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WG3- Phytoplasma control in crop systems

Cost project title: Integrated Management of Phytoplasma Epidemics in Different Crop Systems

STSM Topic: Identification of insect possible phytoplasma vectors in Lithuania

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Introduction

Phytoplasmas are causing many crop diseases all over the world and Lithuania is not an exception. In our Country these pathogens infect plants such as apple-trees, sour and sweet cherries, strawberries, oats, black and red currants, pines and other crops. Phytoplasmas are also infecting a wide range of wild plants and weeds, which are their perfect hosts. From the host plants these bacteria are transmitted by different insect species belonging to the order Hemiptera, mostly leafhoppers, treehoppers and planthoppers. The determination of phytoplasma vectors in Lithuania has never been done, so it is important to define the species that are able to transmit and the variety of phytoplasmas transmitted by them. This will help us to understand the epidemiology and establish suitable phytoplasma disease control measures.

Purpose of visit

The main goals of the STSM were: to learn methods of insect vector identification based on their morphology, basic molecular tools for phytoplasma detection and how to identify possible and confirmed phytoplasma vectors collected in Lithuania.

Insect morphological identification

I learned features needed in morphological identification of insects belonging to the Hemiptera order and I was also taught basic techniques of insect collecting, rearing, storage and examination.

I used the entomological keys for discrimination of insects belonging to infraorders and families of suborder Auchenorrhyncha in order to sort out the specimens collected in Lithuania in to the Aphrophoridae, Cicadellidae families (infraorder Cicadomorpha) and Delphacidae family (infraorder Fulgoromorpha).

Additionally I learned morphological features needed for insect determination belonging to the suborder Sternorrhyncha superfamily Psylloidea and was taught how to distinguish psyllid species vectors of phytoplasmas such as: *Cacopsylla pyri, C. pyricola, C. pyrisuga, C. melanoneura, and C. affinis.*

I used entomological keys to identify insects at genus level, then dissected male genitalia in order to reach the species level. In my samples brought from Lithuania I identified the following species and genera of insects:

1. *Agalia* sp.
2. *Anacertagalia* sp.
3. *Aphrodes* sp.
4. *Aphrophora alni*
5. *Arthaldeus pascuellus*
6. *Arthaldeus striifrons*
7. *Arthysanus* sp.
8. *Balclutha punctata*
9. *Cicadella viridis*
10. *Errastunus ocellaris*
11. *Euscelis incisus*

12. *Graphocraerus ventralis*
13. *Macrosteles sexnotatus*
14. *Macrosteles* sp.
15. *Neophilaenus* sp.
16. *Philaenus spumarius*
17. *Psammotettix alienus*
18. *Sagatus* sp.

Insect molecular characterization and phytoplasma identification

During this STSM I was taught DNA extraction techniques from insects. I used these techniques for DNA extraction from twenty identified insect specimens collected in Lithuania. Extracted DNA was used for phytoplasma detection. For this work I used PCR protocols and techniques that are used in the host laboratory. After electrophoresis of PCR products in agarose gel, fifteen samples were positive and gave 1200 bp size bands that are specific for phytoplasmas.

I got familiar with molecular characterization of insect species performing a few tests. These methods are based on specific primers designed for amplification of particular insect gene fragments (ribosomal internal transcribed spacer (ITS2); cytochrome oxidase I gene (COI); mitochondrial Control Region (CR)) that allow discrimination of morphologically hardly distinguishable insect species.