BIOAg Project Report

Report Type: PROGRESS

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 red raspberries. Since its arrival in 2009, the cost and intensity of insecticide programs for these crops has dramatically increased. SWD adults generally develop and invade crops from unknown alternative host plants, complicating management. Despite great efforts to identify the non-crop hosts of SWD, no reliable method has been devised. In this project, we will address this issue with a novel molecular approach. We hypothesize that previous plant hosts of SWD can be accurately identified via next-generation sequencing (i.e. deep sequencing) of plant DNA remaining the guts of field collected adults. We will test this hypothesis by first attempting to identify known plant species fed to SWD adults and larvae in the lab via deep sequencing. Next, we will determine if the same technique can identify unknown prior host plants from field-collected SWD adults. This project fits the BIOAg mission because it will examine a novel tool that could answer one of the most difficult questions related to a major agricultural pest. If successful, the ability to identify SWD's non-crop hosts will allow growers to implement ecologically based management strategies, such as non-crop host treatment or removal. Furthermore, this work will provide a pivotal new approach for studying insect behavior and ecology at a landscape level.

Project Description:

Introduction:

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 cannot uncover all hosts with any certainty.

Molecular gut content analysis provides a potential solution to identify the 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. With their results, Cooper et al. (2019) inferred patterns of psyllid movement across natural and agricultural landscapes. We propose to test whether the methods used by Cooper et al. (2019) can be adapted to study the dietary history of SWD to determine host plants used as temporary hosts or for development prior to crop invasion.

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 hot-spots, 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.

Procedures:

(From Proposal)

Fly collection and rearing: We will collect SWD adults from the Washington State University Sunrise Research Orchard cherry block in late spring 2020. SWD will be trapped using dry-baited jar traps (one-way entrance jars with a Scentry pheromone lure) hung in cherry trees. Flies will be returned to the lab. We will then anesthetize the flies under CO2 and transfer set amounts to 7 cm vials containing known diet. SWD readily oviposit on diet in the laboratory and will develop from eggs to adults (E. H. Beers, personal communication). After one generation is reared on the standard diet, experiments will be conducted.

Objective 1. Identify plant DNA in SWD fed known laboratory diets (June - September 2020):
First generation laboratory reared adult SWD will be separated into 4 groups of 30 flies each. SWD adults will be introduced into vials containing drosophila diet mixed with 1 of 3 fruits (strawberries, cherries, or wild blackberries) or no added fruit (check). SWD adults will be allowed to oviposit, then will be removed from vials. Vials with SWD eggs/larvae and diet will be checked daily for pupae. Once pupae are found, individual pupae will be transferred to a container containing pureed fruit, different from the fruit on which they were reared, following the methods of Diepenbrock et al. (2018). After 1 day of feeding on puree as an adult, individual SWD will be surface sterilized with ethanol, then frozen at -80°C for molecular analysis (see “Molecular Analysis” below).

Objective 2. Identify plant DNA in SWD collected from the field (June - September 2020):
To assess the dietary history of field-collected SWD adults, samples of SWD adults will be collected from the Washington State University Sunrise Research Orchard cherry block. Collection methods will follow those in Objective 1, but traps will be collected once per week starting from the last week in June until the first week in August, or until at least 60 adults are captured. Adults from traps will be transferred to the lab in coolers on ice packs. Thirty individual SWD adults will be surface sterilized with ethanol and thirty others will not be sterilized. The non-sterilized adults are included here to test the extent that non-food plants may contaminate samples (such as floating pollen or other plant particles in the field or lab). Each adult will be placed into separate containers and stored in a -80°C freezer. If DNA is identified from these flies, we will attempt to locate these hosts in the greater landscape surrounding Sunrise Orchard to verify their potential to be previous hosts. If hosts are found, we will place traps for adults and collect fruits for larvae at these sites to further verify that SWD are using the plants detected in sequences, and determine if those hosts are being used for development.

Molecular Analysis: Following Cooper et al. (2019) we will extract DNA from the individual frozen flies described above and conduct polymerase chain reactions (PCRs) with universal primers to amplify plant DNA sequences from a chloroplast gene (trnL) and a ribosomal gene (ITS) within their gut contents. Both of these genes are well-suited for plant identification (Cooper et al. 2019). The primers for each PCR

reaction will contain a unique barcode region so that amplified DNA from individual flies can be mixed into one sample for DNA sequencing and still be identified to individual flies after sequencing. The DNA will be sent to the Washington State University Laboratory for Biotechnology and Bioanalysis for sequencing with the Pacific Biosciences (PacBio) platform. Resulting sequence data will be processed and cleaned with standard methods, grouped in operational taxonomic units (OTU, groups of sequences with 98% similarity), and each OTU will be searched against the National Center for Biotechnology Information database using the basic local alignment search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify plant species DNA. For each category of larval diet (3 fruit diets and 1 standard laboratory diet × 30 flies) and adult diet (3 fruit diets and 1 set of unfed adults × 30 flies), we will calculate the percentage of flies for which the fruit DNA was detected.

Outputs (2020 – Current)

Work Completed: All experimental procedures leading up to sequencing have been completed. For objective 1, SWD adults and larvae were fed multiple diets, frozen (as described in methods below) and delivered to the USDA ARS for processing. For objective 2, traps were deployed at 10 sites across Chelan, Douglas, Grant, and Benton counties of Washington. SWD were retrieved from traps at five weekly time intervals following deployment, from 22 June to 22 July 2020. All traps caught SWD; most caught SWD every week. SWD were frozen and delivered to the USDA ARS for processing.

Work in Progress: SWD samples are at the USDA ARS being prepared for PacBio sequencing. This involves extracting DNA from SWD samples, barcoding each sample, and combining samples into lanes which will be sent to the PacBio lab at WSU (detailed description in *Molecular Analysis* above). These procedures were delayed because both the USDA and PacBio labs were either closed or operating at limited capacity for COVID 19 safety. Samples should be processed by the USDA lab and sent to WSU by 1 March. We were unable to gain an estimate from the PacBio lab on their currently processing speed because there are too many affecting factors, however their turn-around has historically been within 1-2 months depending on their workload.

Methods used in 2020:

Objective 1.

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

Preliminary experiments. Preliminary experiments were performed to determine how to rear SWD on wild fruits in the lab for feeding experiments. First, we examined if SWD could be reared from egg to adult on pureed fruit alone, or if it would need to be mixed with standard diet. We determined that pureed fruit alone grew fungus quickly and few SWD completed development, so additional tests were performed to determine what ratio of SWD diet to pureed fruit would be optimal. A 50:50 mixture of fruit puree to standard diet (omitting yeast, which is usually part of the standard diet ingredients but produced to much CO_2) produced optimal survivorship with low fungal growth. Since our experimental diets would now contain other ingredients in addition to the fruit, we retrieved a list of ingredients in the SWD diet from Carolina Biological, so these plants would be accounted for as part of the diet in our analysis. The diet consists of potatoes and oats, which were not from facilities where fruit are handled. We performed diet to fruit ratio tests on multiple fruits. We only wanted to use fruits that we could

harvest from farms ourselves, to ensure that they were not contaminated by other fruits during processing or handling. Plum, cherry and blueberry all yielded acceptable survival of SWD using the 50:50 mixture, so these fruits were used in our experiment.

Feeding Experiment: The feeding experiment was performed similar to the proposed methods, with a few adjustments based on preliminary experiments and provide additional contamination checks. Our first goal was to determine if host plant DNA can be detected in SWD adults from larval feeding. For example, if an SWD adult is captured in blueberries, but it developed as a juvenile in cherry, will any cherry DNA from juvenile feeding remain detectable in that adult (even if that SWD did not feed on cherry as adult) using PacBio sequencing? The second, more simple, goal was to determine if we could detect host plant DNA within adults from feeding as adults.

Both goals were achieved through one experiment (Fig. 1). We started with three groups of SWD adults replicated 4 times, with 10 adults per replicate (40 adults per group). Each group (treatment) was added to tubes with one of three diets: A. 100% Carolina Biological SWD diet (herein, 'SWD diet'; negative control), B. 50:50 plum puree to SWD diet (herein, 'plum diet'), C. 50:50 blueberry puree to SWD diet (herein, 'blueberry diet'), or D. SWD diet mixed with plum, blueberry and cherry puree (positive control). Adults remained in these tubes with diets for approximately 48 hours, until larvae were visually detected. For treatments A, B and C, adults were then removed from tubes, surface sterilized in DI water and 5% bleach solution, stored in at -80°C. These adults would be used for the second goal mentioned above, detecting host plant DNA from adult feeding only. For treatment D, SWD adults, larvae and the multiple diet mixture were homogenized together and frozen at -80°C; no other steps were taken for treatment D. For the remaining treatments A, B and C, once pupae were detected in tubes, pupae were removed, washed in a 1% bleach solution (enough to sterilize but not kill) and added to a new sterile tube with Styrofoam pellets. Pellets partially buried the pupae to prevent the adults, once they developed, from interacting with the pupal cases which may have held DNA debris from the previous diet if sterilization was not completely effective. As an additional check, the pupal cases from this step were saved and stored at -80°C for sequencing to verify if sterilization was complete. Once pupae hatched into adults, the adults were moved to a third tube with diet. Adults from treatment A were put into tubes with 100% SWD diet again. Adults from treatment B and C were marked with colored florescent powder (B dyed red, C dyed blue), then these adults from both treatments were combined into a single tube, but keeping replicates separate (four tubes between B and C, 20 adults per tube: 10 from B and 10 from C) 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). If contamination occurred, adults starting in one diet will also produce DNA sequences from the other diet. Adults were left in the third tube with cherry diet for 24 hours, then removed, surface sterilized in 3% bleach and frozen at -80°C. Flies from treatments B and C were reseparated 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 restored at -80°C until further processing.

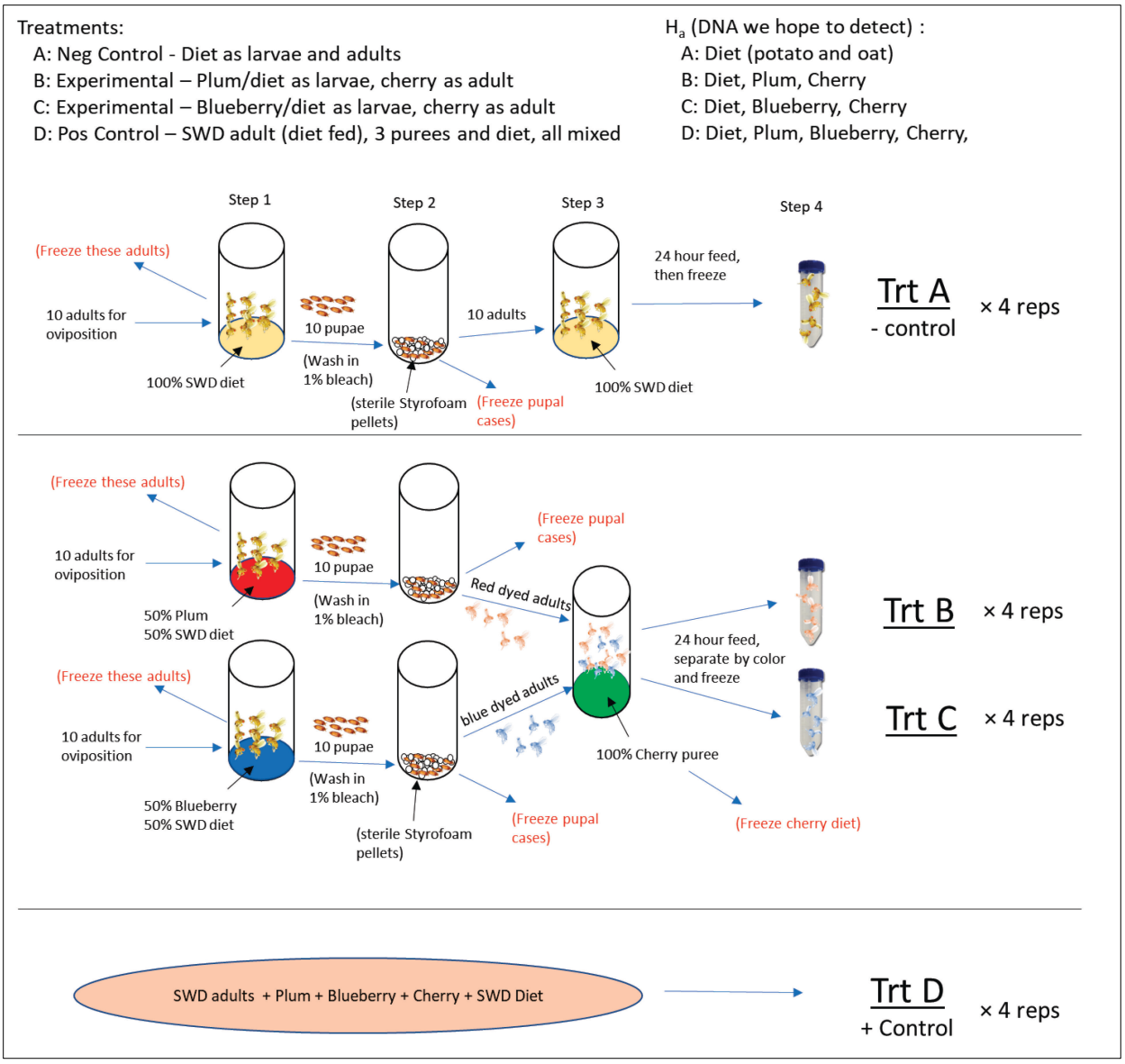

Fig 1. Diagram of experimental design for of Obj. 1, SWD lab feeding experiment.

Objective 2.

Field Trapping. Traps were deployed on 22 Jun 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 (Fig. 2) to reduce the potential for plant host DNA debris blowing into traps and to avoid overlap with orchard operations. Sites 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.). Both traps were Scentry liquid jar traps with 200 ml of propylene glycol to preserve captured SWD and internal plant DNA from feeding. Control traps were used to determine if DNA detected in SWD was in fact, from SWD and not from another source such as windblown pollen. Experimental traps also received a Scentry SWD lure, while control traps did not have a lure. 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, 5 colony-reared SWD adults were

added to control traps only. Their wings were clipped halfway up to identify them as colony adults. Sequencing control adults will tell us if plant DNA may get into SWD samples without it being brought in by the insect (false positives).

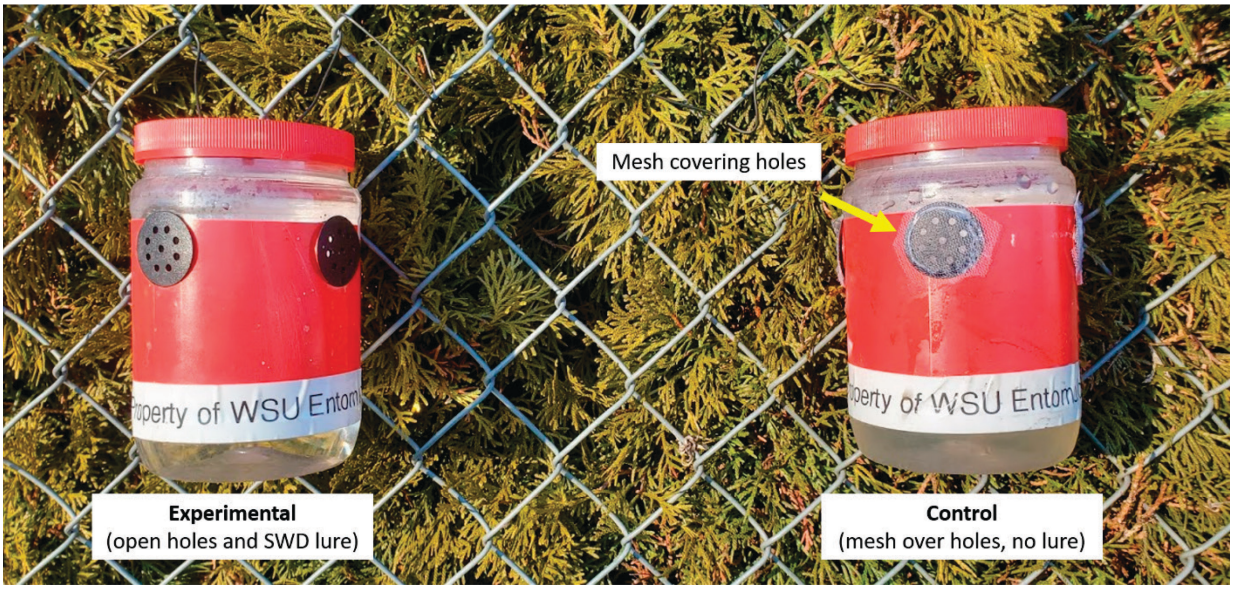

Fig. 2. SWD traps, experimental (left) and control (right), in the field.

Once per week, SWD were collected from experimental and control traps, traps were cleaned and new propylene glycol was added, 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 at the WSU TFREC until August (ca. 1 month), then were transported to the USDA ARS facility in cooler filled with dry ice. Samples were restored at -80°C until further processing.

Trap	Trap coordinates and location (all in WA)	Location in Washington	Adjacent host	Nearby hosts
1	47.4373, -120.34884	Wenatchee TFREC	cherry	Peach, nectarine, plum, cherry, service berry
2	47.43849, -120.35104	Wenatchee TFREC	Wild service berry	Wild current, strawberry, various stone fruit
3	47.48744, -120.39025	Sunnyslope Stutzman Ranch	Plum	Peach, nectarine, apricot, cherry, pluot
4	47.60797, -120.64831	Leavenworth Chumstick Hwy	Blueberry	Cherry, wild service berry, peaches, plum
5	47.44205, -120.33832	Wenatchee, Maple St. Apartments	Ornamental Plum	Peaches, Cherries
6	47.565391, -120.246673	East Wenatchee, Columbia View	Cherry	Apricots, Wild Service Berry
7	47.30603, -120.06483	Rock Island, Sunrise Orchard	Cherry	Apricots
8	46.768265, -119.642751	Mattawa, Zirkle Orchards	Cherry	Blueberries,
9	45.94005, -119.58296	Patterson, Zirkle Orchards	Cherry	Blueberries, grapes
10	45.95706, -119.60261	Patterson Zirkle Orchards	Cherry	Blueberries, grapes

USDA and PacBio Processing. The methods to extract and sequence DNA for both Objectives 1 and 2 will be unchanged from the original methods described above in the original proposal procedures section: *Molecular Analysis*. Exact details and any adjustments will be described in the final report once this portion is completed.

Results and Discussion:

There are currently no results to report because no sequencing has been performed. Results and discussion will be provided in the final report.

Publication, Handout, Other Products:

We have not published any aspect of this project.

Outreach & Extension Activities:

Outreach and extension activities will be provided following acquisition of results. We are currently refraining from publicizing this project to protect the intellectual novelty of these experiments until after publication. SWD is a very high profile pest, and advanced sequencing techniques like PacBio are become more wide-spread, so it is conceivable that our idea could be replicated and published by another group.

Impacts

There are no impacts to report at this time.

Additional funding applied for/secured:

No additional funding has been applied for due to the lack of preliminary results. Due to COVID 19 and reduced operations, it seemed irresponsible to expand lab activities prior to a clear timeline for returning to normal operations.

Graduate students funded:

None. Funding supported a postdoctoral researcher and an ungraduated researcher.

Recommendations for future research:

Future research recommendations will greatly depend on our results. Therefore, it will be more appropriate to wait to provide these recommendations in the final report.