

The aim of the project is to identify the symbiotic and pathogenic bacteria associated with serious pest of cereals – cereal leaf beetle (CLB, *Oulema melanopus*, Chrysomelidae, Coleoptera) and to elucidate how these microorganisms influence defense response of plants exposed to CLB feeding.

Plant has the ability to induce defense response against stress factors by synthesis of proteins, including pathogenesis-related (PR) ones. PRs are related with systemic resistance SAR (*systemic acquired resistance*) which protects plants against infection by wide range of microorganisms and ISR response (*induced systemic resistance*) that is associated in general with defense against pests. In response to pathogen or insect attack, different plant signaling pathways are induced. Defence response induced by damage of plant caused mechanically differs from that caused by insect feeding. During feeding, plant tissue has contact with insect's oral secretion, which contains factors (elicitors) affecting plant response. One of these factors are bacteria, which may modify (suppress) the plant response to insect host benefit – in our case –CLB.

The hypotheses behind this proposed research project are that:

- 1) Orally secreted bacteria of CLB larvae modify plant defense response during insect feeding for the benefit of its insect host, however, the final effect of plant host response depends on the plant variety and the symbiotic content of insect's oral secretions
- 2) CLB is a potential reservoir of pathogenic bacteria – *Pantoea ananatis*.

Currently, there is no data on the presence of bacteria, including symbionts, associated with CLB. The correlation of the *Oulema* spp. origin (host species they feed on, and location they were isolated from) with the composition of these bacteria in insects' oral secretions have not been studied so far, likewise the plant-CLB-microbe interactions. There is also no report on the pathogenic bacteria (*e.g. P. ananatis*) occurrence in CLB. Therefore, in the proposed research we want to fill the gap in this area.

The research to be carried out involves:

- 1) Identification of bacteria from CLB oral secretions. Analysis of symbiotic bacteria composition in relation to two factors, plant host species and geographical locations from which CLB originates;
- 2) Analysis of the presence of pathogenic bacteria *P. ananatis* in CLB populations. Screening for the presence of this bacteria in CLB coming from different geographic locations in Poland and feeding on various host plant species;
- 3) Analysis whether identified strains of *P. ananatis* in CLB might be responsible for development of disease symptoms on cereals;
- 4) Checking if orally secreted bacteria of CLB may suppress plant defense response (analysis of the expression level of marker genes involved in SAR and ISR-type response. Elucidation which type of plant response, SAR and/or ISR, prevails and which type of response is inhibited depending on the factor tested: CLB, CLB and bacteria);
- 5) Search for the wheat variety, in which expression of genes involved in the plant response was the most efficiently suppressed by bacteria (measurements of expression of genes associated with SAR and ISR response).
- 6) Analysis of a global plant host response (total gene expression profile) to CLB feeding as well as checking the modifying role of CLB-associated bacteria for the chosen wheat variety (indicated in the previous stage) by its transcriptome profiling (RNAseq) approach.

The results of these experiments will provide important clue on how insect-associated microbes impact the plant – insect interaction. They will also give an insight into components and the resultant of plant response, resulting from the double challenge by insect and bacteria.