

North American Bramble Growers Research Foundation

Proposal Category: Research, Production- Pest Management Strategies (Management of SWD)

Title: Using molecular tools to incorporate spotted wing drosophila host use and movement patterns towards the development of targeted management programs

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*Note that as of May 2018, Dr. Diepenbrock has moved to the Department of Entomology & Nematology at the University of Florida.

Final report

Drosophila suzukii Matsumura, (spotted wing drosophila, SWD), is a polyphagous invasive pest of soft skinned fruits which has quickly spread throughout North America, Europe, and South America since its initial detection in California raspberries and cherries (Walsh et al. 2011, Calabria et al. 2012, Cini et al. 2014, Asplen et al. 2015). *D. suzukii* has caused significant losses in crop yield and quality throughout its introduced range, and annual loss potential in the United States alone has been estimated at over \$700 million annually (Bolda et al. 2010, Goodhue et al. 2011, Walsh et al. 2011, Cini et al. 2012, eFly Working Group 2012).

Recent studies have described *D. suzukii*'s wide potential host range, including both crops and non crop fruit (Lee et al. 2015, Poyet et al. 2015), and have attempted to documented the movement of *D. suzukii* between natural habitats and commercial host crops using **indirect** methods (Klick et al. 2014, Pelton et al. 2016, Swoboda Bhattarai 2017). It is clear from this work that non crop habitat plays an important role in crop host risk. Gut content analysis provides a method to determine **direct** relationships between *D. suzukii* and hosts, and determining how it may move between them. Improving our understanding of the local movement of *D. suzukii* can lead to improved biologically-based management methods.

Previous research has shown that *D. suzukii* is capable of reproducing using a wide variety of both crop and non crop hosts (Lee et al. 2011, Lee et al. 2015, Poyet et al. 2015). Members of our project team have demonstrated that flies developing in a non crop host (American pokeweed, *Phytolacca americana*) that co-occurs with cultivated blackberry are more likely to infest blackberry fruit than the wild host, suggesting that non crop plants may increase crop risk (Diepenbrock et al. 2016). Recent work has shown that early season *D. suzukii* prevalence is related to the abundance of wooded areas in one host crop (raspberries, Pelton et al. 2016) and that *D. suzukii* move between fields and neighboring natural habitats during the course of a day (blackberry, Swoboda Bhattarai 2017). These studies provide information about potential movement of *D. suzukii* into a crop field from a nearby habitat and suggest that non crop habitat influences crop risk, but they lack the resolution to describe how far adults move and if they move from a crop during the growing season, which is important in developing targeted management programs

D. suzukii are often not readily visible in crop fields unless fly populations are very large, and we do not know where they spend the majority of their time. Unlike other tools used to detect *in situ* movement (e.g. mark-recapture with protein sprays), gut-content analysis ensures that the insects collected directly fed on/interacted with the crop of interest. Further, using gut content analysis allows flies to be collected several ways because the material detected will not wash off in liquid traps such as those most attractive to *D. suzukii*. Because DNA can be detected for several days post-consumption (preliminary data), we are confident the data we collect will inform our understanding of how flies move relative to available food resources.

Understanding local resource use is essential to developing effective pest management programs. Non crop habitat can provide refuge from in-crop pesticide applications (Rusch et al. 2010) and higher temperatures (Evans et al. 2017), and hosts present in non crop habitat can facilitate population growth (Lee et al. 2015, Poyet et al. 2015, Diepenbrock, et al. 2016). Insects may move between crop and non crop habitat frequently or they may move into crops when they become susceptible, spend the majority of their time there, and only return to non crop habitat post-harvest. Different use patterns suggest different management tactics for mobile crop pests.

Given the high value of berry and tree fruit crops and the risk posed by *D. suzukii* damage, the current reliance on pesticides as their primary management tool (Isaacs et al. 2013, Diepenbrock et al. 2016, 2017) and the demonstrated influence of natural habitats (Pelton et al. 2016, Swoboda Bhattarai 2017), it is necessary to develop tools that further enable researchers and IPM practitioners to understand the ecology of this polyphagous, highly mobile pest. The use of qPCR for gut content analysis will enable us to decipher local patterns of movement that can be incorporated into effective integrated pest management programs.

Progress to date

Molecular gut content has been widely used to study predatory insects (Sheppard and Harwood 2005) but to date has never been applied to plant feeding insects in the field with the goal of improving pest management practices. We are confident that this powerful tool can be used to aid in tracking the local movement of *D. suzukii* and enhance management recommendations. To date, we have tested published fruit-specific DNA primers and primers for an internal DNA extraction control specific to *D. suzukii*. We have identified a pair of primers that consistently amplify strawberry (*Fragaria sp.*) DNA (Fig. 1A; via Dong *et al.*, 2015) and primers for *D. suzukii* (Fig. 1B; via Dhami and Kumarasinghe, 2014). Using quantitative PCR

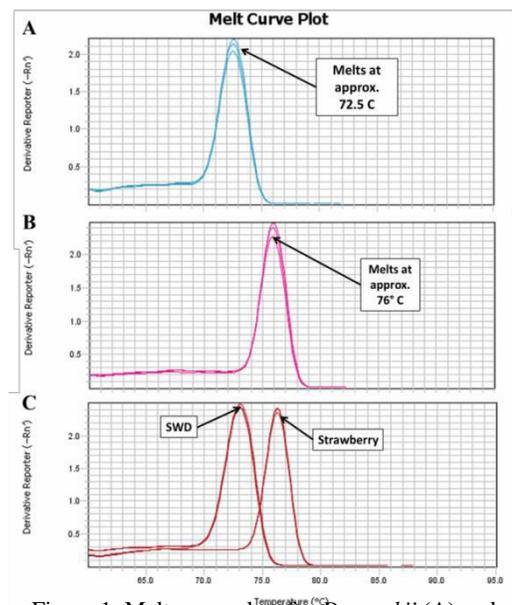


Figure 1: Melt curve plots for *D. suzukii* (A) and strawberry (B) DNA from qPCR amplification and melt curve analysis. Specific temperatures (A, B, C) enable differentiation of amplified product.

(qPCR), we have determined that DNA from a target host, strawberry, can be detected in the DNA extracted from the *D. suzukii* (gut content) for up to 7 days post-consumption under laboratory conditions (Diepenbrock et al. 2018).

Research is currently underway to determine optimal methods for trapping and preserving flies for DNA extraction within a field setting for strawberry. Initial work focuses on this crop as primers are currently available for purchase to validate our procedures. Because blackberry is a highly impacted crop in North Carolina and needs more options for managing this pest, we need to be able to use these tools to describe SWD movement in relation to this crop. However, none of the blackberry primers that are published and currently available were capable of amplifying blackberry DNA within our samples. Therefore, the **goal** of our project is to design and validate primer pairs which can amplify blackberry DNA extracted from spotted wing drosophila. This data will be used to develop management strategies focused on feeding resources of spotted wing drosophila and its movement between these resources.

Objectives

- (1) To develop blackberry specific DNA primers for the detection of blackberry DNA from the spotted wing drosophila after consumption. - **completed**
- (2) To determine how long post-consumption that blackberry DNA can be detected. – **in progress**
- (3) To determine how temperature impacts duration of blackberry DNA detection. – **dropped due to reduced funding**

Results

Objective 1: Develop blackberry specific DNA primers for the detection of blackberry DNA from the spotted wing drosophila after consumption

We have worked with Dr. Hamid Ashrafi, NCSU Department of Horticulture, to identify a unique section of the blackberry chloroplast genome to use as a candidate primer. This primer is in the process of being screened for amplification within SWD and non redundancy with strawberry.

Objective 2: Determine how long post-consumption that blackberry DNA can be detected.

Activities for Objective 2 will be completed during January 2019.