AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2016 – 2018

TITLE: Molecular diet analysis of insects that vector vegetable pathogens

RESEARCH LEADER: Kenneth Frost, Assistant Professor and Plant Pathologist, Department of Botany and Plant Pathology, Hermiston Agricultural Research and Extension Center, Hermiston, OR

COOPERATORS: Sandra DeBano, Associate Professor, Department of Fisheries and Wildlife, Hermiston Agricultural Research and Extension Center (HAREC), Hermiston, OR

David Wooster, Sandra DeBano Assistant Professor, Department of Fisheries and Wildlife, Hermiston Agricultural Research and Extension Center (HAREC), Hermiston, OR

SUMMARY: Each year, vegetable crops are threatened by the occurrence of multiple plant pathogens that are transmitted by insects. The feeding host species used by the insect-vectors prior to arrival in the crop field is rarely known. Previous studies have tried to determine how crop or non-crop habitats surrounding a potato field influence insect abundance within the field. Often those studies rely on positional placement of traps in the field and infer relationships between habitats surrounding the crop and insect captures within the crop. However, pest captures in traps often do not accurately reflect the influence of surrounding landscapes on pest populations, and the approach limits inferences that can be made about the role that non-crop host plants play in supporting pest populations. Often, it is unknown whether host plants in the surrounding habitat were utilized by the insects dispersing into the crop of interest. The development of molecular tools for diet analysis can offer a refinement to landscape studies by identifying plant species in the landscape that are directly utilized for feeding by insect vectors captured in the crop field.

Information about the identity of specific plant taxa can be can be obtained by amplifying, sequencing, and comparing small fragments of genomic or chloroplast DNA to sequence information from known plant species present in GenBank. These small fragments of genomic and chloroplast DNA were originally identified as good target regions for DNA barcoding of plant taxa. More recently, these techniques have been adapted to identify which plants are found in the diets of a number of phytophagous animal species, including crop pests. The primary objectives of the proposed research will be to develop methods to conduct molecular diet analyses on phytophagous insect species with a focus on insect species known to vector vegetable pathogens.

The short-term goal of this project is to determine if plant DNA barcoding regions can be detected and amplified from gut extracts of different insect species that exhibit different feeding behavior. A secondary goal is to determine the length of time that different plant DNA barcoding regions can be detected in insect bodies after transfer to a different feeding host or insect diet. Finally, we would like to

use sequence information generated from barcoding to identify all plant species that may be present in samples with mixtures of DNA. For example, we would like to be able to make inferences about feeding at the individual insect level and at the population level by identifying plant taxa in gut extract mixtures that occur 1) when a single insect feeds on multiple plants and 2) when multiple insects feed on multiple plants. A long-term goal of this project will be to learn how agricultural fields are affected by their landscape context, which will allow growers to better manage those landscapes to reduce pest pressure.

OBJECTIVES: Specifically, we propose to:

1) Develop and refine methods to detect and amplify the chloroplast trnL (UAA) intron present in the bodies of three insect species with different feeding behavior and which are known vectors of plant pathogens in the Pacific Northwest.
2) Use no-choice feeding studies to determine the length of time chloroplast DNA is detectible in the insect body.
3) Identify plant taxa using ctDNA from mixtures of plant species using PCR amplification of the chloroplast trnL (UAA) intron followed by cloning of the amplicon(s) into a plasmid vector, transforming *E. coli* with the plasmid, and sequencing the resulting DNA library.

PROCEDURES: As proof of concept and to test primer sets designed to target chloroplast DNA, a population of thrips were collected from dandelion, *Taraxacum officinale*, flowers around the HAREC in non-crop areas. From the collection of thrips, DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) methodology. Quantity and quality of the extracted DNA was assessed by scanning 1.5 µl of each sample in a NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc. Waltham, MA). Extracted DNAs were used as template in polymerase chain reaction that used primer sets that targeting the chloroplast trnL (UAA) intron. Amplicons generated from PCR reactions were cloned into a plasmid (pGEM, Promega Madison, WI) and plasmids were transformed into E. coli. Bacterial colonies from the resulting library were selected and plasmids were purified and inserts were sequenced. The BLAST was used to compare insert sequences to sequence present in GenBank.

Gut Passage Experiment: In 2016, beet leafhoppers were be collected from three different field sites in Umatilla County of northeastern Oregon. Briefly, field margins were sampled by conducting approximately 300 pendulum sweeps with a standard (15” diameter) sweep net. Contents in the sweep net were emptied into a see through bug tent. Beet leafhoppers were aspirated, and placed in plastic bags in a cooler for transport to the lab. For each location, a subset of five individuals were immediately euthanized and placed in a 2 ml screw-cap vial and placed in the freezer, 30 individuals were placed in an arena with access to a sachet containing 3% sucrose, and 30 individuals were placed in an arena with access to sugar beet plants. The gut passage experiment involve two diets crossed with three sampling periods (i.e. 6 treatments = 1 insect species x 2 diets x 3 time points). Five leafhoppers were sampled (i.e., euthanized by placing in sample jars of ethanol) at 24, 72, and 168 hours after placement on either sugar beets or allowed to feed on a sachet of sugar water (3% sucrose + green food color). Nucleic acids were extracted from the leafhoppers generated in the gut passage experiment and are currently stored

in the freezer. Library preparation for sequencing is ongoing and DNA sequencing of libraries will occur in spring 2017.

Weed survey work and thrips gut passage experiments will be conducted in 2017.

SIGNIFICANT ACCOMPLISHMENTS TO DATE: This report represents the first 6 months of this project and the proposed work is on schedule. With funding from ARF and other sources a graduate student is being recruited continue work on this project in 2017. This individual will be responsible for

Proof of concept: We have identified several primer sets that target plant DNA and have had success amplifying target sequences extracted from insects. We are currently using the trnL (UAA) intron but will start work with the internal transcribed spacer region (i.e. ITS-S2F and ITS4R) in 2017. From thrips collected from dandelion, we detected DNA sequences that matched sequences from the genera *Taraxicum* (50%), Pstacia (25%), Solanum (6%), and Medicago (6%). Approximately 13% of the sequences did not match known sequences in GenBank.

Gut Passage Experiment: A no-choice feeding experiment was carried out on beet leafhopper individuals collected from weedy field margins at three locations near Hermiston, OR. Nucleic acid extracts of the insects generated from the no-choice feeding experiments are currently stored in the freezer and library preparation for sequencing and sequencing will occur in spring 2017.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: Additional funding ($20,000) was received from the Northwest Potato Reserarch Consortium (FY 2016-17) to conduct diet analysis of Lygus spp. affecting potato crops. A similar proposal was resubmitted to the NPRC for funding in FY 2017-18, but awards have not been announced.

Wooster received funding ($9,886) from the General Research Fund (FY 2016-18) to use molecular diet analysis to study predator-prey linkages in wetland ecosystems.

FUTURE FUNDING POSSIBILITIES: In 2016, Frost, DeBano, and Wooster submitted a proposal to the USDA AFRI Foundational Program (*Pests and Beneficial Species in Agricultural Production Systems* program area) that was not selected for funding. The proposal will be modified and resubmitted in 2017.

Both Wooster and DeBano have submitted proposals to ARF 2017-19 competitive grant cycle to conduct research that uses molecular tools related to those being developed in this proposal.

Currently, Wooster has a preproposal submitted to NSF Division of Environmental Biology to study the relationship between morphology and behavior type in native crayfish. A component of the study is to learn how morphology and behavior influences the diets of individuals in the population.