



Grower Summary

SF 145

Understanding and developing methods for managing spotted wing drosophila (SWD) in the UK: Vital research to maintain the viability of the UK fruit industry

Interim 2016

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Project title: Characterisation of winter SWD (*Drosophila suzukii*) gut flora for the development of future control measures

Project number: SF 145

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Report: Final report

Previous report: No

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Date project commenced: January 2016

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(or expected completion date):

GROWER SUMMARY

Headline

- This study has developed a suitable experimental framework for the analysis of microorganisms in the gut contents of the winter-form *Drosophila suzukii*.

Background and expected deliverables

Drosophila suzukii, commonly known as spotted wing drosophila (SWD), was first detected in the UK in 2012 (Harris and Shaw 2014). It is a recently invasive pest of soft- and stone- fruit that causes major challenges to the UK fruit industry. Endemic to Asia, *D. suzukii* has spread rapidly throughout the temperate regions of North America and Europe since 2008 (CABI 2015). Control currently relies upon an integrated approach that involves strict crop hygiene measures, exclusion netting, novel approaches and the use of traditional crop protection products.

The female *D. suzukii* has a serrated ovipositor that punctures the fruit epidermis allowing it to insert its eggs under the skin. A single female can lay up to 60 eggs per day and 200–600 eggs in her lifetime, randomly distributed on fruits. Larval development in the fruit results in its collapse rendering it unmarketable. If uncontrolled, total crop loss can occur in susceptible fruits like cherry, plum, strawberry, raspberry, blackberry and blueberry.

The aim of this project was to produce a replicated, robust pilot dataset that can be investigated to determine whether there are microorganisms found in the digestive system of *D. suzukii* that could be developed as natural attractants, eventually leading to improved monitoring and control tools for growers.

Summary of the project and main conclusions

The rationale behind this study was to explore the microorganism diversity in the gut contents of *D. suzukii* to determine if there are suitable candidate microorganisms for use in future bait development for the attraction of the winter-form of *D. suzukii*. The work would also provide scientific contributions towards the understanding of the ecology and behaviour of the organism.

There were two aims of this study: the first was to determine how *D. suzukii* gut contents varied across a range of sites; the second was to identify bacterial and fungal groups in the digestive tract of *D. suzukii* to identify microbial winter food sources.

Through the use of a next generation sequencing approach known as ‘metagenomics’, we investigated the gut contents of *D. suzukii* collected across four farm locations in Southern

England and found that they contain diverse microorganism communities in their guts. Analysis of these samples at the Genus and Family taxonomic levels showed high levels of diversity in the *D. suzukii* gut flora samples with no significant differences between individuals or between sites for either bacterial or fungal biota in the alimentary canal of winter-forms. This outcome was consistent regardless of the sex of the insect and to our knowledge has not been documented before for this species.

This result provides the first evidence that the UK winter-form of *D. suzukii* is a potentially opportunistic feeder during the winter period and probably exploits food sources associated with moisture on decomposing vegetation during this time. This corresponds with evidence demonstrating that *D. suzukii* is known to overwinter in sheltered habitats such as woodlands and is associated with a wide range of egg laying host plants and overwintering habitats, including ivy, nettle, leaf litter, bramble, elder, bracket fungus, scrub, compost heaps and pond debris.

In this study, we were able to identify a 'core' set of bacterial and fungal genera that are present across all sites in all the gut samples of the winter-form. Our results show that the genera we identified include some of those found previously in other studies investigating the gut flora of fruit fly species, namely *Bactrocera zonata* (Naaz et al. 2016) and *D. suzukii* (Hamby et al. 2012). These studies isolated cultivatable bacteria and yeast species (Naaz et al., 2016; and Hamby et al., 2012; respectively) from gut contents. Hamby et al. (2012) reported that both larvae and adult *D. suzukii* are associated with specific yeasts, predominantly *Hanseniaspora uvarum*; however, this yeast species, though identified in several samples in our study, was not found to be a part of our core microbiome. A related, but unidentified Operational Taxonomic Unit (OTU) of the *Hanseniaspora* genus was identified at all sites.

Owing to the experimental method employed in the studies by Naaz et al. (2016) and Hamby et al. (2012), the full complement of microorganisms in the gut flora community was not fully explored, as the studies were restricted to those species that could be cultivated under laboratory conditions. Our metagenomic approach does not rely on cultivating the microorganisms, thereby providing a fuller picture of the gut microbiome.

In our research, several genera in the *D. suzukii* core microbiome were found to be present in the gut flora in successive years through independent metagenomic analyses.

The core bacterial genera were *Pseudomonas* and *Sphingomonas*. *Pseudomonas* species play a role in the decomposition of organic matter. They are favoured by moisture and are ubiquitous in soil and water. They also give rise to plant diseases such as the bacterial tree canker which is caused by *Pseudomonas syringae*. *Sphingomonas* are found in a range of land and water habitats, as well as in plant root systems.

The fungal genera were *Guehomyces* (widespread distribution, predominantly in cold climates) and *Cryptococcus* (the majority of these species live in the soil).

The phylum Ascomycota were also present in both years. This higher level taxonomic group are the largest phylum of fungi, with over 64,000 species, including many plant-pathogenic ascomycetes, such as apple scab, rice blast, the ergot fungi, black knot and the powdery mildews. It is therefore unsurprising that it appeared in large numbers in our studies in both years.

A larger sampling strategy during the fruit growing season and covering additional locations in the UK, would enable a more comprehensive picture of overwintering and summer hosts. Food requirements of *D. suzukii* would also be determined providing a complete representation of the natural gut flora through the year.

In a related study, Chandler et al. (2012) found that yeast communities are dominated by a small number of abundant taxa, that the same yeast lineages are associated with different host species and populations and that host diet has a greater effect than host species on yeast community composition. Therefore, based on the evidence presented in the Chandler study, we would predict the genera found in the core microbiome to be present in a wider sampling of gut flora of *D. suzukii* from around the UK.

Recently published research to develop fruit fly control strategies using metabolites produced by bacteria or yeasts found in association with gut contents, have been reported for the peach fruit fly, *Bactrocera zona* (Naaz et al. 2016). The results from our study have provided a list of candidate genera that, with further investigation, could enable a similar approach to be undertaken for use in future bait development to attract the winter-form of *D. suzukii*.

Main conclusions

This short study has provided a suitable experimental framework for the analysis of microorganisms in the gut contents of *D. suzukii*. The integration of molecular methods with classical entomological procedures has been a successful approach to address important questions underlying *D. suzukii* winter-feeding behaviour and over-wintering habitat niches. The relatively small number of sampling locations, all occurring in southern England, and sampling time (winter) were a limiting factor to this study and a more expansive sampling strategy covering the United Kingdom would provide an enhanced and more comprehensive analysis of *D. suzukii* gut contents.

This study has developed and produced:

- Laboratory protocols for gut dissection from the fruit fly *D. suzukii*;

- An amplicon-based metagenomics approach to characterise bacterial and fungal genera in the *D. suzukii* gut;
- Bioinformatic analysis pipelines for next generation sequence data processing;
- A library of core gut microflora for use in future studies.

The main conclusions and outputs coming from this study are:

- Compared to previous studies, high levels of microorganism diversity were present in the gut flora of *D. suzukii* (winter form) with no significant differences within or between sites for either Family or Genera of bacterial or fungal biota, regardless of insect gender;
- New evidence suggests that the UK winter-form of *D. suzukii* are potential opportunistic feeders during the winter period and are exploiting food sources associated with moisture and decomposing vegetation that are available during this time;
- A core gut microbiome of both bacterial and fungal genera common across all sites has been identified.
- Bacterial and fungal genera common in *D. suzukii* gut contents between different years were identified.

In addition, there are several noteworthy pieces of information which were gained from attendance at two *D. suzukii* international conferences:

- The data generated through the methodologies used in this study are a 'world-first'. NIAB EMR are currently the global leaders in SWD research in this area. Many other international research groups are applying for funding to begin this avenue of work.
- Researchers at NIAB EMR are the first to identify and report a core gut microbiome for *D. suzukii*.

Financial benefits

- No financial benefits for growers could be identified from this research study, the results of which will feed into future SWD research.

Action points for growers

- No action points relevant to growers can be offered from a study of this nature.