

Phytoplasmas are wall-less, plant pathogenic bacteria (~500 nm) that form a threat to agronomic crops, including high-value crops such as oilseed rape, wheat and maize. They have the unique capability to replicate in plants and insects e.g. leafhoppers (Hemiptera, Cicadellidae) and to manipulate both organisms for optimizing their own dispersal. They occur worldwide, but in many regions, such as the temperate climate zone, the epidemiology of phytoplasma-caused diseases remains to be understood. There is a general concern that phytoplasma disease incidences will increase in the future, due to global warming and the higher leafhopper abundance in the temperate regions of the world. The project aims to study the role of two selected leafhopper species in the dispersal of '*Ca. Phytoplasma asteris*' in central Europe.

Phytoplasmas colonize the sieve cells of the plant, where they replicate and migrate to other parts of the plant. Generally, a healthy adult leafhopper or a nymph acquires the phytoplasma by acquisition feeding from the phloem. But on top of that, the rare phenomenon of transovarial transmission may occur. We hypothesize that '*Ca. Phytoplasma asteris*' may be vertically transmitted to the insects' progeny.

The Northern American Aster Yellows Witches' Broom (AY-WB) phytoplasma strain can interfere with the plant host defence response to its vector *Macrostelus quadrilineatus* by secreting virulence proteins (effectors) into host cells. The research will be also focused on providing the experimental evidence that the '*Ca. phytoplasma asteris*' strains endangering rapeseed, wheat and maize crops in central Europe, also can modulate the plant development and defence responses by secreting effector proteins.

The phytoplasmas studied in this proposal are '*Ca. Phytoplasma asteris*' strains 16SrI-B and 16SrI-C, known to have a broad plant host range. Phytoplasmas cannot be grown in artificial media, therefore the pathogen strains will be maintained in infective insect colonies. Following leafhoppers *Macrostelus laevis* and *Psammotettix alienus* will be tested for the transmission of the phytoplasma strains to oilseed rape, wheat and maize, three widely cultivated crops in central Europe. Phytoplasma detection in all experiments will be carried out by PCR method using phytoplasma-specific primers, followed by sequencing of amplified products.

Moreover, the insects will be examined for the rare phenomenon of vertical transmission. To confirm the hypothesis that '*Ca. Phytoplasma asteris*' enters the oocyte of female leafhopper, we will use electron microscopy and fluorescence *in situ* hybridization (FISH) to trace the phytoplasma cells within the insect body.

To provide experimental evidence that phytoplasmas in nature can promote the insects feeding and development on infected plants the putative effector genes derived from studied phytoplasma strains will be integrated into the genome of the *Arabidopsis thaliana* model plant and used to perform insect fecundity tests and choice assays.

The proposed research fits perfectly into the contemporary demand for effective diagnostics, early prediction of epidemiological threats and a quick response to producers' concerns about emerging diseases of crops and methods of plant protection. The results of the project are addressed not only to agricultural practice but also to all its recipients, and thus consumers. The research will be carried out with the use of phytoplasma strains obtained from Wielkopolska province, hence they make an important contribution to knowledge about the central Europe region and phytopathogenic threats occurring in the temperate climate zone.

Expanding the knowledge about insect vectors and plant defence mechanism is an important aspect of research that allows implementing integrated plant protection in the production. Integrated methods are based on biodiversity and preservation of the natural relationships between organisms in the environment, and thus help to reduce the use of chemicals such as pesticides.

The research will bring new, yet unpublished in the world literature, information about insect vectors of the '*Ca. Phytoplasma asteris*' species, subgroups 16SrI-B and 16SrI-C strains. It will also characterise novel bacterial effectors that the strains 16SrI-B and 16SrI-C are armed with. Testing the leafhopper's maternal pathway occupation by phytoplasmas and the impact of its effector proteins on plants and insects will shed light on the disease dynamics.