

# QBOL

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

[www.qbol.org](http://www.qbol.org)

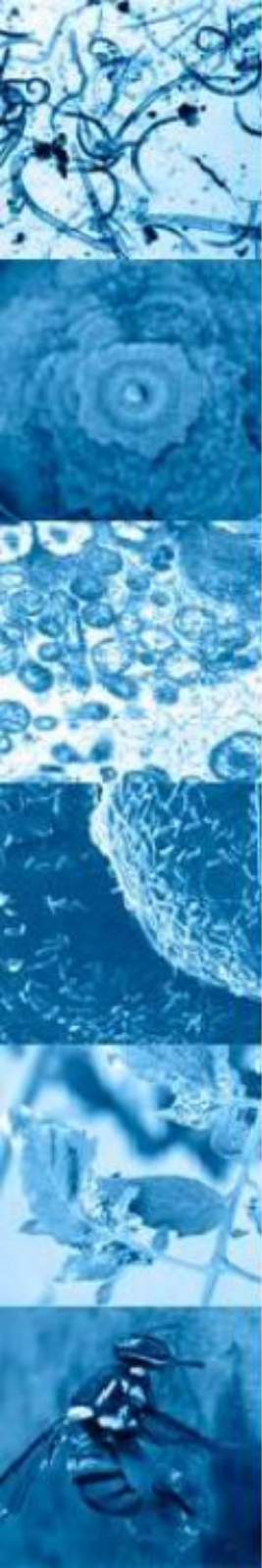
[peter.bonants@wur.nl](mailto:peter.bonants@wur.nl)



# What is QBOL?

- > 20 organizations (universities, research institutes and phytosanitary organizations) in 15 countries
- Financed by EU 7th Framework Program
  - 3 M€ EU + 1 M€ own contribution for 3 years
- Developing DNA barcoding to identify quarantine organisms in support of plant health





## Why DNA barcoding?

- Increasing world wide trading of plants enhances risk of spreading harmful organisms
- Decreasing taxonomic knowledge to identify Q-organisms
- Result in significant possible economic damage
- **DNA barcoding** offers **accurate identification** and focuses on strengthening the link between traditional and molecular taxonomy

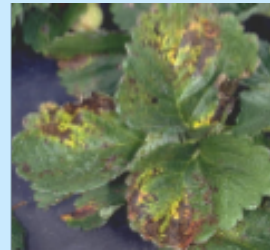
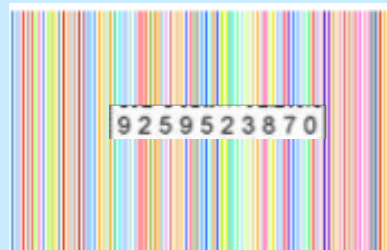
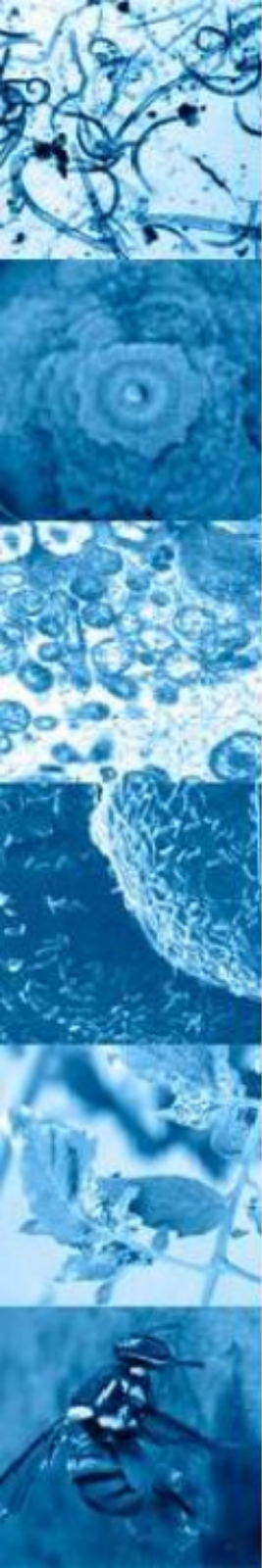




# Three principle QBOL Objectives



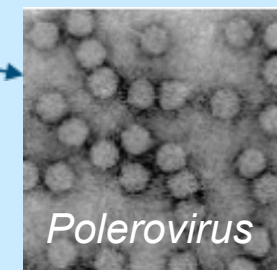
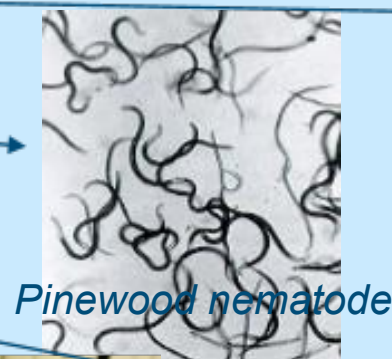
- *to DNA barcode relevant Q-organisms + morphologically and/or taxonomically related organisms*
- *to develop a database of DNA barcode sequences plus relevant taxonomic/geographic/host data*
- *to develop a DNA bank for the selected set of Q-organisms + morphologically and/or taxonomically related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



# Targets Quarantine

## Which?

- Fungi
- Arthropods
- Bacteria
- Nematodes
- Viruses
- Phytoplasmas

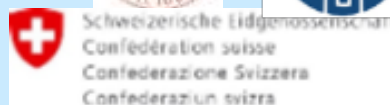


- Council Directive 2000/29/EC



# Partners QBOL

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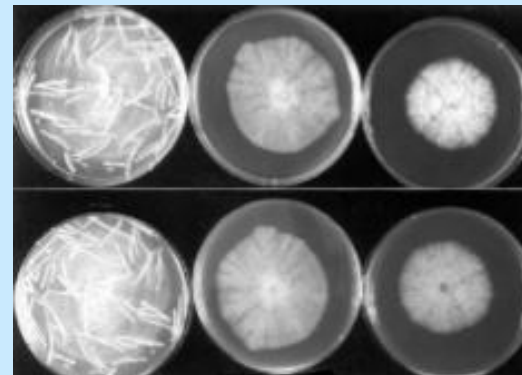
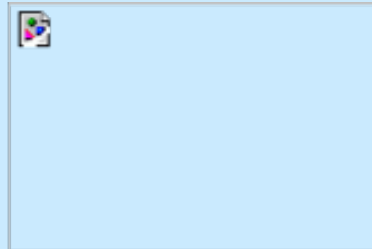
WP2 leader: Ewald Groenewald (KNAW-CBS)

# WP2: Fungi

Which:

*Monilinia, Ceratocystis, Melampsora, Puccinia, Thecaphora and Mycosphaerella*

List Q- species and relatives on website





# WP2: Fungi: potential barcode loci

- Internal transcribed spacers of rDNA operon (ITS):

V9G, ITS1, ITS1F / ITS4, LR6

- Beta-tubulin (TUB):

T1, Bt1a / Bt1b, Bt2b

- Cytochrome oxidase I (COI):

PenF1 / PenR1, AspR1

- Histone H3 (HIS):

CYLH3F / CYLH3R

- Translation elongation factor 1-alpha (TEF):

EF1-728F / EF1-986R, EF-2

- Calmodulin (CAL):

CAL-228F / CAL-737R

- Actin (ACT):

ACT-512F / ACT-783R

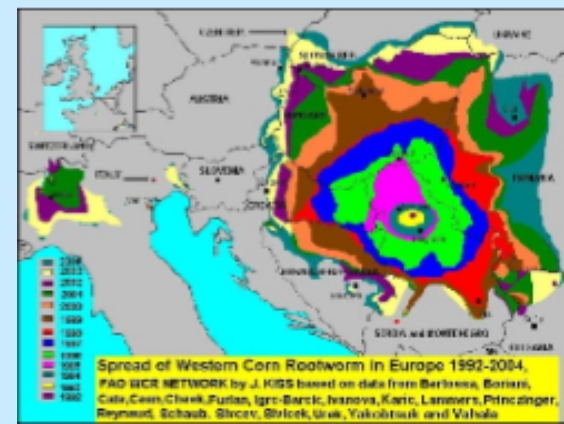
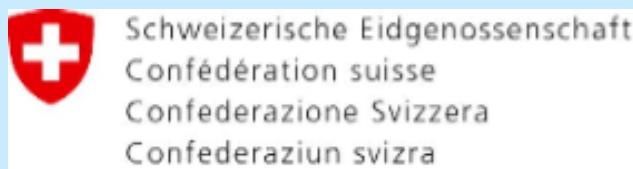




## WP3: Arthropods

### ○ Which?

1. Number of species (198), multiple target crops (Agriculture also forests, ornamentals etc.)
2. Example : *Diabrotica* spp. on Maize
3. Billion \$ cost in US, introduced in Europe
4. Pesticide use (20 to 25 million acres in US) □ indirect costs hardly estimated
5. Species complex (i.e. *Ips*, *Gonipterus*, *Epitrix*, *Bemisia* globally poorly known)

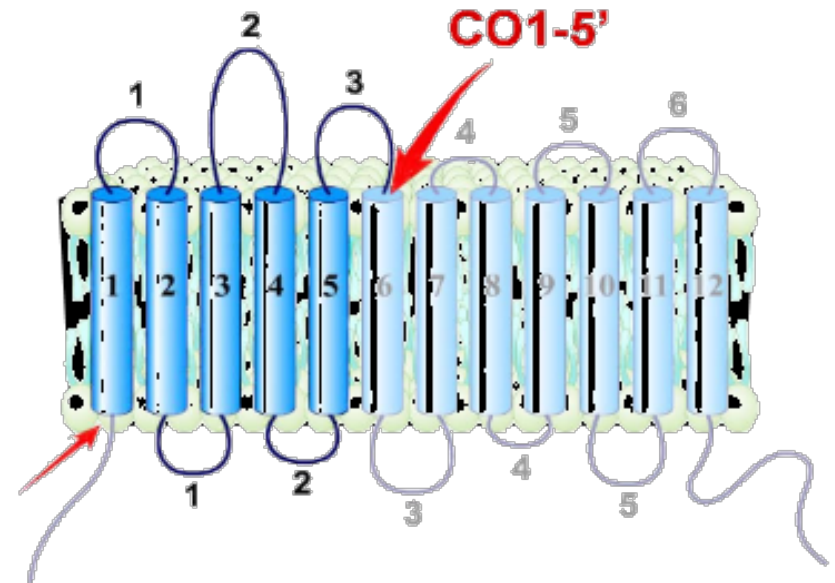
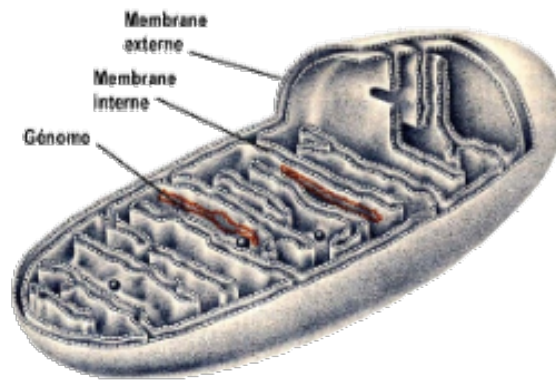
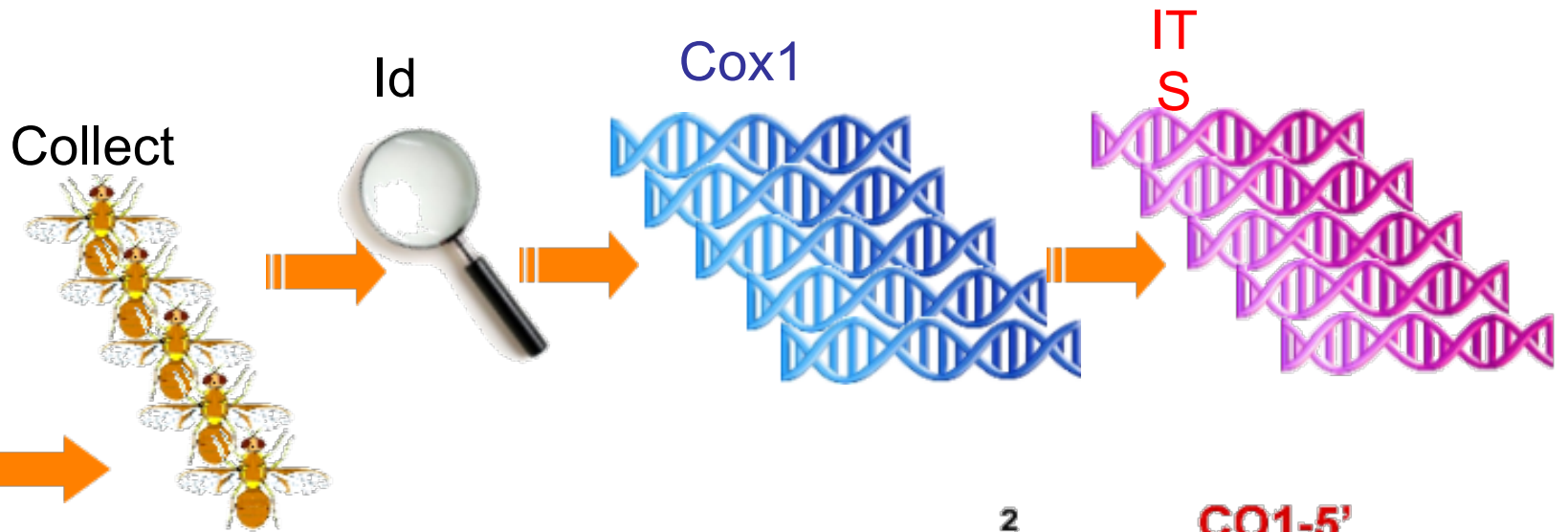


## Species list

*Aleurocanthus spiniferus* (Quaintance)  
*Aleurocanthus woglumi* Ashby  
*Anastrepha obliqua* (Macquart)  
*Anastrepha fraterculus* Wiedmann  
*Anastrepha ludens* (Loew)  
*Anastrepha suspensa* Loew  
*Blitopertha orientalis* (Waterhouse)  
*Anoplophora chinensis* (Thomson)  
*Anoplophora glabripennis* (Motschulsky)  
*Anthonomus bisignifer* Schenck  
*Anthonomus eugenii* Cano  
*Anthonomus grandis* Boheman  
*Anthonomus quadrigibbus* Say  
*Anthonomus signatus* Say  
*Aonidiella citrina* (Coquillett)  
*Arrhenodes minutus* (Drury)  
*Aschistonyx eppoi* Inouye  
*Aulacaspis yasumatsui* Takagi  
*Bactrocera cucumis* (French)  
*Bactrocera cucurbitae* Coquillett  
*Bactrocera dorsalis* (Hendel)  
*Bactrocera invadens* (Hendel)  
*Bactrocera minax* (Enderlein)  
*Bactrocera tryoni* (Froggatt)  
*Bactrocera tsuneonis* (Miyake)  
*Bactrocera zonata* (Saunders)  
*Bemisia tabaci* (Gennadius) & biotype B  
*Cacoecimorpha pronubana* Hübner  
*Cacysreus marshalli* Butler  
*Carneocephala fulgida* Nottingham  
*Carposina niponensis* (Walsingham)  
*Cephalcia lariciphila* (Wachtl)  
*Ceratitis capitata* Wiedemann  
*Ceratitis quinaria* (Bezzi)  
*Ceratitis rosa* Karsch  
*Ceratitis cosyra* (Walker)  
*Choristoneura conflictana* (Walker)  
*Choristoneura fumiferana* (Gahan)



## WP3 Barcoding Arthropods OBJECTIVES



QBOL : Barcoding Quarantine Pests

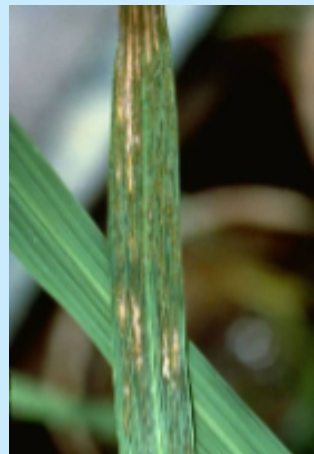
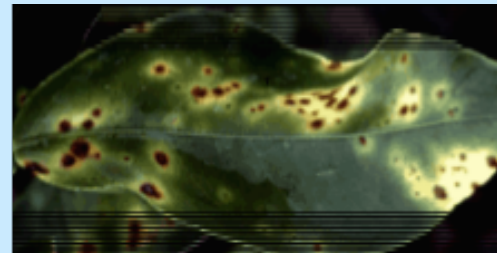


## WP4: Bacteria: potential barcode loci

- 16S

- GyrB : DNA gyrase, subunit  $\beta$ , which unwinds double stranded DNA

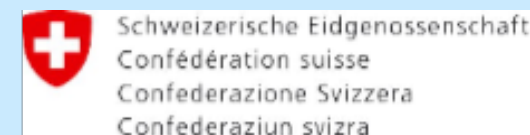
rpoB : RNA polymerase, subunit  $\beta$ , involved in RNA biosynthesis



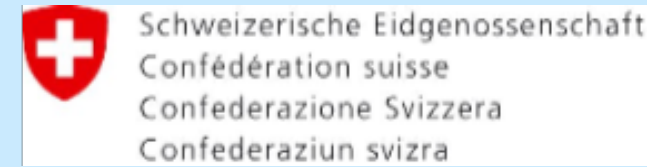
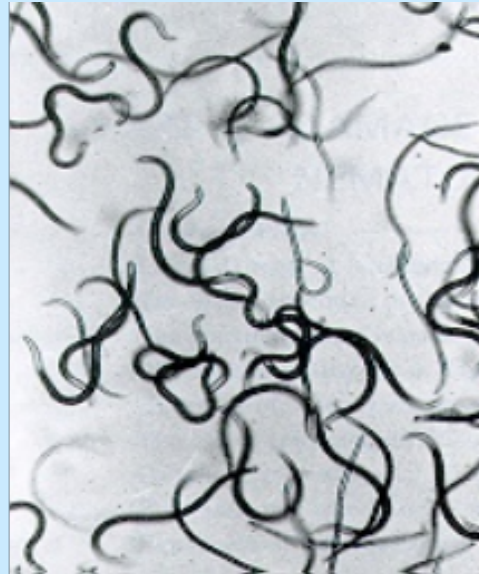
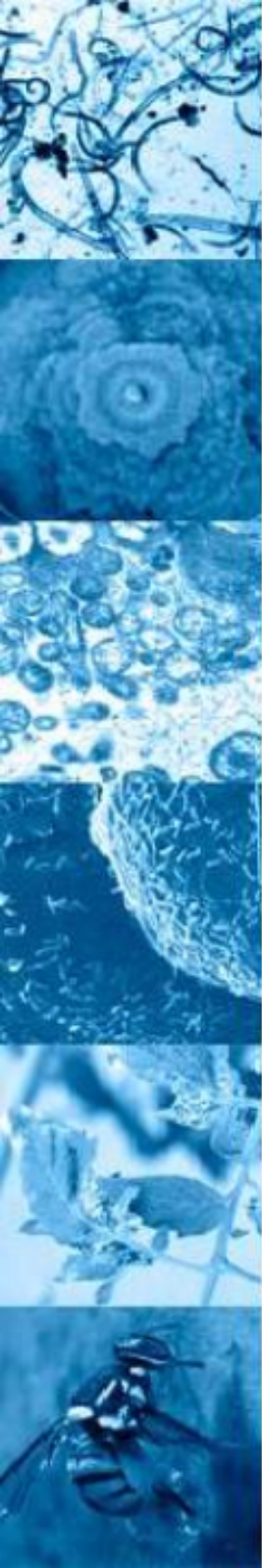


# WP4: Bacteria

	QBOL Priority group 1	QBOL Priority group 2
<i>Xylella fastidiosa</i>	X	
<i>Clavibacter michiganensis</i> subsp. <i>sepedonicus</i>	X	
<i>Pseudomonas solanacearum</i> = <i>Ralstonia solanacearum</i>	X 3 phylotypes	
<i>Xanthomonas</i> strains pathogenic to Citrus	X X X 3 subspp.	
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> & <i>oryzicola</i>	X X 2 pvs	
<i>Clavibacter michiganensis</i> subsp. <i>insidiosus</i>	X	
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	X	
<i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i>		X
<i>Xanthomonas vesicatoria</i> & <i>X.</i> <i>axonopodis</i> pv. <i>vesicatoria</i>		X X
<i>Xanthomonas fragariae</i>		X
<i>Xanthomonas translucens</i>		X
<i>Xanthomonas axonopodis</i> pv. <i>dieffenbachiae</i>		X
<i>Xanthomonas axonopodis</i> pv. <i>allii</i>		X



# WP5: Nematodes





# D 5.1 List of selected nematode Q-organisms (Month 6)

SG1	SG2	SG3	1
X			Meloidogyne arenaria
X			Meloidogyne incognita
X			Meloidogyne javanica
X	X		Meloidogyne enterolobii/mayaguensis
X	X		Meloidogyne chitwoodi
X	X		Meloidogyne fallax
X			Meloidogyne hapla – race A
		X	Meloidogyne minor
		X	Meloidogyne naasi
	X		Bursaphelenchus xylophilus
		X	Bursaphelenchus mucronatus
		X	Bursaphelenchus chengi
		X	Bursaphelenchus doui
		X	Bursaphelenchus thailandae
		X	Xiphinema americanum sensu lato
		X	Xiphinema diversicaudatum
		X	Xiphinema rivesi

2
Aphelenchoides besseyi
Ditylenchus destructor
Ditylenchus dipsaci
Globodera pallida
Globodera rostochiensis
Heterodera glycines
Hirschmaniella spp.
Longidorus diadecturus
Meloidogyne ardenensis
Meloidogyne maritima
Nacobbus aberrans
Radopholus similis (attacking citrus, formerly R. citrophilus)
Radopholus similis (not attacking citrus)
Xiphinema bricolense
Xiphinema californicum

3	
Anguina tritici	Longidorus macrosoma
Aphelenchoides ritzemabosi	Meloidogyne exigua
Aphelenchoides bicaudatus	Paratrichodorus porosus
Aphelenchoides saprophilus	Paratrichodorus anemones
Bursaphelenchus conicaudatus	Paratrichodorus pachydermus
Bursaphelenchus fraudulentus	Pratylenchus loosi
Ditylenchus adasi	Pratylenchus mediterraneus
Globodera achilleae	Pratylenchus crenatus
Globodera tabacum	Pratylenchus neglectus
Heterodera avenae	Pratylenchus penetrans
Heterodera filipjevi	Pratylenchus thornei
Heterodera latipons	Pratylenchus vulnus
Heterodera schachtii	Radopholus arabocoffeae
Helicotylenchus dihystra	Radopholus duriophilus
Helicotylenchus pseudorobustus	Subanguina radiculicola
Helicotylenchus varicaudatus	Trichodorus cedarus
Helicotylenchus vulgaris	Trichodorus cylindricus
Hirschmaniella gracilis	Trichodorus primitivus
Laimaphelenchus penardi	Xiphinema incognitum
Longidorus arthensis	Xiphinema index
Longidorus dunensis	Xiphinema simile
Longidorus elongatus	

nematodes supplied by:  
ACW, WU-Nematology, FERA,  
ILVO



## WP5: Nematodes

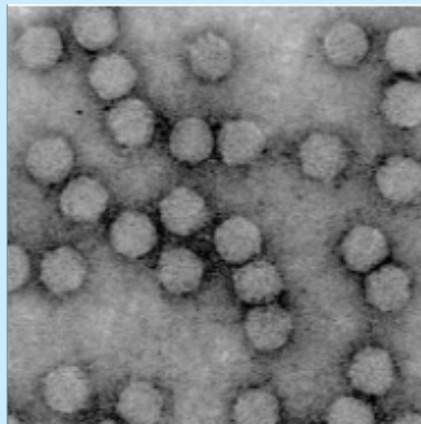
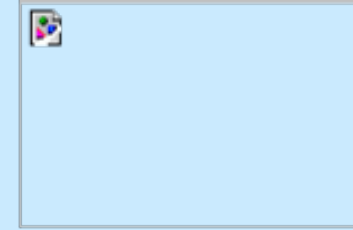
Seven barcode regions will be screened in Priority Group 1:

- SSU rDNA, D1-D2 region
- LSU rDNA, D2-D3 region
- IGS2
- COI
- COII
- RNA polymerase II



## WP6: Viruses

- No standard region
- Not culturable
- Relative small genomes
- 90% RNA viruses
- Change of WP description: whole genome seq



# WP6: Viruses

Species	Genus	List	Genome sequence available	Partner responsible	Isolates available within WP
Arracacha virus B, oca strain	Nepovirus	IAI	No	Fera	Yes
Potato black ringspot virus	Nepovirus	IAI / EPPO A1	No	Fera	Yes
Potato virus T	Trichovirus	IAI / EPPO A1	Yes x2	CIP	Yes
Chrysanthemum stem necrosis virus	Tospovirus	EPPO A1	No	PRI	Yes
Potato yellow dwarf virus	Rhabdovirus	EPPO A1	No		
Potato yellowing virus	Alphamovirus	EPPO A1	No	CIP	Yes
Tomato infectious chlorosis virus	Crinivirus	EPPO A2	No	Fera	Yes
Iris yellow spot virus	Tospovirus	EPPO Alert	No	PRI	Yes
Tomato torrado virus	Toradovirus	N/A <sup>1</sup>	Yes	PRI	Yes
Tomato marchitez virus	Toradovirus	N/A <sup>1</sup>	Yes	PRI	Yes



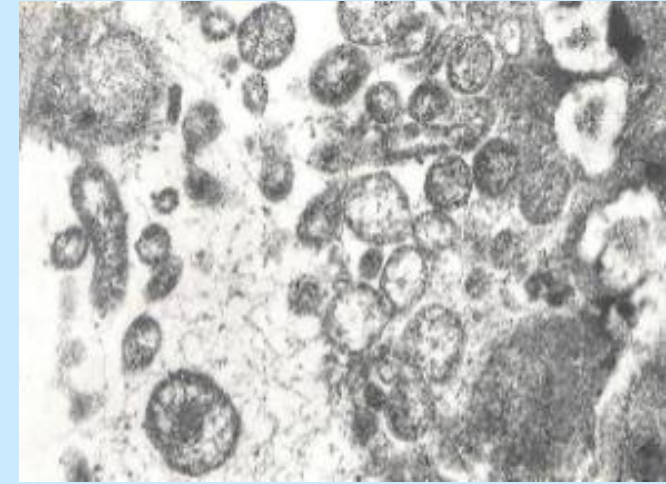
## WP7: Phytoplasmas

### Quarantine Phytoplasma species

- Strawberry witches' broom (16SrI)
- 'Witches broom' *Citrus* and few other *Ca. P. aurantifolia*' (WBDL, 16SrII)
- Peach rosette (16SrIII)
- Peach X (16SrIV) strains CX and WX)
- Peach yellows (16SrIII)
- Palm lethal yellowing (16SrIV)
- Grapevine flavescence *Vitis* (16SrV and 16SrVI)
- Elm phloem necrosis (16SrV strain EY)
- Apple proliferation *Ca. P. mali*' (16SrIX strains AP, AT)
- Apricot chlorotic leafroll *Ca. P. prunorum* (16SrXB, ESFY)
- Pear decline *Ca. P. pyri*' (16SrX, PD)
- Potato stolbur (16SrXI)
- Potato purple top wilt (aster yellows) (EPPO list)

## WP7: Phytoplasmas: potential barcode loci

- 16S
- Tuf
- SecA
- 16S-23S spacer
- Ribosomal proteins
- SecY
- rpoC



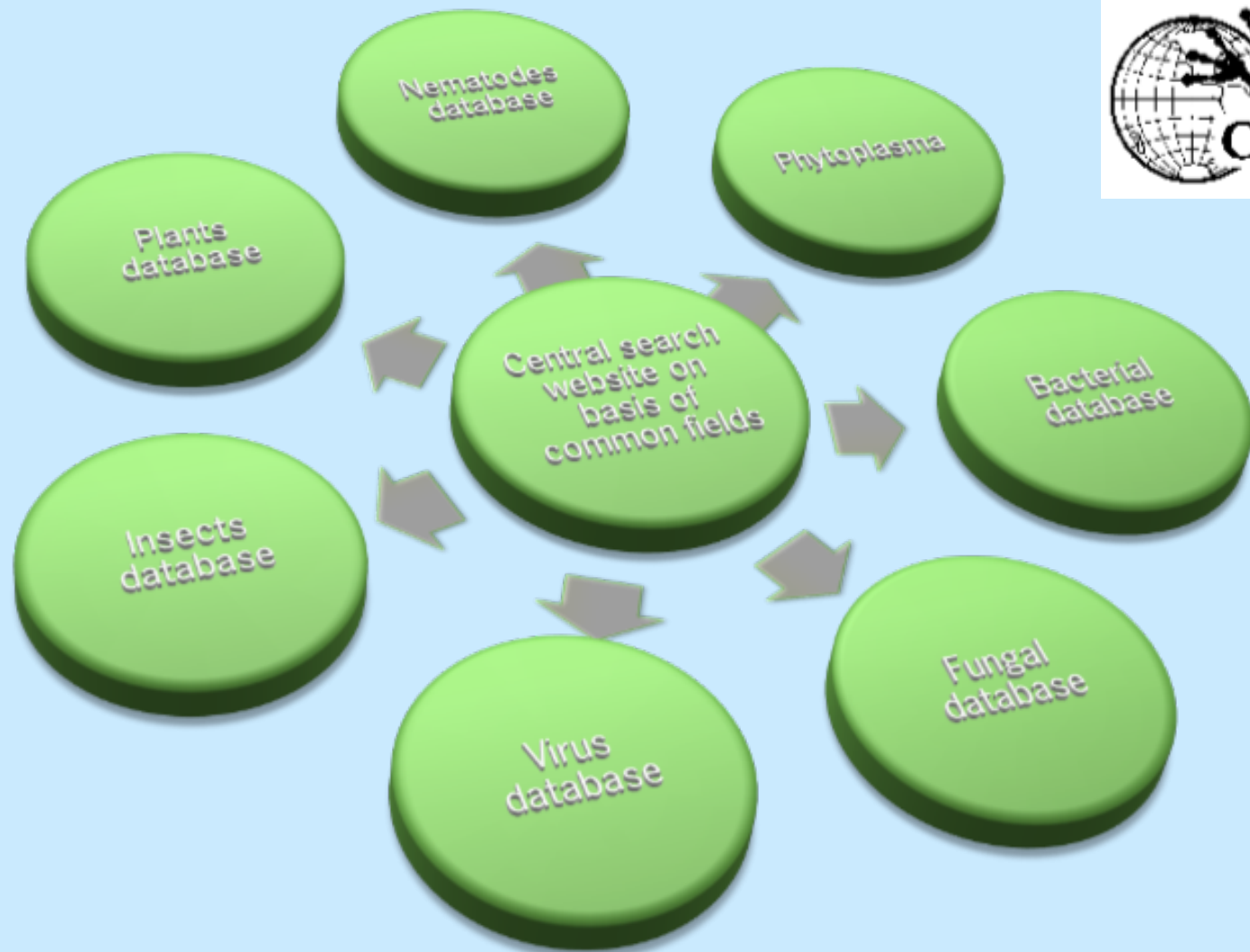
## WP2-7: 'DNA barcodes'

QBOL	sequences obtained		3-12-2010
		# sequences	Remark
WP2	FUNGI	3923	7 loci
WP3	ARTHROPODS	1221	2 loci
WP4	BACTERIA	1646	20 loci
WP5	NEMATODES	720	6 loci
WP6	VIRUSES	11	whole genome se
WP7	PHYTOPLASMS	126	2 loci
	TOTAL	7647	



WP9 leader: Vincent Robert (KNAW-CBS)

# WP9. Database: BioLomics



Q-bank: [www.q-bank.eu](http://www.q-bank.eu)





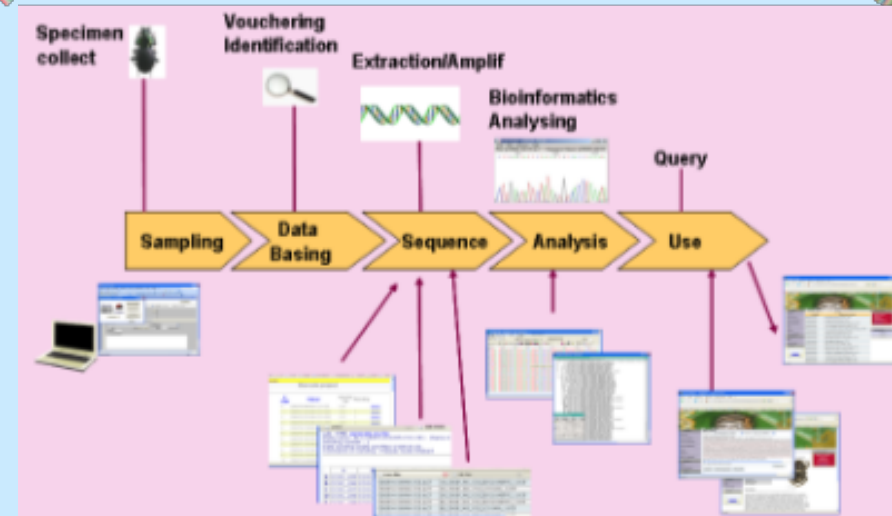
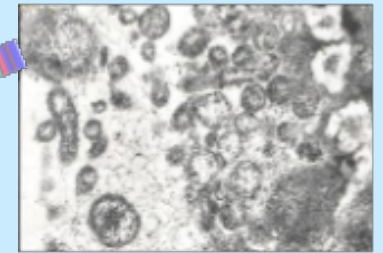
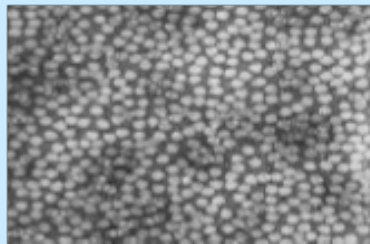
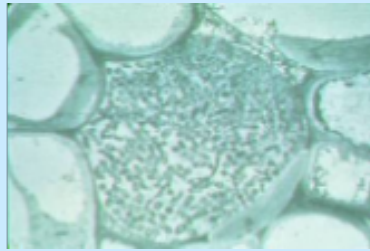
## QBOL: Barcoding for Plant Health

- 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 – NPPO's





# DNA barcode identification





# QBOL: Acknowledgements

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Advisory Board: Paul Hebert, Francoise Petter, André Levesque, Paul vd Boogert





Thank you for your attention!

[www.qbol.org](http://www.qbol.org)

[peter.bonants@wur.nl](mailto:peter.bonants@wur.nl)

