Title: Using bacteria and fungi to develop sustainable control methods for *Drosophila suzukii.*

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SUMMARY

Many *Drosophila* spp. have close relationships with microbes. They feed upon yeast as both adults and larvae, and other fungi and bacteria can influence female egg laying behavior. The spotted wing drosophila (SWD), is a devastating invasive pest of soft-skinned fruit crops. Previous studies have characterized adult SWD gut bacteria and gut-associated fungi, but there is no information on the complete array of microbes carried by SWD or those found on their crop hosts. ***The goal of our proposal is to use next generation sequencing to characterize the microbial community found on SWD and their preferred and non-preferred crop hosts to help develop targeted control methods.*** We believe this information will aid in the development and refinement of selective baits, infestation indicators, identify possible repulsive microorganisms, and determine if SWD infestation results in greater pathogen risk to fruit. Additionally, this research will provide insight into how microbes are used in SWD host location and attraction. Because there has been no comprehensive assessment of the microbes present on SWD host crops, with or without flies present, we also expect that data may yield valuable information for disease management and post harvest storage research.

OBJECTIVES

Objective 1) Track changes in the microbial community of SWD host plants as a function of fruit ripeness, time of year, and level of SWD infestation using high throughput sequencing (HTS).

Objective 2: Identify microbes that may deter or attract SWD based on sequencing data.

WORK COMPLETED

The following life stages and treatments were identified and sampled:
Vegetative type: Leaf or fruit
Fruit ripeness stage: Green, pink, red (underripe), ripe, overripe
Netting (for insect exclusion): Present or absent

Samples were collected in a full factorial design, with three field replicates per sample, when available.

Samples were collected at the following locations: blackberry and blueberry at the Piedmont Research Station (Salisbury, NC), blackberry at the Sandhills R. S. (Jackson Springs, NC), and blueberry and raspberry at the Upper Mountain R. S. (Laurel Springs, NC). Plots at all locations were managed by the Burrack lab and were not pruned or treated with any pesticides for the duration of the growing season.

Samples were collected weekly May through August 2016, beginning at green fruit stage and continuing through senescence. To collect microbial samples aseptically (to the extent that can be expected for field collection), we wore sterile gloves, sterile cotton swabs, and changed each between samples.

Fly sample collection: A single collection of four male and eight female SWD were collected individually from blackberry plants on August 18. Flies were surface washed to remove microbes and saved in a separate container. The flies and liquid were saved at -80C for further processing.

Weather data: Temperature and relative humidity data were collected using data loggers for the entirety of the experiment.

Fruit infestation data: During each visit, fruit from each ripeness stage (except overripe) were collected and examined under a stereomicroscope for SWD infestation. The number of eggs per fruit was enumerated.

Monitoring traps: Yeast, sugar and water traps were deployed and refreshed weekly to monitor for SWD activity within each field. Two traps/planting were placed less than 1 foot away from the canopy. The traps were sorted in the lab and the number of male and female SWD collected were recorded.

DNA Extraction and Quantification

Bacterial and fungal DNA was extracted from each sample using a microbial-specific isolation kit (MoBio Powersoil DNA Isolation Kit). Thus far, control samples yielded no amplification, meaning thus far, any contamination has been minimal. The next steps for this project include using PCR to amplify and sequence species-specific regions of bacteria (16S) and fungi (ITS), and to analyze sequence information. This will be accomplished during 2017.