BIOAg Project Report

Report Type: **Final**

Title: Deep sequencing SWD gut contents to reveal non-crop alternative host plants

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Abstract:

Spotted wing drosophila (SWD) is a devastating pest of Washington soft fruits including cherries, blueberries, and raspberries. Since its arrival in 2009, the cost and intensity of insecticide programs for these crops has dramatically increased. SWD adults generally invade crops from unknown alternative host plants, complicating management. Despite many efforts to identify the non-crop hosts of SWD, no reliable method has been devised. The goal of this project was to determine if a molecular method known as deep-sequencing can identify unknown hosts of field-trapped SWD adults. Universal primers were used to amplify plant genes, trnF and ITS, using PacBio platform, and sequences were organized and identified to species using the National Center for Biotechnology Information database. We tested this method on lab-reared SWD fed known diets and field trapped SWD with unknown dietary histories. Deep-sequencing of lab-fed SWD produced sequences for potato, cherry, and plum, but not blueberry, all of which were primary components of known diets. Sequencing of field collected SWD adults detected DNA of most known close-range hosts (cherry, plum, apple, blueberry), known distant hosts (apricot, peach, cherry) and unknown hosts (mahaleb cherry, raspberry, elderberry, and various weeds and wild trees). This study confirms the utility of deep sequencing for identifying previous hosts of SWD. We also discuss limitations of the method that will improve future studies.

Project Description:

Literature Review and Rationale:

Spotted wing drosophila (SWD), *Drosophila suzukii*, is an invasive pest of soft fruit, such as cherries blueberries, and red raspberries, and was first detected in Washington State in 2009 (Walsh et al. 2011). Most fly species only feed on overripe fruit, but SWD has a high capacity to cause damage because it attacks fresh soft fruit prior to ripening. Yield loss potential in the U.S. is estimated at over $700 million (Asplen et al. 2015). SWD can undergo over 10 generations per season and adults can live several weeks, so they have considerable opportunity to disperse and feed on multiple plants (Walsh et al. 2011). Furthermore, this pest can overwinter and develop early generations on non-crop host plants before dispersing to crops. Although studies have identified certain non-crop hosts via scouting (Kenis et al. 2016) and lab rearing (Poyet et al. 2015, Stockton et al. 2019), these methods are labor-intensive and only detected anticipated hosts, not unknown hosts.

Molecular gut content analysis provides a potential solution to identify unknown dietary history of pests in the field. Cooper et al. (2019) developed methods to identify plant DNA in the stomachs of five field-collected psyllid species, which like SWD, can feed on multiple plant species across the landscape.

Cooper et al. (2019) detected multiple unknown hosts, then determine patterns of psyllid movement across natural and agricultural landscapes. This project tested the methods used by Cooper et al. (2019) to study the dietary history of SWD for the same purpose.

Greater understanding of SWD host plants and movements obtained with molecular techniques will provide valuable information to improve management of the pest. For example, coupled with currently available data (Poyet et al. 2015) and future studies on host plant suitability, information on SWD dietary history could be used to identify highly fed upon but poor-quality hosts to use as trap plants. Alternatively, highly fed upon and suitable hosts could be removed from habitat within and near crop zones to reduce pest pressure. Management strategies could be broadened to a regional scale by identifying non-crop refugia hotspots, which could then be targeted for management. Finally, more precise regional scouting of refugia could be implemented to develop invasion models, allowing growers better predict when to begin management within their crops.

Objectives:
Objective 1. Deep sequence lab reared SWD with known lab diets.
Objective 2. Deep sequence field collected SWD with unknown dietary history.

Outputs

Overview of Work:
Experimental procedures involving SWD rearing, feeding, and trapping were successfully completed in 2020. DNA sequencing was delayed due to COVID-related shutdowns and molecular supplies shortages; however, these procedures were completed by September 2021. Data processing and analyses were completed to sufficient degree for general reporting, but further data processing will occur to achieve publishable visualizations and summaries. Any major changes or additional findings occurring between this report and publication will be provided to BIOAg.

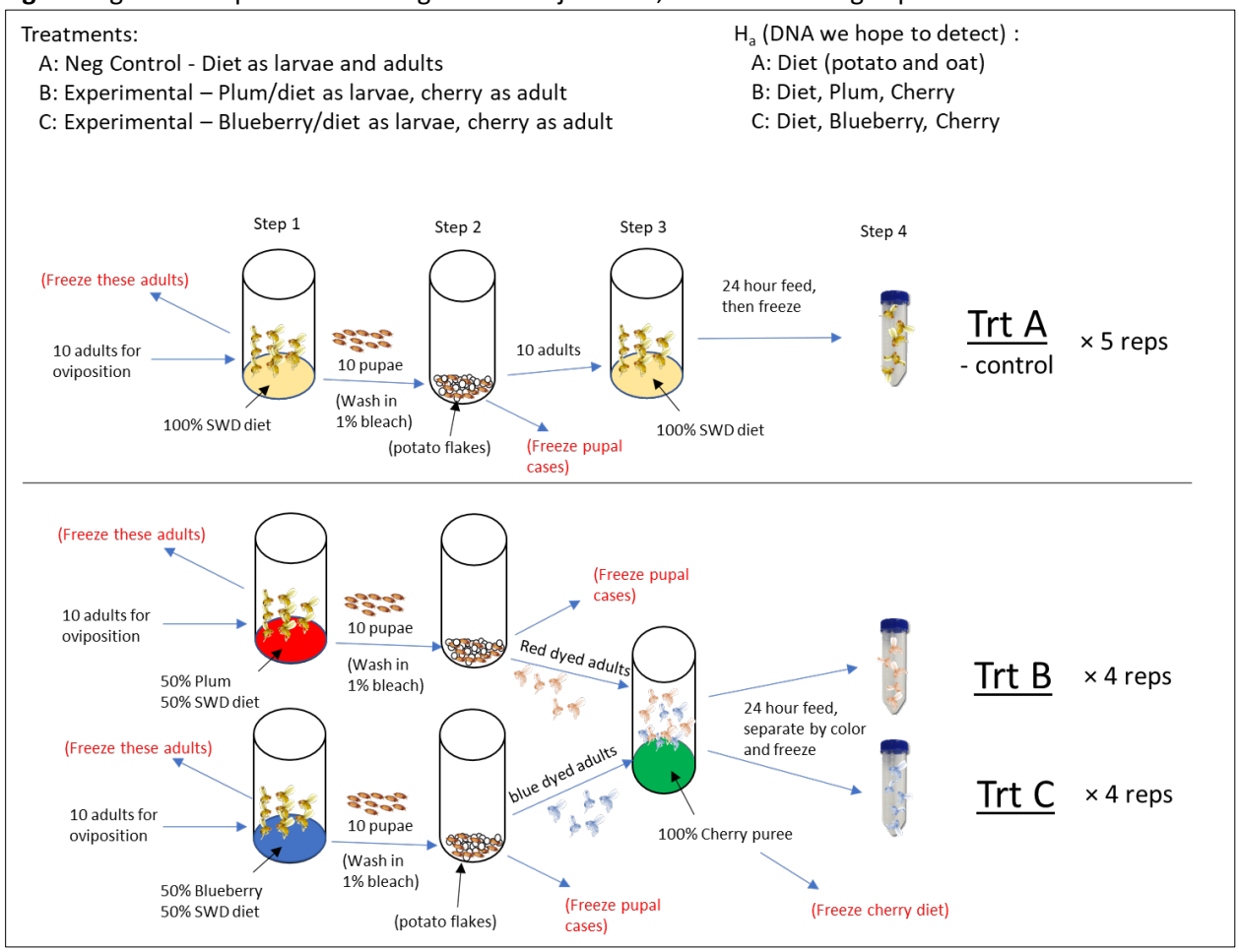
Methods:
Rearing. A colony of SWD was started in April 2020 from a previous lab colony (E. Beers, WSU TFREC) which was initiated in 2017 from SWD caught in a research cherry orchard near Wenatchee, WA. This colony was augmented with wild SWD each summer to prevent a genetic bottleneck. The colony was maintained using a standard drosophila diet (Formula 4-24 Drosophila Diet, Carolina Biological) and methods designated by the company.

Objective 1. Lab Feeding Experiment.
Overall: The goal of lab experiments was to determine if SWD's larval and/or adult feeding hosts could be detected via the PacBio deep sequencing technique. SWD adults and larvae were fed known fruit diets in the lab, then processed and sequenced to see if known plant DNA would be identified.

Methods: Diets were prepared with cherry, plum and blueberry puree mixed with standard SWD diet (50:50). Mixing puree with standard diet was necessary for SWD to complete development. SWD adults were added to tubes having one of three treatments: A. 100% Carolina Biological SWD diet (SWD diet; negative control), B. 50:50 plum puree to SWD diet (plum diet), or C. 50:50 blueberry puree to SWD diet (blueberry diet). Each treatment had four replicates with 10 adults each (40 adults per treatment). Adults remained in tubes with diets for approximately 48 hours, until larvae were visually detected. Adults were then removed from tubes, surface sterilized in DI water and 3% bleach solution, and stored at -80 °C. An additional group of adults fed cherry diet (50:50 cherry: SWD diet) was also frozen. All these adults were used to test detection potential for host plant DNA from adult feeding.

Once pupae were detected in tubes, pupae were removed, washed in a 1% bleach solution to surface sterilize, and added to a new sterile tube with just standard SWD diet. Once pupae hatched into adults, adults from treatment A were put into tubes with 100% SWD diet, treatments B and C were marked with colored florescent powders (B dyed red, C dyed blue), then combined into a single tube containing 100% cherry puree (Fig. 1). The dying and mixing procedure was developed to be certain that if DNA from the larval feeding stage was detected in adults, it was not due to contamination at the cherry diet stage (e.g., from the cherry diet itself or airborne contamination). Adults were left in the tube with cherry puree for 24 hours, then removed, surface sterilized in 3% bleach and frozen at -80 °C. Flies from treatments B and C were separated by color prior to being frozen. All samples remained in a -80 °C freezer at the WSU TFREC until August (ca. 1 month), then were transported to the USDA ARS Wapato Washington facility in cooler filled with dry ice. Samples were stored at -80 °C until further processing.

Fig 1. Diagram of experimental design for of Objective 1, SWD lab feeding experiment.



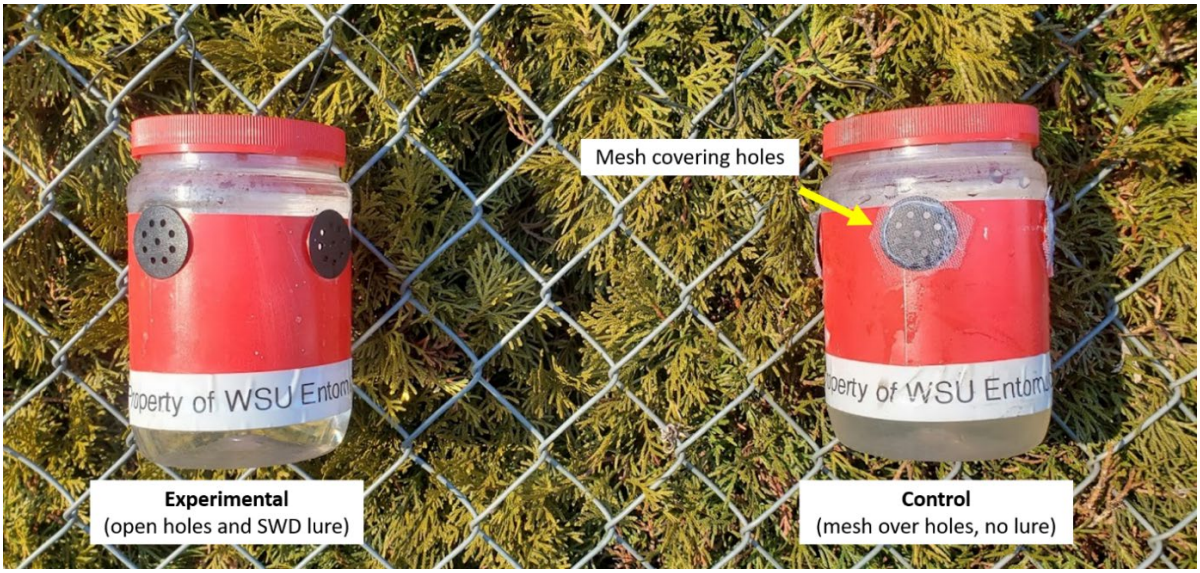
Molecular Analysis (Objectives 1 and 2): Following Cooper et al. (2019), DNA was extracted from the frozen SWD adults. SWD adults were surface sterilized, then homogenized for DNA extraction for polymerase chain reaction (PCR) with universal primers to amplify plant DNA sequences from a chloroplast gene (trnL) and a ribosomal gene (ITS). PCR reaction from no-template controls were included in sequencing analysis to measure levels of contamination. The primers for each PCR reaction were given a unique barcode region ("tag") so that amplified DNA from individual flies could be mixed into one sample for DNA sequencing and still be identified to individual flies after sequencing. The DNA

was then sent to Washington State University Laboratory for Biotechnology and Bioanalysis for sequencing with the PacBio platform. Sequences gained from each sample were grouped in operational taxonomic units (OTU, groups of sequences with 98% similarity), then identified to species using National Center for Biotechnology Information database using the basic local alignment search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Objective 2. Field Trapping.

Traps were deployed on 22 June 2020 at 10 sites across Chelan, Douglas, Grant, and Benton counties in a variety of crop and wild hosts (Table 1). When possible, traps were hung in non-host trees or fences directly adjacent to the host to reduce the potential for plant host DNA debris blowing into traps and to avoid overlap with orchard operations (Fig. 2). Most traps were all within a 1-mile radius of at least two potential hosts (e.g., traps placed next to blueberry were within one mile a of cherry orchard). At each site, two traps were deployed, one experimental and control (Fig. 2.). Control traps were used to determine if DNA detected in SWD was from SWD and not from other sources like other insect or windblown pollen. Both traps were Scentry liquid jar traps with 200 ml of propylene glycol to preserve captured SWD. Control traps used mesh screen (1 mm holes) to cover SWD entrance holes, thus preventing wild SWD from enter traps while allowing passage of particles and smaller insects. Prior to each deployment, five colony reared SWD adults were added to control traps only.

Fig. 2. SWD traps, experimental (left) and control (right), in the field (Photo: L. Nottingham).



Once per week, SWD were collected from experimental and control traps. Traps were cleaned and new colony reared SWD adults were added to control traps. Contents of traps were returned to the lab for processing. Processing involved identifying SWD adults among other insects, surface sterilizing SWD adults in a 3% bleach solution, transferring SWD adults into sterile 1.5 ml centrifuge tubes for storage in a -80 °C freezer. All samples remained in a -80 °C freezer for about 1 month until molecular analysis was performed as described in Objective 1.

Results and Discussion:

Objective 1. Lab Feeding.
Results: Low levels of molecular processing contamination were detected, and mainly included potato and lyceum. Potato was detected in nearly all samples, which was expected because potato was a primary component of the standard SWD diet. Only detections containing multiple amplifications of taxon are reported to omit contamination reads that may have occurred prior to molecular processing.

Adult Feeding: Sweet cherry DNA was detected in multiple replicates of adults fed cherry. Almond DNA was detected in one replicate of adults fed plum. We suspect "almond" reads may actually be plum, because it is same genus as almond, and "almond" reads were also amplified in field samples near plums. Blueberry was not detected in any adults fed blueberry.

Larval feeding: No detections of blueberry or plum resulted from larval feeding on those diets.

Discussion: *Adult feeding*: This experiment demonstrated that adult host food sources can be detected through deep sequencing; however, there are some limitations to this technique and our methods. Potato was the most readily detected, followed by cherry, then plum (detected as almond), then blueberry (not detected). All fruits were boiled to remove moisture when making puree, which may have denatured some DNA. Cherry was fed to adults as a 100% puree, as opposed to plum and blueberry which where 50:50 mix with SWD diet. In future experiments, it would be wise to test samples with genomic PCR, which is much faster, to tease out experimental design issues (such as "should diets be cooked and or mixed with SWD diet?"). Deep sequencing took nearly 1 year following the completion of physical experiments, so this technique was not the most efficient starting point. It would also be wise to select plants that are more taxonomically distant. Plum and cherry are both in the genus *Prunus*, which was likely a poor choice because sequencing may only be reliable to genus level.

Larval feeding: No DNA from larval feeding was detected, which was expected. Larvae will clear their gut contents during the pupal stage, so it was unlikely to detect plants from larval feeding, but we were hopeful for a useful discovery, nonetheless. Because this method barely detected plum DNA and did not detect blueberry from adults, it is likely that this experiment was not a reliable test on the larval-feeding hypothesis. Future studies should use diets that are more concentrated with the target DNA, take care to avoid processes that may denature DNA, and attempt to amplify known diets with conventional PCR prior to deep-sequencing.

Objective 2. Field Trapping.
Results: About 80% of reads were gained from open (non-control) SWD traps. Controls rarely produced DNA from known adjacent trees (only in one location on one date), suggesting that DNA reads from adjacent trees were brought in by SWD, not by pollen or other insects.

Plants detected at each open trap are shown in Table 1. Only plants with sufficient reads are shown. Nearly all sites amplified DNA from known adjacent hosts. Many amplified DNA from known distant hosts and various unknown hosts. There were a few particularly interesting unknown plant detections: 1. elderberry was detected at two sites which we later determined had nearby riparian areas containing elderberry plants; 2. raspberry was detected at a site, which we later learned was about a mile from a small raspberry plot in a neighbor's yard; 3. mahaleb cherry was detected in the second TFREC block, and we later discovered the tree about 40 feet of trap. Similar to the lab experiment, blueberry was surprisingly absent, except for one detection occurring in a blueberry plot.

Table 1. Plant DNA detections from SWD caught in open traps. Trap locations known hosts within the surround areas are described in three left columns, detected plants are in the far-right column.

Trap			SWD
Name/Location	**Adjacent host**	**Distant Hosts**	**Plant DNA Detected (# of detections)**
TFREC (photo block), Wenatchee	Cherry, peach, plum	Apricot, sweet cherry	Peach (6), almond/plum (4), apricot (2), ash (2)
TFREC (hill), Wenatchee	Serviceberry, mahaleb cherry	Apricot, sweet cherry	Mahaleb cherry (12)
Stutzman U-Pick, Sunnyslope	Peach, nectarine	Plum, raspberry, sweet cherry	Peach (7), raspberry (3)
Chumstick Hwy Leavenworth	Blueberry	-	Dogwood (4), potato (2), aster (3), blueberry (1)
Maple St. Apartments, Wenatchee	Ornamental plum (cherry-plum)	Peach, cherry	Almond/plum (17), cherry-plum (6), common plum (4), sweet cherry (4)
Columbia View, East Wenatchee	Cherry	Apricot, huckleberry	Sweet cherry (14), elderberry (6), chickweed (5), mahaleb cherry (3)
Sunrise Orchard, Rock Island	Cherry	Apricot	Sweet cherry (16), elderberry (2)
Zirkle 1, Patterson	Poplar, sweet cherry	Blueberry, grape, apple, cut flowers	Ash (18), caper (2),
Zirkle 2, Patterson	Poplar, apple	Blueberry, grape, cut flowers, sweet cherry	Willow (7), sweet cherry (3), maple (3), apple (2), almond/plum (3), purslane (2)

Discussion: Due to numerous detections of expected hosts from field collected adults, this study provides confidence that this method can accurately detect previous hosts of SWD. However, it is possible that many hosts are not being detected either due to DNA degradation over time or failure of the method to detect certain plants. We also cannot be certain that detected plant DNA is coming from SWD feeding (i.e., gut contents), and not on external parts of the insect. The surface sterilization process adds confidence that DNA was from SWD gut contents, but no true check was performed to confirm. In any case, the functional outcome is not compromised whether DNA is detected from external contact or within the gut.

SWD from control traps (reared on the potato-based diet) rarely amplified *Solanum* DNA, suggesting that DNA in guts of deceased insects likely degrades within a week. Future studies may benefit from using fewer sites and more frequent collection of insects.

Publications, Handouts, Other Text & Web Products:
Due to the delays in completion, no publications or other products have occurred. One manuscript is in progress.

Outreach:
No outreach has been conducted for the same reason as above; however, finalized information will be disseminated through Extension collaborators, the WSU Tree Fruit Website, and Extension seminars beginning in the summer of 2022.

Impacts

Short-term: These findings are a first step in understanding the utility of this method for identifying non-crop hosts of SWD. Next-generation sequencing can be used to effectively study landscape-level movements of insects; however, more work is needed to optimize this method.

Intermediate-term: Once this method has been used to identify non-crop hosts on a broader scale, researchers and growers may start using knowledge of SWD's non-crop host plants to develop more ecologically based management strategies. Knowledge of local refugia will allow growers to better predict invasion potential in different locations and/or treat those spots by either performing perimeter sprays or removing the refugia.

Long-term: Greater knowledge of SWD plant utilization and movement could transform management of this pest on a regional basis. Non-crop host plant hot spots can be identified and targeted for treatment by local agencies, thus reducing areawide populations. Using crops and non-crop hosts for regional scouting sites could be used to develop models to forecast seasonal dispersal and invasions of SWD.

Additional funding applied for/secured:

Additional funding was sought through the Washington Tree Fruit Research Commission Cherry Group in 2021, however the project was not moved beyond the preproposal stage. Although the project was met with positive reviews, the emergence of a serious insect vectored disease (x-disease) was the top priority of this group.

Graduate students funded: None

Recommendations for future research:

- Continue trapping studies with greater frequency trap checking.
- Compare results to conventional PCR.
- Determine how long DNA from fed upon hosts last within guts.
- Determine if larva are fed only certain diets (i.e., raw fruit without added SWD diet), will those fruit amplify in adults?
- Understand if certain plants amplify better than others by PacBio and/or certain genes (i.e., why was blueberry only amplified once in the entire experiment, even though it was directly fed to adults from some samples?).
- Does boiling fruit to make diet denature the DNA, and should this method be avoided in the future?
- How much do adult SWD actually feed? Do they use stopover sites to feed or should sequencing target points of contact, such as legs, instead of guts?
- Locate host plants from which DNA was amplified for the unknown hosts, such as elderberry, and mahaleb cherry.

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