PHILIB - ILVO

Liberibacter research in Belgium, an update

Kris De Jonghe
Rachid Tahzima

Instituut voor Landbouw- en Visserijonderzoek
Eenheid Plant
www.ilvo.vlaanderen.be

Ca. Liberibacter and potato phytoplasmas

Planning Ca. Liberibacter and potato *Phytoplasma* research
ILVO – 2013 & 2014

PHYLIB:

Ring testing – validation of detection/identification methods

Additional survey – potato, carrot, (celery ?), weeds, other ?
Vector monitoring (phytoplasma, Liberibacter)

Project proposal: FYLIBER – 2012 call & approval –
Foreseen start: spring 2013

still no signature from the government (transit government)

➡ Project start date ???

Ca. Liberibacter and potato phytoplasmas

FYLIBER Research questions

1. Phytoplasma and Liberibacter status (and their insect vectors) in potato & carrot ?
2. Is there a genetic diversity in P & L populations that may compromise the available diagnostic tools?
3. Does the validation of a selection of diagnostic protocols and the results of interlaboratory comparison of these protocols lead to the implementation of a standardized high quality diagnostic test?
4. Is there a possible role for crops other than potato and carrot (e.g. celery), based on the knowledge on alternative hosts of the respective vectors ?
5. Does the presence of plant-, leafhoppers and psyllids pose a threat to new introduction and spread of Liberibacter and phytoplasma diseases in potato and carrot ?

Ca. Liberibacter and potato phytoplasmas

FYLIBER Project

WP1 – diagnostics - incl. LAMP PCR validation & proficiency test (both P & L)

WP2 – Pathogen survey in potato and carrot (Phytoplasma & *Ca*. Liberibacter)

WP3 - Insect survey & monitoring (potato & carrot)

Reporting: status according to ISPM8

Ca. Liberibacter

So far: no funding – cfr. Fyliber - only preliminary survey

Screening potato samples – same samples as for phytoplasma screening 2012-2013-2014

⇨ No *Ca*. Liberibacter solanacearum found

Carrot:

Sensibilising field research station staff and extension workers (meeting, leaflets and sample call)

Several samples with leaf yellowing/reddening + hairy root formation

⇨ No *Ca*. Liberibacter solanacearum found
yet, Aster Yellows Phytoplasma present

Liberibacter

Detection: CTAB + nested PCR

Characterization: RFLP + sequencing

Identification: Carrot yellows phytoplasma 16SrI-C

Liberibacter

May 2014 – Start bilateral co-operation with INRA Morocco

- Carrot sampling at the El Jadida area (Gharb region)

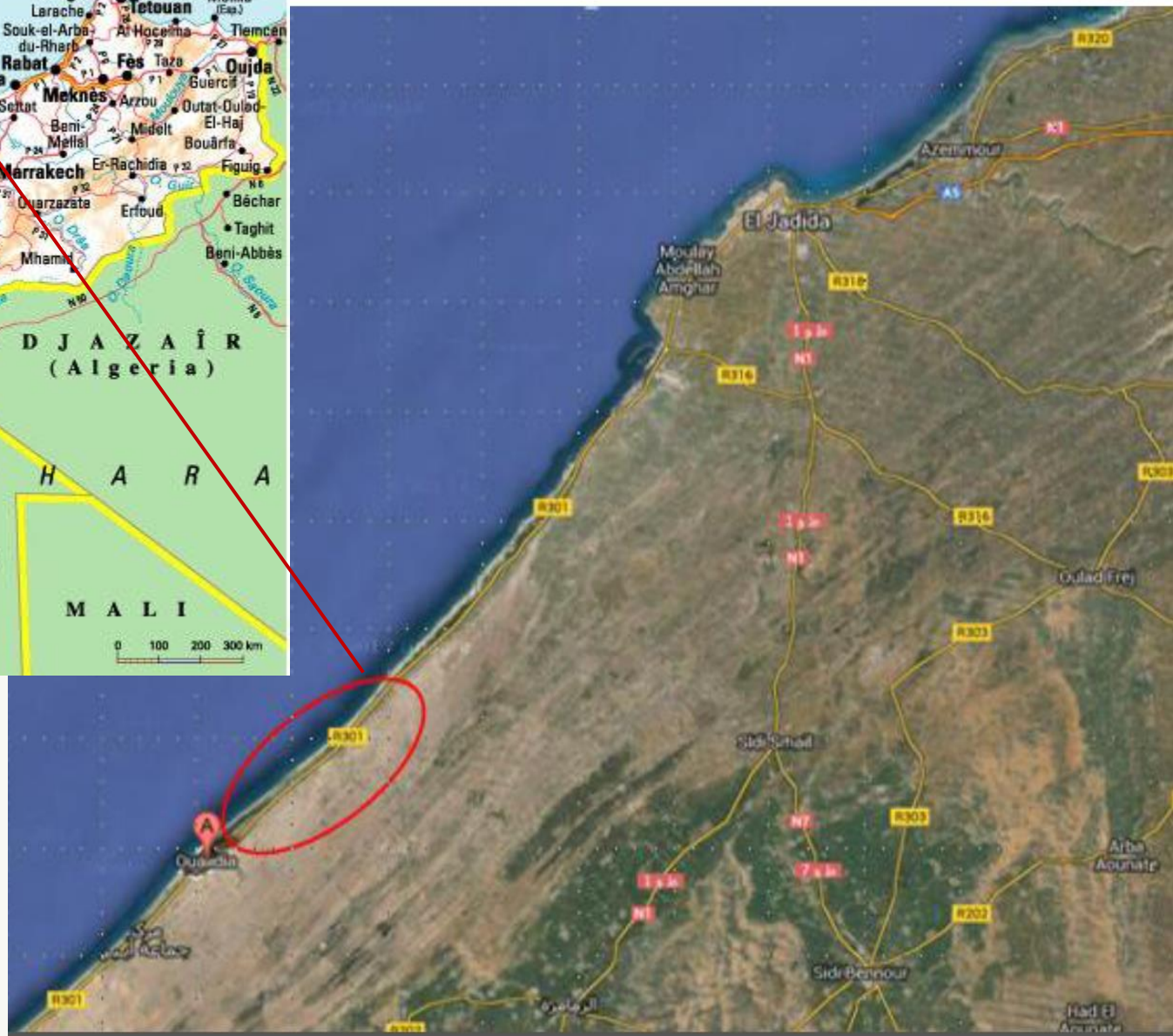
  yellowing, purpling, leaf curling,
  hairy sec. roots, general stunting

- Symptomatic plants and symptomless plants where sampled from three fields

Tests for: Phytoplasma - all negative
Spiroplasma - all negative
Liberibacter

Symptomatic plants positive => characterisation

Maroc - Résultats Prospections
Régions El Jadida – Site Oualidia



1

2

3

4

5

6

7

8

9

Liberibacter

Characterisation:

- 16S rDNA - OA2/OI2c
  (Liefting et al. 2009)

- adenylate kinasegene - Lso *adk*F/R
  (Munyaneza et al. 2009)

- rDNA ribosomal protein genes – *rpIJ*/*rpIL*50S
  (Ravindran et al. 2011)

Liberibacter

Characterisation:

- 16S – *adk* – 50S

  All three obtained fragments revealed 2 distinct sequences

- Identification: in all three fields *Ca*. Liberbacter solanacearum was confirmed (Accession Nos. PD2014, 98(10), p1426)

- 2 different haplotypes present: D & E
  Nelson et al 2013 & Teresani et al 2014

- First confirmation of *Ca*. Lso in Morocco

- Pathogen &vector survey in progress

- Regulation and action plan under review

Liberibacter

PHYLIB project end – however…

- Still partner interest in proficiency testing for Lso ?
- If yes: What format ?
    - Protocol/method comparison ?
        - Which protocols ? Real-time – endpoint ?
    - Alternatively: 1 protocol – fixed reagents
        - Inter laboratory comparison
- Preferred period ?

Who will organise ?

If no candidate, ILVO but…

- Only strains from carrot available (not enough tissue – starting from DNA would be more convenient)
- Additional carrot strains (N, S, Sp, F, other ?)
- Potato, celery strains ?
- L. africanus and L. asiaticus

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Potato Phytoplasma research in Belgium, an update

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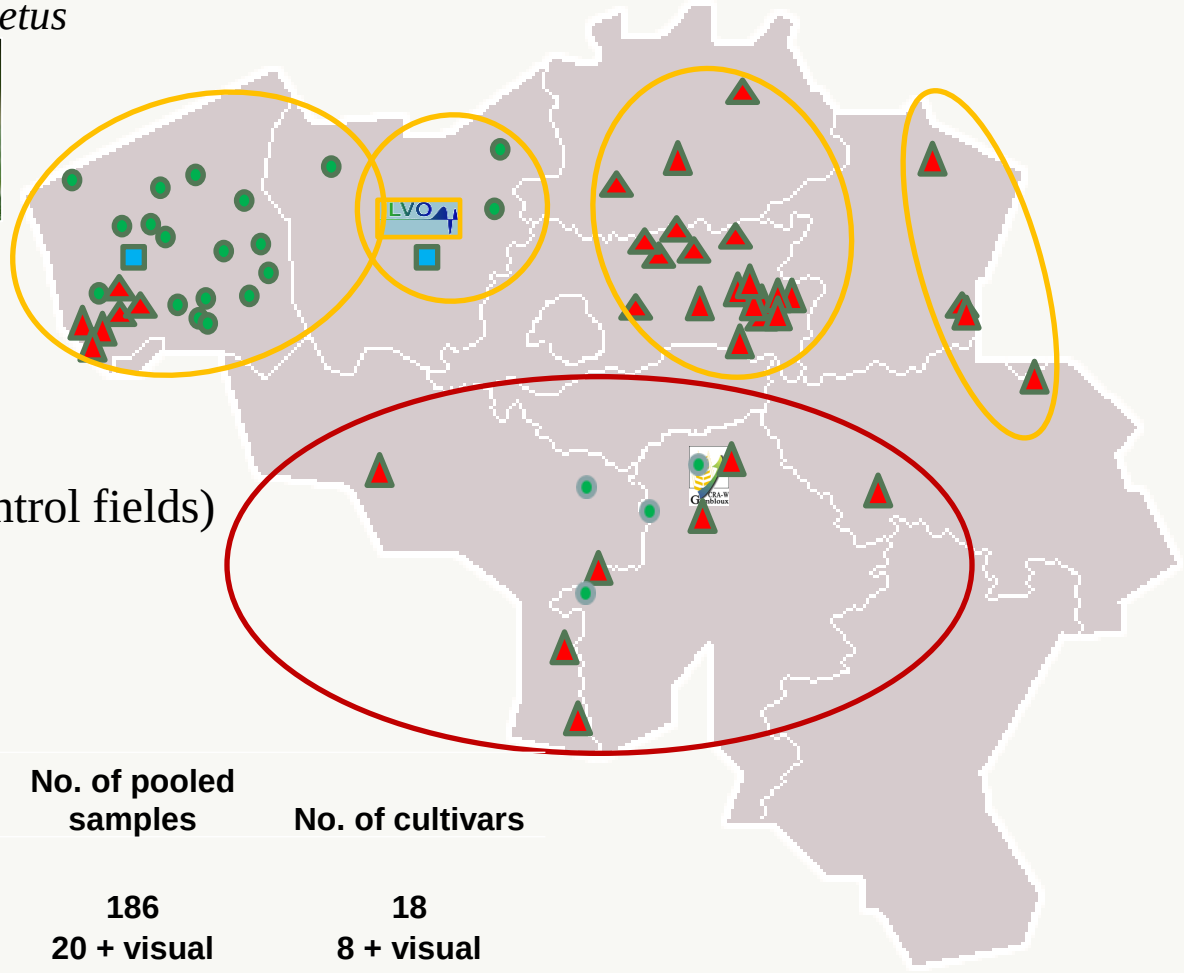
FYQUARSTAT: Potato stolbur phytoplasma survey

H. obsoletus

Sampling :

- Grapevine
- Strawberry
- Potato (pre/post control fields)



	No. of producers	No. of pooled samples	No. of cultivars
Grapevine	**27**	**186**	**18**
Potato	**Pre/post control field + 2**	**20 + visual inspection**	**8 + visual inspection (102)**
Strawberry/*Rubus*	**19**	**153**	**3**

+ sampling of *Convolvulus* and *Urtica*

No positive samples

Potato phytoplasmas

2012-2014: – PHYLIB
2012: MSc thesis – Rachid Tahzima

WP1. Collections: isolates

Ca. Phytoplasma solani
- *Rubus* and *Convolvulus* isolate
- On potato tubers (limited)

WP2. Detection and identification

Extraction
CTAB
DNeasy or Invitek Spin Plant

Phytoplasma
P1/P7 (or Tint) – R16F2/R2 – stol11 – fu5/ru3
real-time PCR (Christensen & Hodgets)
Qbol protocol
- TufAY/Tuf cocktail
- 16S (P1-ATT/P625r)

Ring testing ?

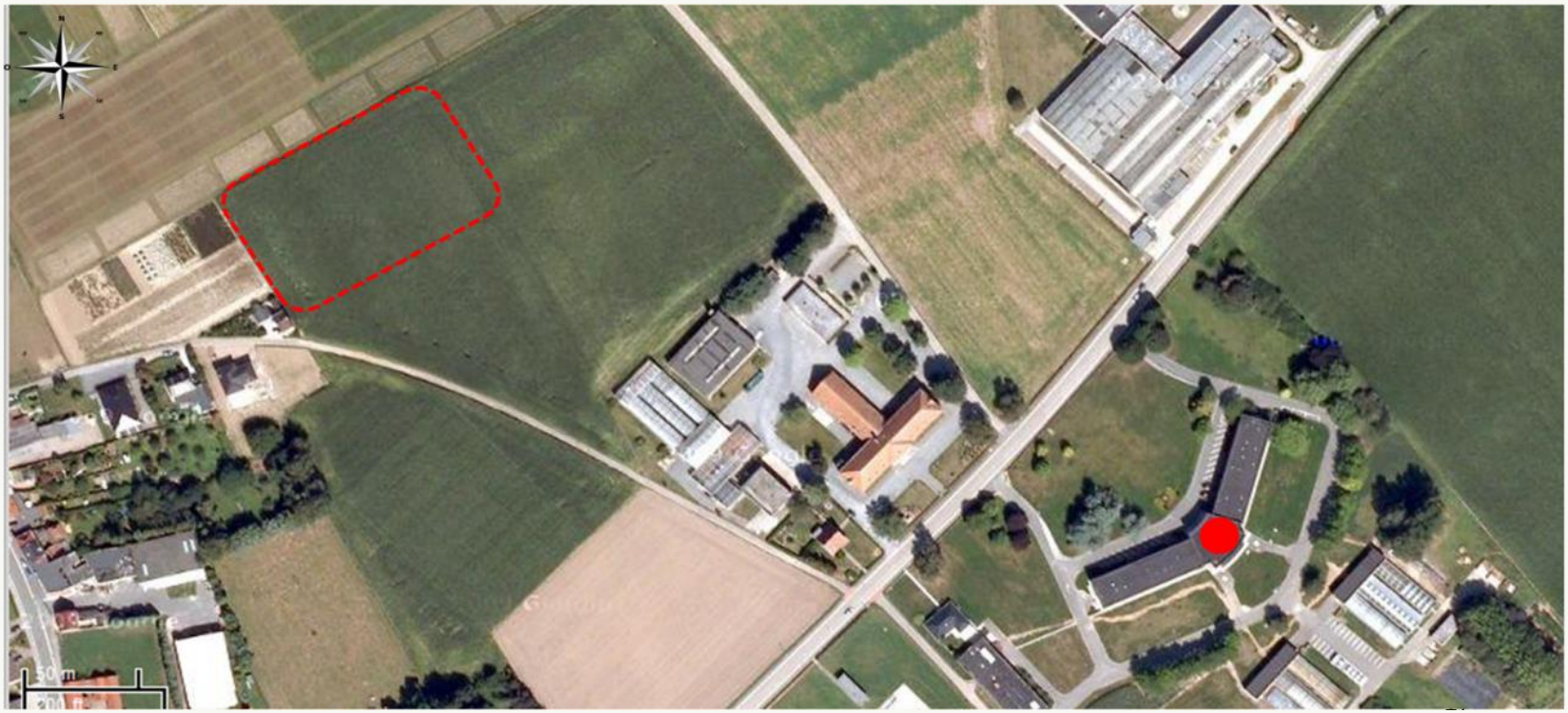
Liberibacter
LsoF/OI2c (Li et al 2009 & Jagoueix et al 1996)

Potato phytoplasmas

WP3. Epidemiology

Seed potato survey (2012)

- Pre and post control field visual inspection
- Sampling: 10 leaves/seed potato lot (spec. cv. And class)
- Inspection/sampling: vectors and possible source plants (weeds)



Potato phytoplasmas

WP3. Epidemiology

Post control field 2012

Potato phytoplasmas

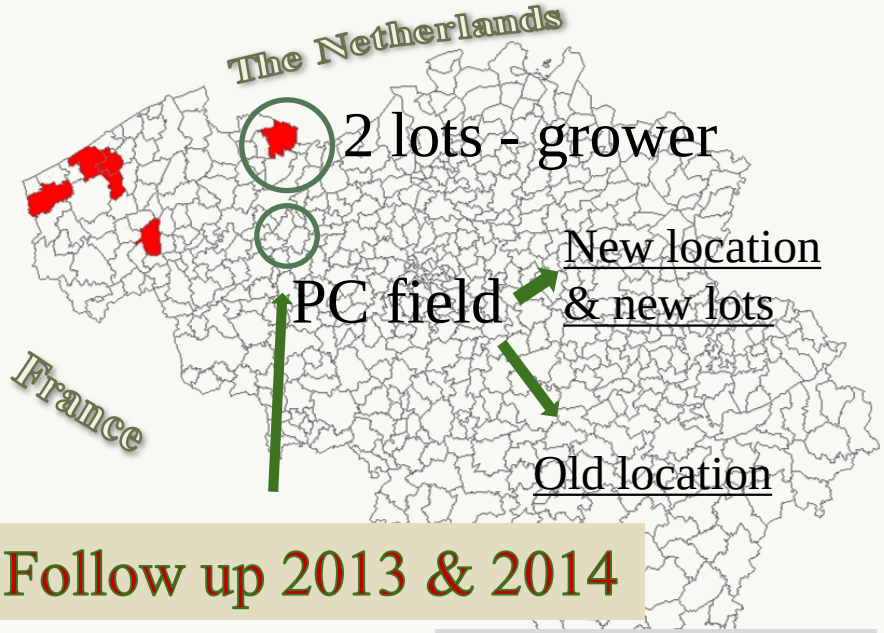
Survey results:

Ca Phytoplasma solani – identified based on tuf sequence results

	Plot	Variety	Class	Symptoms	R16F2/R2 (10+2/250) PCR Score	tuf band	16S band	lot ID	potato type	Origin
1	021	PREMIERE	E2	Leaf	1 (2/26)	1	1	11 027057 003	early	Assenede
2		***Buffer plant Fontane***		*Aerial Tubers*	*1*	1	1	_		*2011*
3	029	PREMIERE	A2	Leaf	1	1	1	11 027129 009	early	Koekelaere
4	040	AGATA	SE	Leaf	1 (1/2)	1	1	11 084066 011	early	Middelkerke
5	041	AMORA	E2	Leaf	1 (1/1)	1	1	11 028544 012	intermediate	Veurne
6	042	ANOSTA	E2	Leaf	1 (2/13)	1	1	11 027057 011	early	Assenede
7	048	ANOSTA	A2	Aerial Tubers	1	1	1	11 053890 017	early	Roesselaere
8	061	CILENA	SE	Leaf	1 (1/1)	1	1	11 027057 006	early	Assenede
9	083	BALLERINA	B	Aerial Tubers	1 (1/1)	1	1	11 084066 014	early	Middelkerke
10	194	BINTJE	A2	Aerial Tubers	1 (2/73)	1	1	11 010752 011	late	Waterland-Oud.
11	247	BINTJE	A2	Aerial Tubers	1	1	1	11 027057 029	late	Assenede
12		***Buffer plant Fontane***		*Start aerial tuber formation*	1	1	1	_		*2011*

Potato phytoplasmas

Origin of the infected seed potato lots



Follow up 2013 & 2014

NO STOLBUR found anymore

Leaf and stem symptoms

Potato phytoplasmas

Tuber symptoms

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