Contribution to the PHYLIB project:

Activities in Hungary in 2012-2013-2014

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EUROPHRESCO – PHYLIB project

Activities of Genlogs Ltd. in 2012 and 2013/2014

1 PHYTOPLASMAS AND VECTORS

1.2 Phytoplasmas

WP1 Collections – providing tubers and symptomatic shoots from highly infected potato field for SASA *(2012, 2013*)

WP2 Detection and Diagnosis/identification - Participation in the phytoplasma ring test *(September 2014)*

WP 3 Epidemiology - Surveillance of phytoplasmas (*2012-2013)*
- Tuber transmission studies *(2012-2014)*

1.3. Phytoplasma vectors

WP 3 Epidemiology - Monitoring of Auchenorrhyncha species on potato field *(2012, 2013*)
- Determination of abundance & seasonality of stolbur vectors *(2012, 2013)*

2 LIBERIBACTER SOLANACEARUM AND VECTORS

WP 2 Epidemiology - Participation in Liberibacter (Lsol) ringtest (Autumn 2012)

WP 3 Epidemiology

Surveillance of Phytoplasmas (*2012-2013)*

Objective: Follow the symptom development & infection dynamics of stolbur on the potato field during vegetation

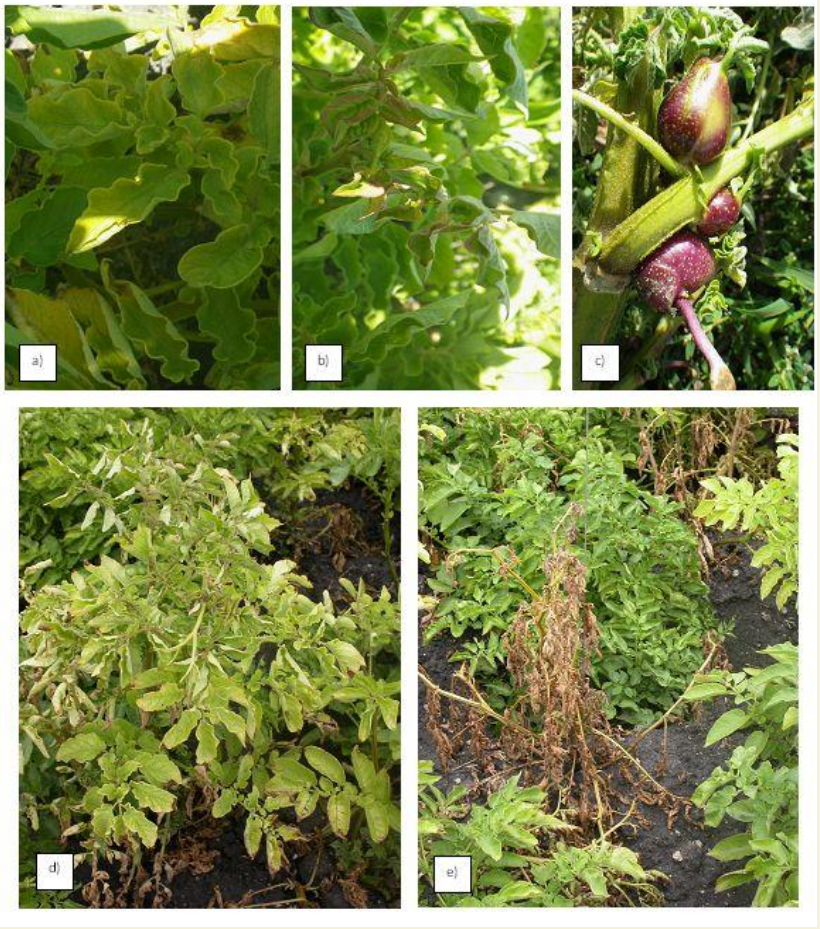
Method:

- Potato trial field - high infection pressure recorded by stolbur in the 3 previous years.
- Visual assessments of 11 varieties in 2012 (1920 plants/variety) at 3 dates (55th, 70th & 85th day after emergence.
- Visual assessments of one variety in 2013 (800 plants) at 4 dates (45^{th}, 70^{th}, 75^{th} & 85^{th} day after emergence.
- Types of symptoms & % of symptomatic plants were recorded.
- Plants were sampled and the infection rate was determined by nested PCR+RFLP

Results and conclusions:

- First mild symptoms around the 50^{th} day after emergence (<1 % of the plants)
- Infection rate increased as the vegetation progressed
- Highest infection rate: 30-40% in both years
- Based on symptom expession, differences among varieties in susceptibility to stolbur infection (5-33%).
- Typical symptoms:
  - Purple top , mild leaf rolling, yellows of foliage, aerial tubers; wilting & death of plants;
  - Spongy, shrinking tubers & healthy-looking tubers on the infected plants;
  - Stolbur could be often detected in healthy-looking tubers.

Typical symptoms of stolbur infection



on potato plants

a) mild upward rolling of leaves with yellows on shoot tips;
b) mild purple top on the shoot tips;
c) aerial tubers on the above-ground shoots;
d) wilting plant;
e) dead plants

on potato tubers

a) potato plants with tubers: infected *(left*), healthy *(right*)
b) close up of tubers: healthy *(left*), infected (*right*)
c) tubers of a symptomless plant; d) tubers of a symptomatic plant
e) tubers from under a severely infected plant (early infection)
f) fresh cut symptomless surface of an infected, PCR-positive tuber

Tuber transmission studies *(2012-2014)*

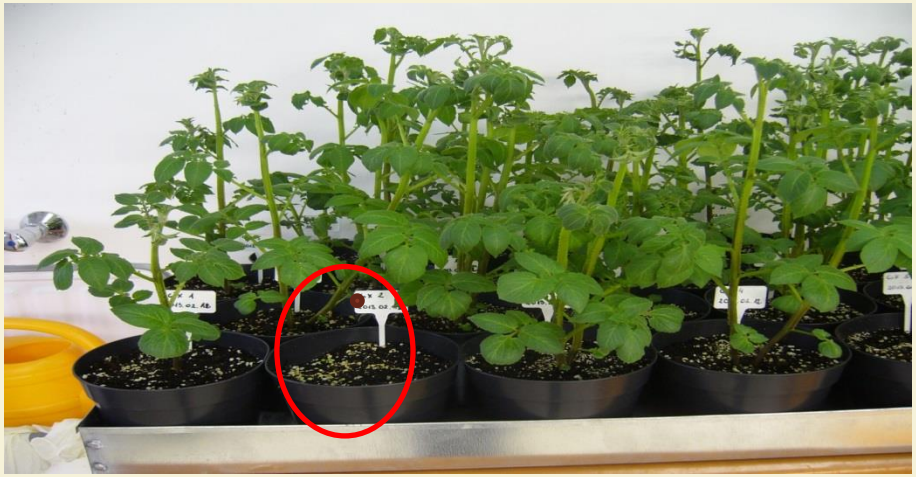
Objective: To determine the role of transmission through tubers in phytoplasma epidemics

Method:

- Autumn 2012 seeds of two varieties were collected from a highly infected potato field.
- Before planting their infection rate was determined by real-time PCR in February 2013.
- One hundred tubers per variety were planted out under controlled conditions in plant growing room on 12th February 2013.
- Progeny plants were regularly surveyed for symptom appearance
- On 29th March 2013 they were sampled, and then stored at 20^{0}C below zero until extraction.
- The samples were tested for stolbur infection using nested PCR+RFLP in summer 2014.

Results and conclusions:

- Before planting 5 of 100 and 4 of 100 tubers from 2 varieties were stolbur positive;
- Germination of the 9 infected tubers delayed approximately one month;



Tuber transmission studies *(2012-2014)*

Results and conclusions:

- Progeny plants of infected tubers:
  - were weak, developed slowly
  - had liana-like, runner appearance
  - did not develop flowers
  - could catch up with the healthy-looking ones 2 months after emergence
- All the 200 progeny plants proved negative in PCR
- Probability of stolbur transmission through tuber can be very low if any occurs
- In previous three-year studies 0.5% stolbur transmission through tuber was obtained when 84% of the mother tubers were infected

PHYTOPLASMA VECTORS

WP3 Epidemiology

Monitoring of Auchenorrhyncha species on potato field in 2012 and 2013

Objectives:

- Survey of the vector and potential vector species to study their abundance & flight activity
- Determine their phytoplasma infestation rate.

Method:

- Insects were weekly collected by yellow sticky traps and sweep netting.
- Species was identified,
- Stolbur infestation rate of sweep netted insects was determined by nested PCR+RFLP.

Monitoring of Auchenorrhyncha species in 2012 and 2013

Results and conclusions:

- In 2012 from beginning of June until 1st week of September altogether **27 879** insect specimens were identified on yellow sticky traps and *425* individuals were collected by sweep netting
- Insects belonged to 30 Auchenorrhyncha species: leafhoppers (16), Cixiidae planthoppers (3), psyllids (3), bugs (6) and aphids (2)
- Stolbur vectors and potential vectors belong to Cixiidae. *Hyalesthes obsoletus* & *Reptalus quinquecostatus* are considered as stolbur vectors

Seasonality of the potential Stolbur phytoplasma insect vectors on the potato field in 2012 and 2013

- In 2012 adult cixiids flew from the 1st week of June until the 1st week of August.
- In 2013 flight period of cixiids started on the 2nd week of June and lasted until the 1st week of September
- In both years *H. obsoletus* started to fly earlier than *R. quinquecostatus*

Monitoring of Auchenorrhyncha species

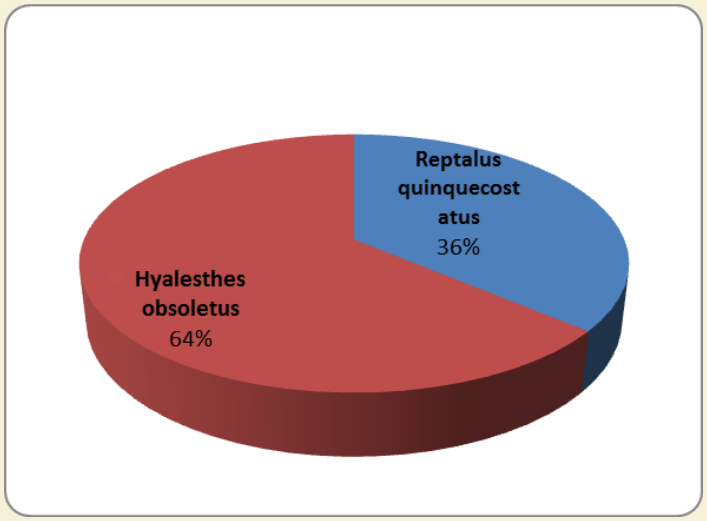
Abundance of Cixiidae on potato in Hungary in 2012 and 2013

H. obsoletus

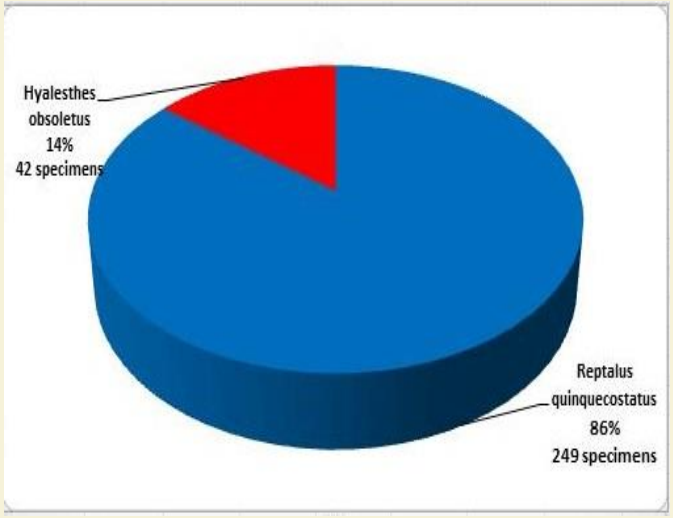
R. quinquecostatus

- In 2012 *Hyalesthes obsoletus* was the most abundant (64%)
- In 2013 *R. quinquecostatus* proved the most abundant (86%).

Abundance of Cixiidae in 2012



Abundance of Cixiidae in 2013



H. obsoletus and *R. quinquecostatus* were found to be infested by stolbur phytoplasma in both years.

Based on these results we may assume that mainly Cixiids can be considered responsible for stolbur epidemics of certain years in Hungary .

It was a real privilage and pleasure for us

to cooperate with the members of the PHYLIB project and contribute a bit to the phytoplasma work.

Thank you very much for the opportunity!

Sorry that I could not be there

Maria

Genlogs lab