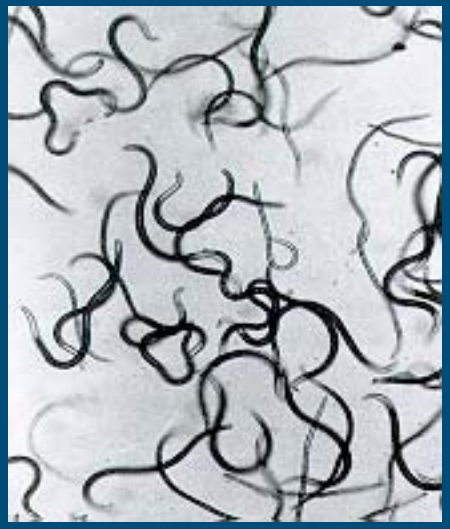
PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Bacteria

Citrus Canker, Xanthomonas citri subsp. citri



Nematodes



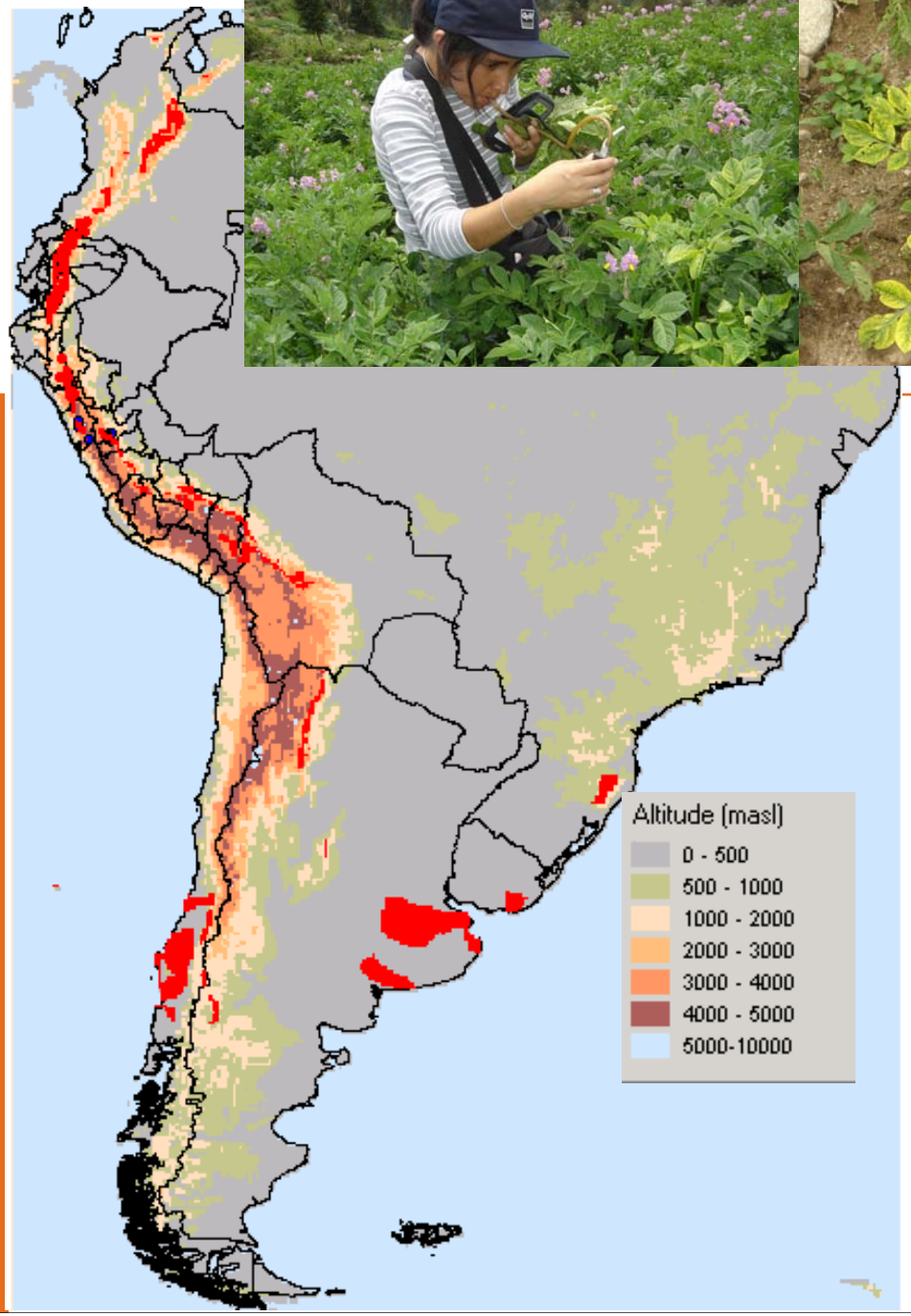
Pine wood nematode

Root knot nematode

Viruses

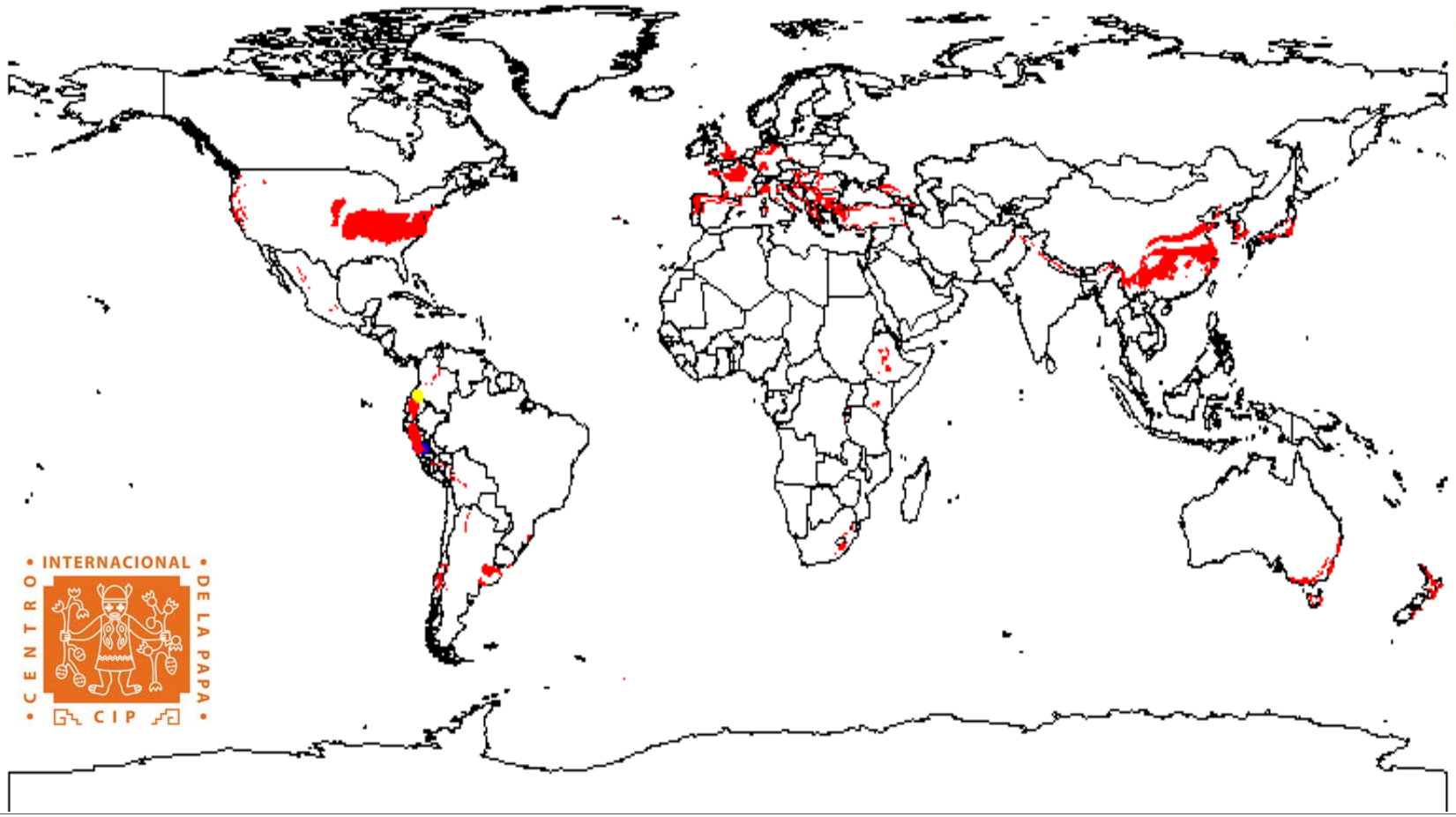
Alstroemeria Yellow Spot Topovirus

Prunus Necrotic Ringspot Virus



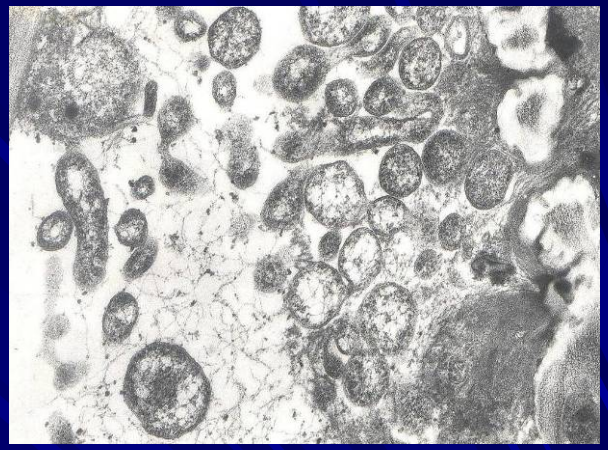
- GPS survey work, laboratory diagnosis and GIS modeling predicts *Potato yellow vein virus (*a model system for emergent disease) threat to Southern Peru and Bolivia.
- Relevant National bodies alerted.
- Climate change scenarios indicate increase in range

Current global potential threat of establishment of PYVV.

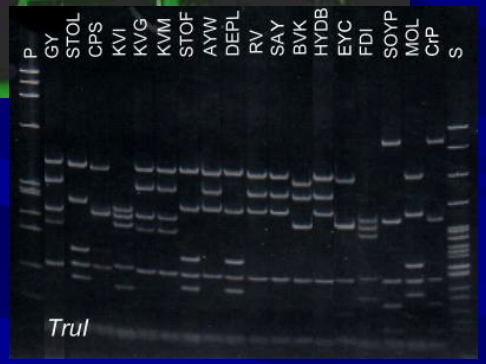


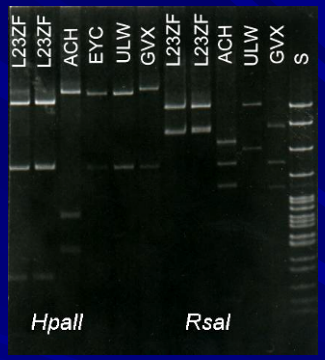
1. What are Phytoplasmas?

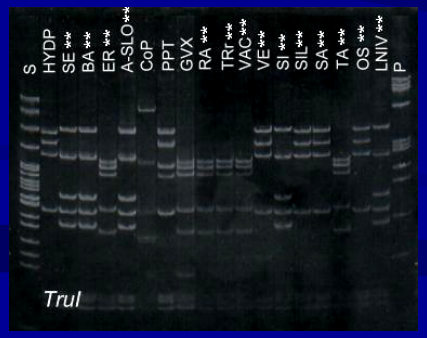
- Phloem-limited insect-transmitted plant pathogenic bacteria that lack a cell wall
- Related to Gram positive bacteria (*Bacillus*) do not encode typical genes related to pathogenicity present in other plant pathogenic bacteria
- Can not be grown in axenic culture
- Genomes of some have been sequenced (sizes range from 530 – 1200 kb) they are the smallest known self-replicating life form
- Symptoms include yellowing, phyllody, proliferation, stunting, general decline, witches' broom

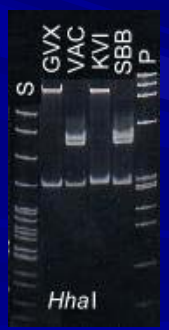


Collection of in periwinkle micropropagated phytoplasma strains



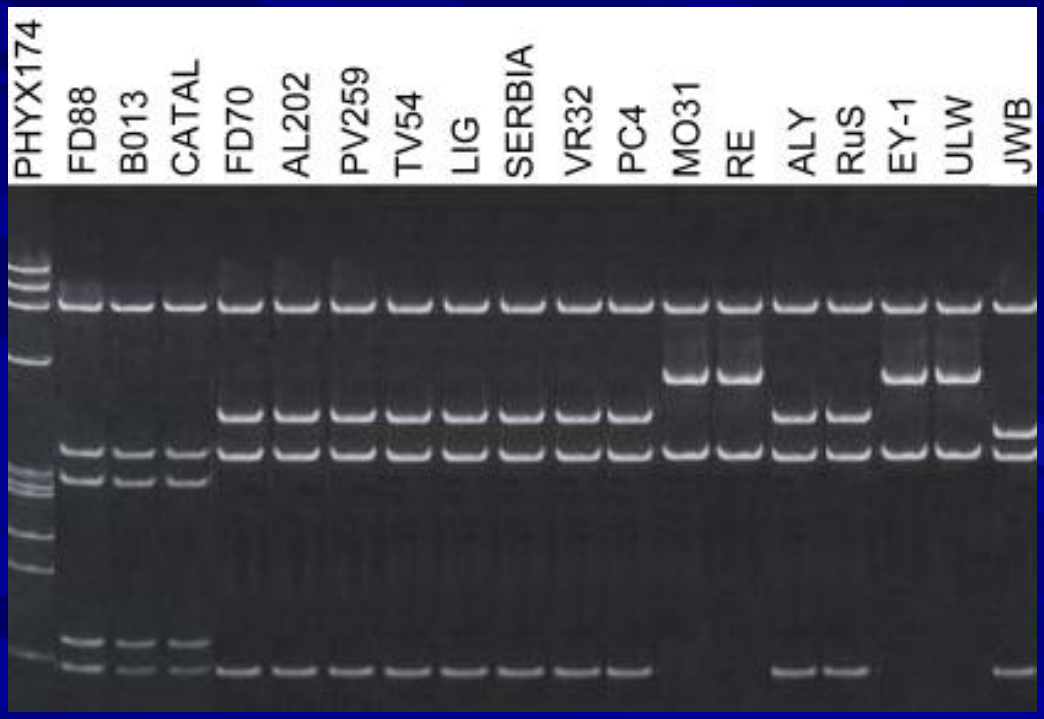




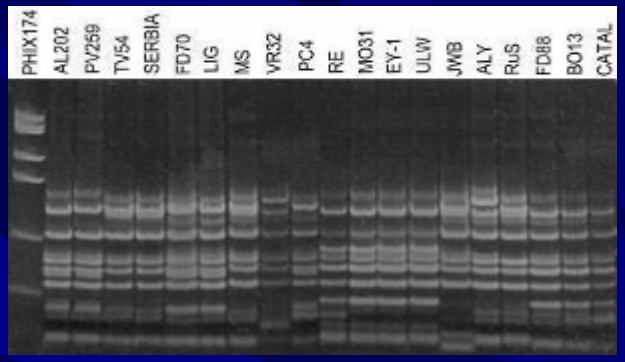


http://www.dista.unibo.it/person/collectionseptember_2003.pdf

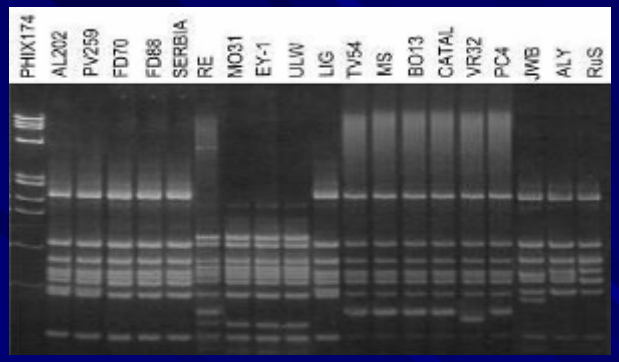
Polymorphisms as shown by RFLP analyses with selected restriction enzymes on three genes of different strains of phytoplasmas related to Flavescence dorée



***TaqI* on 16Sr gene**

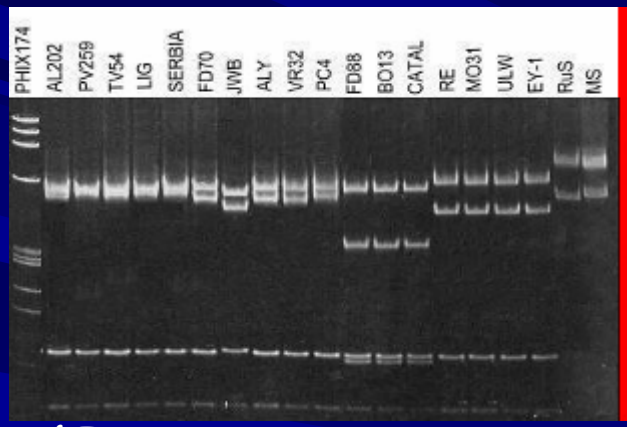


TruI

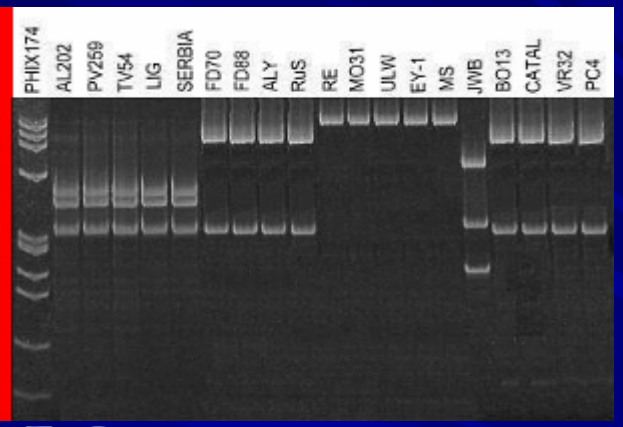


Tsp509I

rpS3 gene

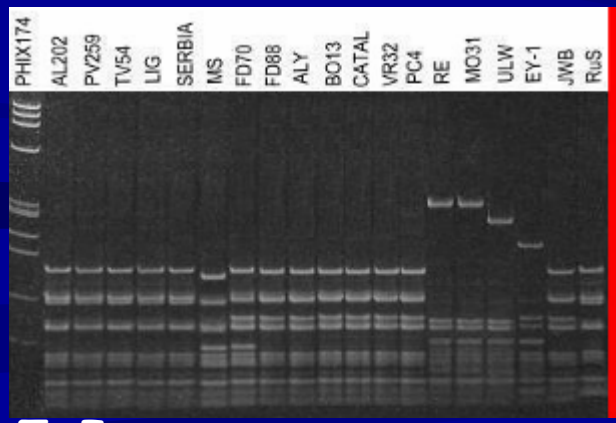


AluI

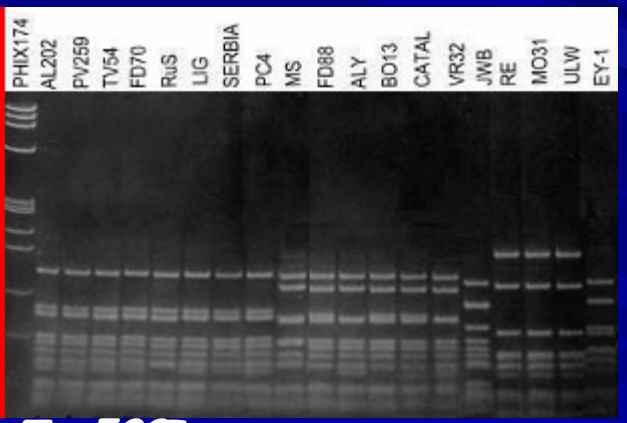


TaqI

SecY gene



TruI



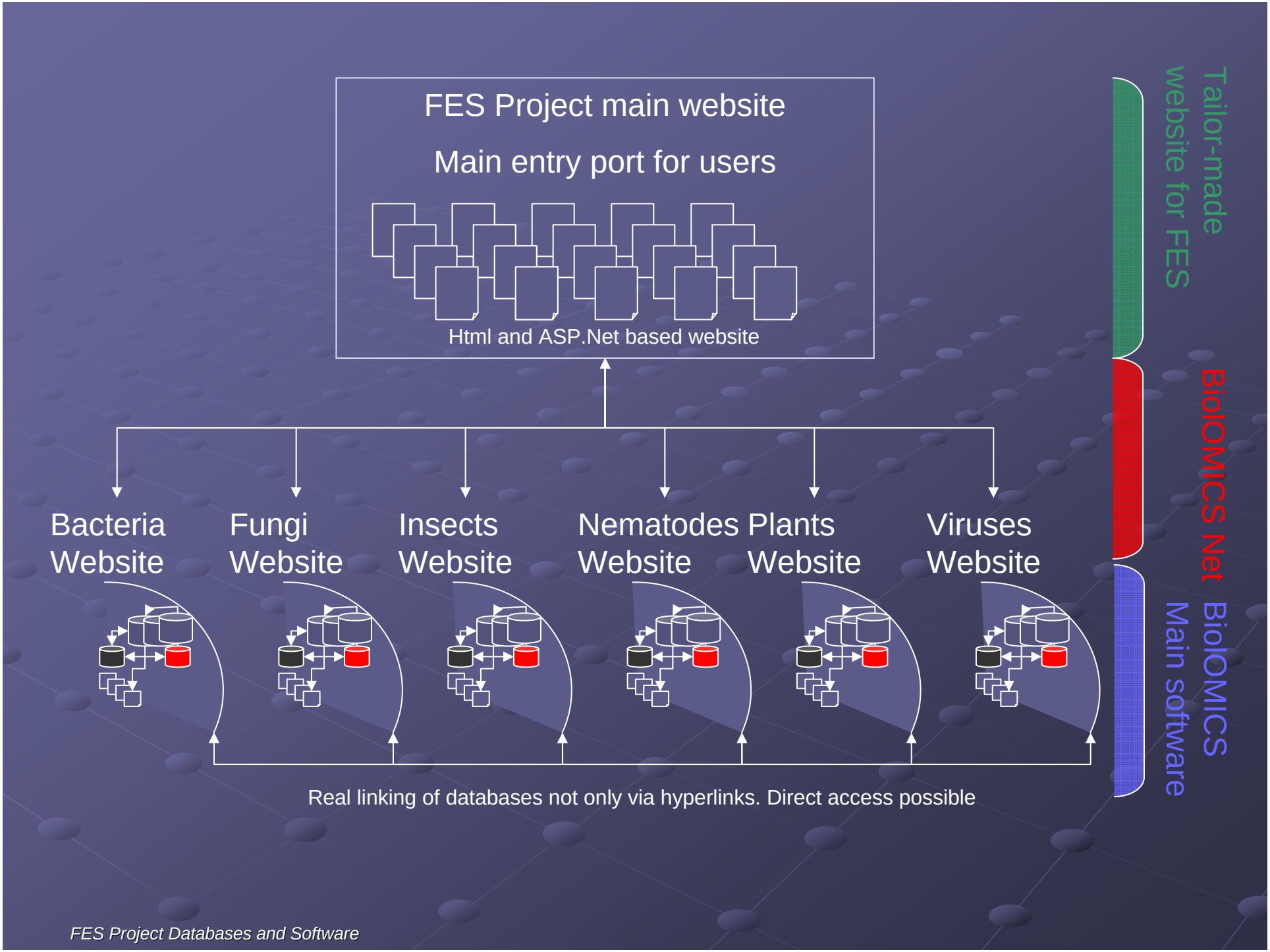
Tsp509I

Three principle QBOL Objectives

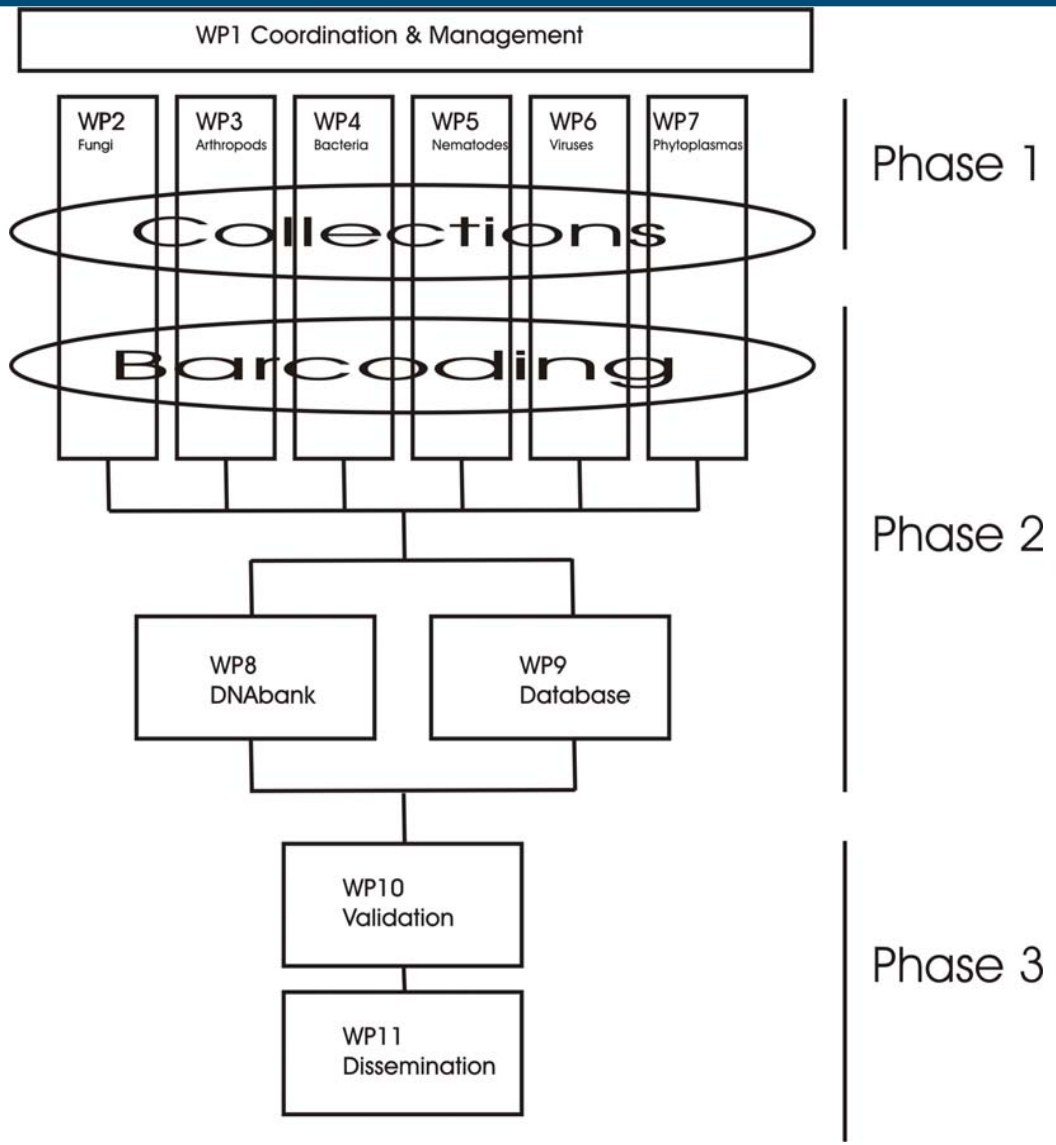
- *to DNA barcode relevant Q-organisms + morphologically and/or taxonomically related organisms*
- *to develop a database of DNA barcode sequences plus relevant taxonomic/geographic/host data*
- *to develop a DNA bank for the selected set of Q-organisms + morphologically and/or taxonomically related organisms*

Databases

- BOLD
- GenBank, EMBL
- FES Programme (NL)
  - *BioloMICS*
  - *MycoBank (www.mycobank.org)*


FES Project main website
Main entry port for users
Html and ASP.Net based website
Bacteria Website
Fungi Website
Insects Website
Nematodes Website
Plants Website
Viruses Website
Real linking of databases not only via hyperlinks. Direct access possible
Tailor-made website for FES
BioIOMICS Net
BioIOMICS Main software

Work Packages QBOL



PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

QBOL

Partners QBOL 1

Plant Research International	(PRI, The Netherlands)
Central Science Laboratory	(CSL, United Kingdom)
Institute for Agricultural and Fisheries Research	(ILVO, Belgium))
Laboratory of Microbiology, Univ Gent	(LM-UG, Belgium)
Agroscope Changins-Wädenswil Research Station	(ACW, Switzerland)
INRA - Centre de Biologie et de Gestion des Populations	(INRA, France)
University of Aarhus	(UA, Denmark)
University of Bologna	(UB, Italy)
Centraalbureau voor Schimmelcultures	(CBS, The Netherlands)
International Potato Center	(CIP, Peru)
Plant Protection Service	(PPS, The Netherlands)

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

QBOL

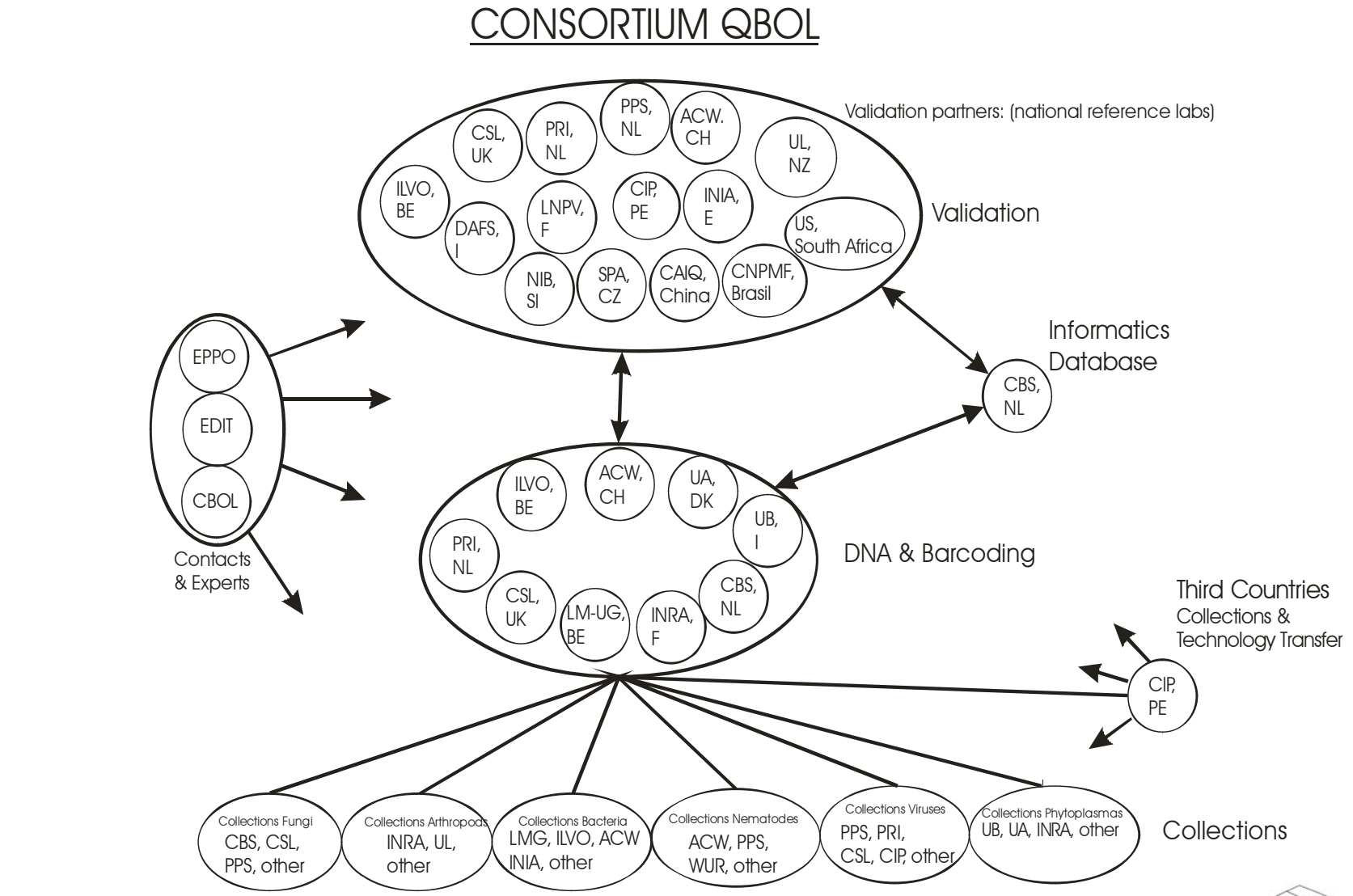
Partners QBOL 2

National Institute of Biology	(NIB, Slovenia))
State Phytosanitary Administration	(SPA, Czech Republic)
French national laboratory for plant health	(LNPV, France)
Chinese Academy of Inspection and Quarantine	(CAIQ, China)
Lincoln University, Bio-Protection Research Centre	(LU, New Zealand)
Embrapa Mandioca e Fruticultura Tropical	(CNPMF, Brasil)
Dept. Agricultural and Food Sciences	(DAFS, Italy)
	(INIA, Spain)
University of Stellenbosch	(US, South Africa)

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

QBOL

Consortium QBOL



PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

QBOL

Advisory Board QBOL

EPPO: European Plant Protection Organisation

Dr. Francoise Petter

BOLD: Barcoding of Life Database

Dr. Paul Hebert

Deliverables QBOL

Culture Collections and Taxonomic Experience

A worldwide network of relevant culture collections containing selected Q-organisms and the taxonomic expertise related to those collections.

Quarantine organisms EU Directive, EPPO list (the EPPO pest lists include 298 pests)

Many of the quarantine or regulated organisms of the EU Directive / EPPO list will be addressed. Priorities will be made based upon availability of target specimens and closest relatives in different regions in the world.

Deliverables QBOL

Sequencing of Q-organisms

Multi gene analysis will be executed on selected quarantine/ regulated organisms

Barcoding projects BOLD

Close contact with BOLD is guaranteed and data will be shared with BOLD

DNAbank

A DNA bank will be build containing DNA samples of barcoded quarantine/regulated organisms and their taxonomically related species

Deliverables QBOL

Database

A web based Database system will be build containing all relevant DNA barcodes and freely accessible by the end-users

Validation

Developed protocols and the database containing relevant DNA barcodes + taxonomic data will be validated by end-users

Implementation- Dissemination

End-users will be trained, developed protocols and the database will be distributed, a workshop/symposium will be organized.

Effort will be put on implementation of Database into an (inter)national platform within and outside EU laboratories.

QBOL: Barcoding for Plant Health

- Genes with sequence difference between Q-organisms and closely related organisms
- Easy to amplify with generic primers
- Culture collections
- Taxonomic experience
- Accessible Database
- Development of ID and DET methods
- Validation – EPPO/IPPC
- Implementation – PPS

The Future

Acknowledgements

- Karen Armstrong, Lincoln University, New Zealand
- Jean-Yves Rasplus, INRA, France
- Martine Maes, ILVO and Paul de Vos, LM-UG, Belgium
- Neil Boonham, CSL, UK
- Hans de Gruyter/Linda Kox, PPS, The Netherlands
- Jaime Cubero, INIA, Spain
- Juerg Frey/Sebastian Kiewnick , ACW, Switzerland
- Assunta Bertaccini, UB, Italy
- Ian Barker, CIP, Peru
- QBOL partners

Thank you very much for your attention

© Wageningen UR

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR