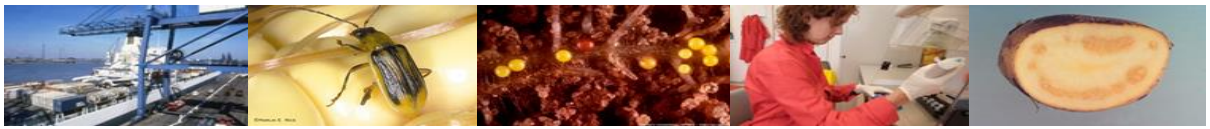
EUPHRESCO PHYLIB II project



The Netherlands' CLso* survey up to 2016 and Next Generation Sequencing (NGS) in carrot survey

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* '*Candidatus* Liberibacter solanacearum' (CLso) and Zebra chip (ZC)

Overview

- Introduction: inducement
- Survey CLso/ Stolbur Phyotplasma 2010-2016
- Exploring, Next Gerneration Sequencing (NGS) versus current method

Introduction: Inducement for activities on CLso

From Last three decades of past Millenium:

- "Zebra Chip" Central America/USA potato
- 2006/2008 Bc/CLso New Zealand
- 2008/2009 Association Clso/CLsp*
- From 2010 reports of CLso, Apiaceae within EPPO region
- 2012 inclusion CLso + Bc on EPPO A1 list

* '*Candidatus* Liberibacter psyllaurous' (CLps); Syn. CLso

National survey on CaLso and vectors 2010-16

Inspections: in tomato and carrots

CLso, psyllids, Stolbur

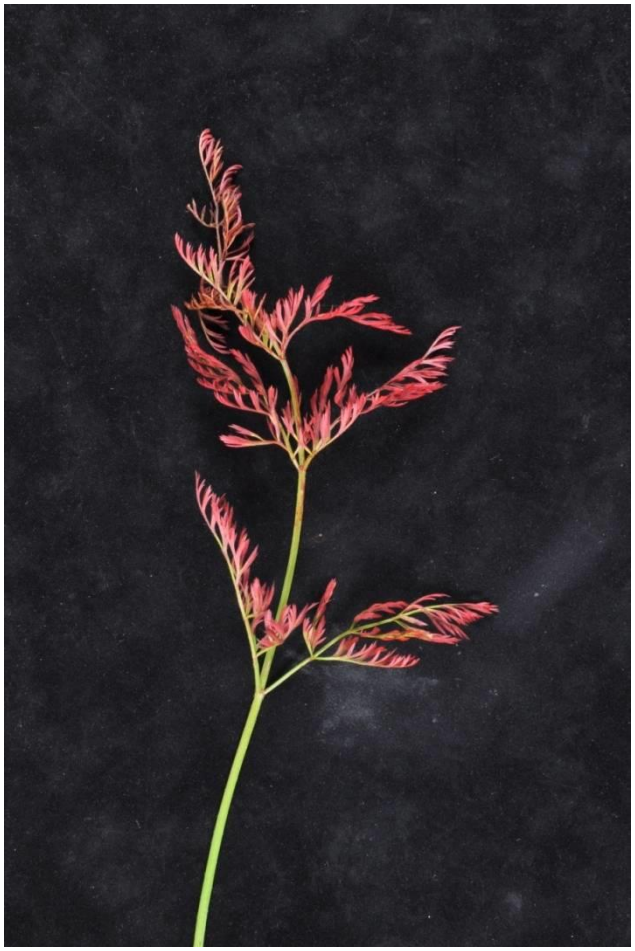
No suspect symptoms for CLso observed in solanaceous crops

Suspect and unusual carrot plants were sent to NVWA-NRC to test for CLso (Li *et al*., 2009; 16S rDNA)

Suspect insects send to NVWA-NRC for determination (and for CLso testing if vector found)

No carrot psyllid nor potato psyllid identified so far

Suspect carrot plants in national survey NL



Evaluation: Survey, routine diagnosis; CLso

Tomato + Carrot (yearly 2x 75-125 sites inspected)

symptomatic samples (2010-2016):

tested: 170 over survey period (only carrot)

3 samples received from seed companies in two other EU counties (non NL survey samples)

samples positive: 3; the foreign samples. Perform.

Tested Stolbur phytoplasma. (found: Aster Yellows)

Carrot survey samples CLso 2011 - 2016 were simultaneously tested on phytoplasma in general, and specific on stolbur phytoplasma. The same DNA extracts used.

phytoplasma was detected (>50%); No stolbur phytoplasma.

ID by sequence analysis (c. 20% of those positive samples)

Target fragments: 16S rDNA and ISR.

Blast results (NCBI):

all highly matching '*Candidatus* Phytoplasma asteris'

Those findings support effective DNA extraction using: automated QuickPick Plant DNA kit (Bionobile) (with additionally PVPP purification). Same for 3 non survey, CLso+

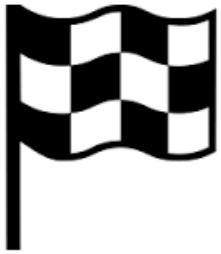
Can Next Generation Sequencing (NGS) replace current detection tests in *Daucus carota* survey?

A wide array of diagnostic tests are used to detect and identify infections

Can NGS outcompete traditional diagnostics in terms of:

Costs

Labour
Consumables
Generating data



Turnover time

From sample receipt to answer

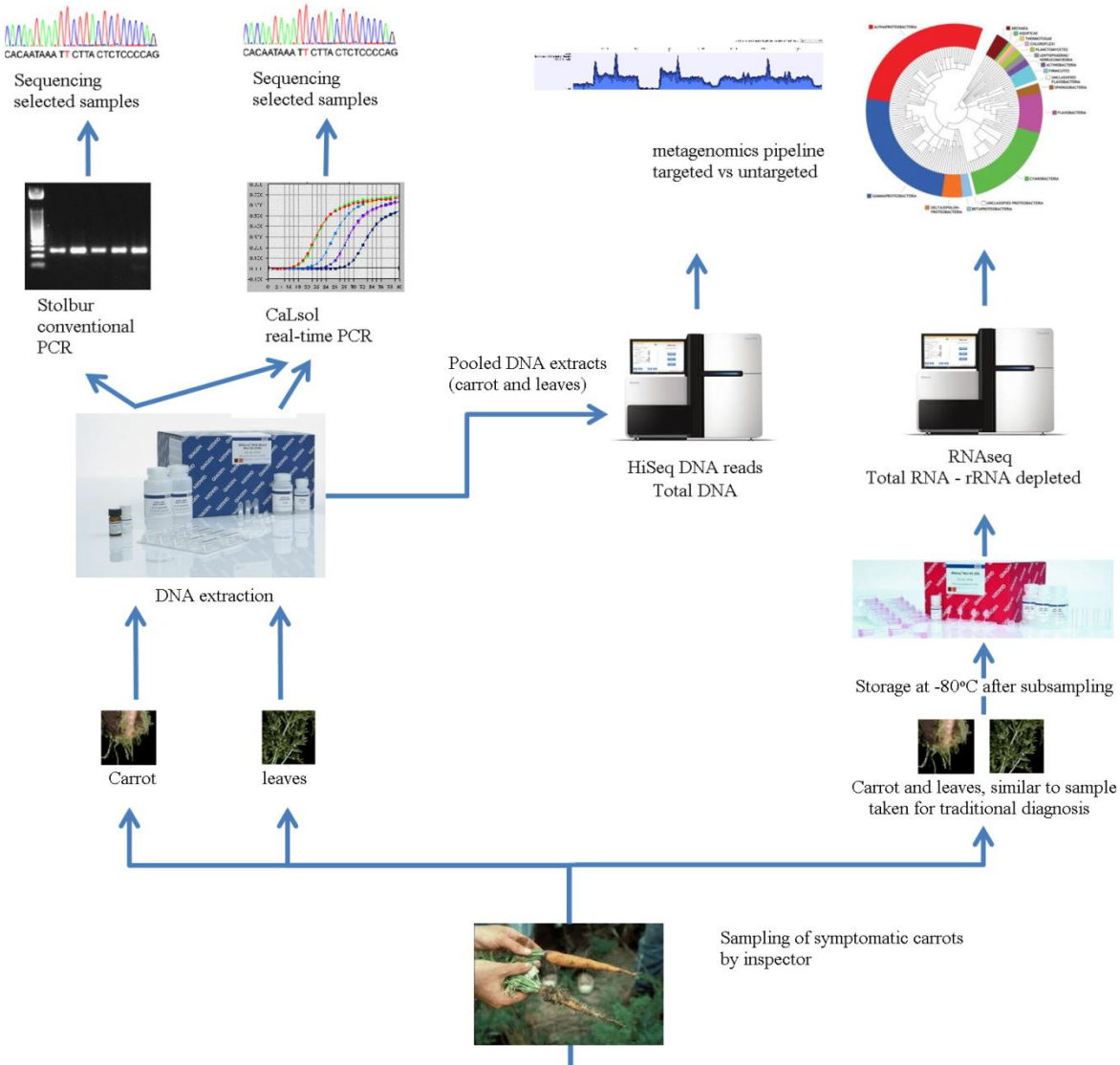
Hands-on time

Will it free time for technicians to do other things

Results

What type of results do you get from the analysis strategy

Traditional *versus* NGS



Traditional *versus* NGS summary

- Traditional: real-time and conventional PCR tests followed by Sanger Sequencing

- Two NGS data types:
  - DNA data (HiSeq 150bp, paired-end) – makes, for good comparison to currently used molecular methods, we know what to expect
  - RNAseq (150bp, PE) – allows detection of viruses and viroids, less comparable to currently used molecular methods, RNA expression selected reference genes could introduce difficulties in detection of the pest

- Three detection pipelines

NGS detection pipelines (three)

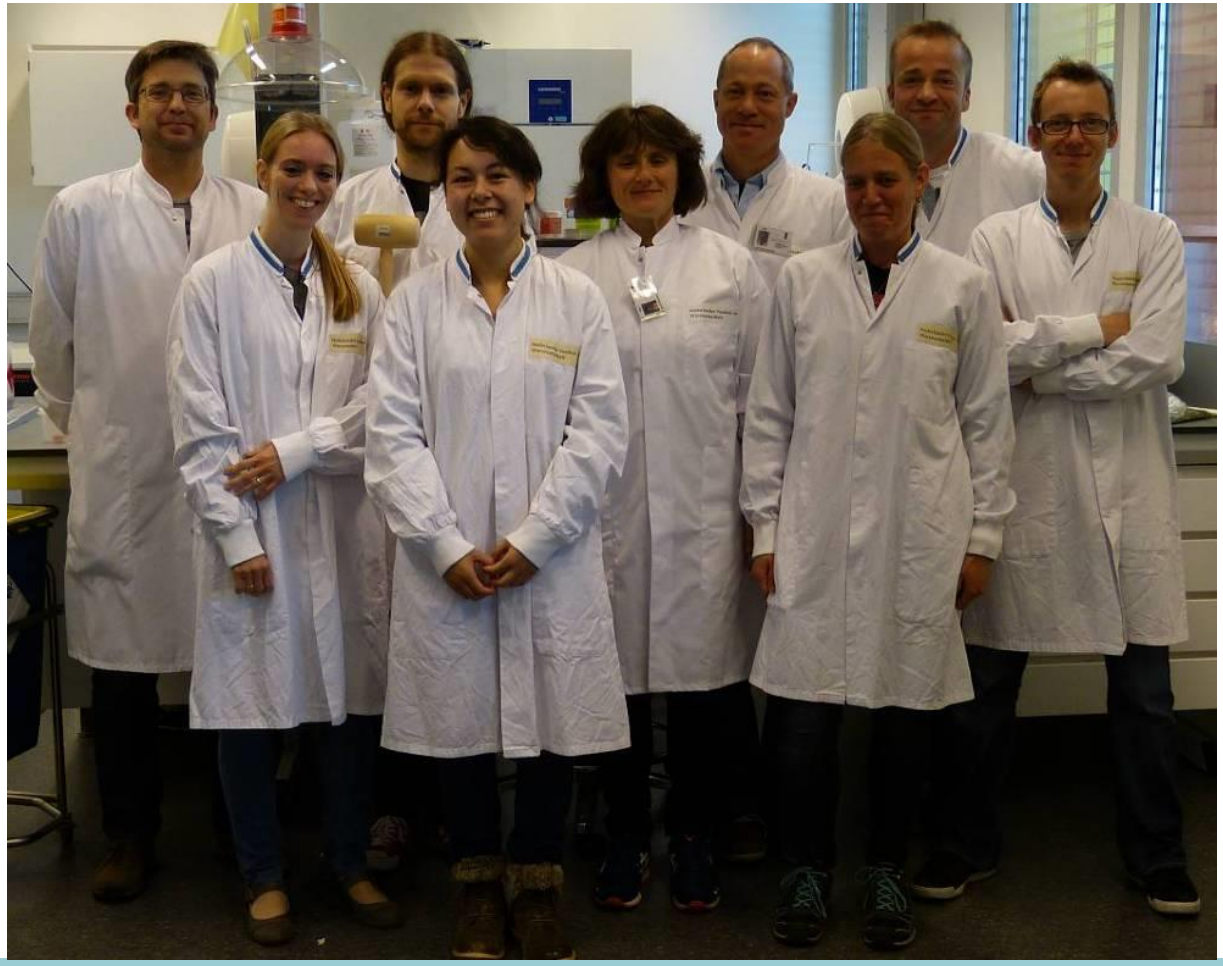
- Targeted: full genomes (core analysis: scope of the survey)
  - CaLsol, StolBur, Aster Yellows
  - High confidence level
  - Result: detected yes/no
- Targeted: mixed references (carrot pathogens)
  - bacteria (5), viruses (40), fungi (53), nematodes (6)
  - Indicative
- Untargeted: blastn and blastp based putative detection
  - Indicative (lowest confidence level)

How can you contribute?

- We want to share selected datasets with the questions
  - *Are CaLsol, StolBur or AY present in this sample?*
  - *Which relevant carrot pathogens are present in this sample?*
  - *Can you put a confidence level to this finding?*

- Compare analysis strategies and outcomes, and to have an open discussion on the use of NGS in diagnostics

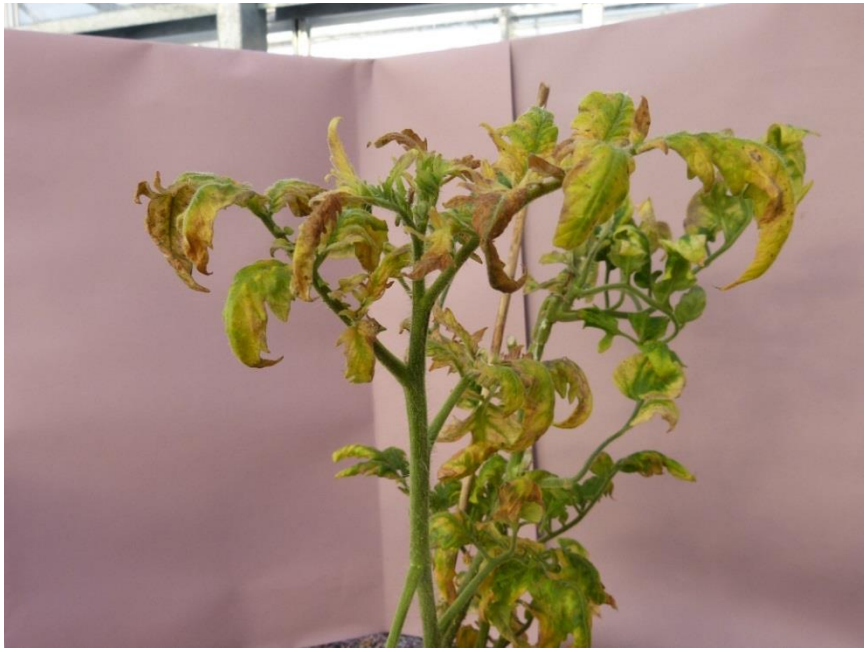
Thank you for your attention. Questions?



Collection Maintainance; grafted tomato plants

2012 accession USA (West distribution area)

tomato



potato