Project title:	Enhancing control of the soft- and stone- fruit pest *Drosophila suzukii* (Spotted Wing *Drosophila*) by exploiting its activity patterns in the field.
Project number:	CP142
Project leader:	Michelle Fountain, NIAB EMR, KENT. Herman Wijnen, University of Southampton
Report:	Final report. Year 3, September 2018
Previous report:	Annual report. Year 2, October 2017 Annual report. Year 1, October 2016
Key staff:	Bethan Shaw
Location of project:	NIAB EMR, Kent and University of Southampton
Industry Representative:	Harriet Duncalfe
Date project commenced:	October 2015
Date project completed (or expected completion date):	September 2018

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Bethan Shaw

PhD student

NIAB EMR

Signature .... Date ....

Michelle Fountain

Research Leader in Entomology

NIAB EMR

Signature .... Date..

Report authorised by:

Harriet Duncalfe

Industry representative

Signature .... Date ....

[Name]

[Position]

[Organisation]

Signature .... Date ....

CONTENTS

GROWER SUMMARY

Headline

- New knowledge gained about the daily activity of spotted wing drosophila

Background and expected deliverables

Daily behavioural and physiological rhythms, such as activity and sleep, are exhibited by a wide range of organisms as a result of the interaction between environmental cycles and an internal timekeeping mechanism known as the circadian clock (Allada and Chung, 2010, Bollinger and Schibler, 2014). The clock can be regulated by environmental cues including temperature and daylight and enables synchronicity to dynamic daily conditions (Dubowy and Sehgal, 2017, Dubruille and Emery, 2008).

D. suzukii is an invasive species that damages ripening fruit, unlike other Drosophila species which only feed on overripe or spoiled fruit (Rota-Stabelli et al., 2013). *D. suzukii* was found for the first time in the UK in an area of wild blackberry in Kent in August 2012 (Harris and Shaw, 2014) and is now a serious pest in protected and outdoor cherry, strawberry, raspberry, blackberry and blueberry. Female *D. suzukii* are able to insert eggs into the skin of ripening fruits with a serrated ovipositor. Once the eggs hatch, the larval stages consume the fruit from within causing the fruit to collapse rendering fruit unmarketable.

Further insight into the daily behavioural and physiological rhythms of *D. suzukii*, as determined by its internal circadian clock and environmental cues, may help to predict the times of day when *D. suzukii* poses the greatest threat to crops and when they would be most vulnerable to control measures. The research that would be invaluable to British soft- and stone-fruit growers would include exploitation of the behaviour and physiology of *D. suzukii* to enable more effective control within the UK growing season.

In recent years there has been a surge in the numbers of studies (in the USA and Europe) on the behaviour and control of *D. suzukii*. On-going studies in the UK (e.g. AHDB project SF/TF 145a) are demonstrating peaks of seasonal activity. However, little is known about the daily and seasonal rhythmicity in the behaviour and physiology of *D. suzukii*. Current work on behavioural rhythms of this pest has been done in other countries under different environmental conditions, which, due to the nature of the clock, may result in different behavioural outputs.

Hamby et al. (2013) described diurnal fluctuations in *D. suzukii* locomotor activity under laboratory conditions mimicking summer and winter days and Evans et al. (2017)

investigated the oviposition rhythms of *D. suzukii* in outdoor field studies. However, both these examples were performed under temperature and light cycles that we would not experience in the UK and used unrealistic social grouping to reach their conclusions. More expansive studies of clock-controlled daily rhythms have been done on the *D. suzukii* sister species, *D. melanogaster*. The latter exhibits not only circadian locomotor behaviour, gene expression and metabolism, but also daily clock-controlled oscillations in processes such as feeding, egg laying and eclosion (adult emerging from pupae) (Xu et al., 2008). Behavioural patterns and rhythms have been observed in *D. suzukii* in constant conditions and so the influence of environmental and social conditions have not been evaluated (Lin et al., 2014).

To tailor control methods to specific behavioural events, a greater understanding of what drives or influences these behaviours is needed. In this project, rhythms in activity (locomotion), oviposition and susceptibility to plant protection products is investigated. The parameters that influence or disrupt these physiological processes will also be explored to identify the correct methods, which can be used to predict 'wild type' behavioural patterns in an artificial setting. This will enable researchers to repeatedly run simulations in a controlled, laboratory environment and could then be used as a tool to predict patterns of key behaviours at specific times of the cropping year.

Aims and methods

The overall aim of this research was to investigate daily and seasonal rhythms of *D. suzukii* locomotion activity and oviposition and formulate recommendations for UK growers in regard to field detection, trapping and crop protection.

Locomotion activity was investigated using an electronic device that monitors movement of Drosophila under various environmental conditions in the laboratory. Individual males, individual females, groups of males, groups of females and mixed sex groups were monitored under different seasonal conditions to determine an average locomotion pattern. The effects of removing environmental cues on the locomotion activity were investigated to understand the mechanisms that drive the internal circadian clock. Locomotion behaviour was also observed under natural light and temperature conditions in a semi-field setting. These environmental conditions were recreated in the laboratory to confirm whether laboratory locomotion patterns would correlate with those collected under natural conditions in the field when under the same temperature and light cycles.

Oviposition patterns of wild *D. suzukii* populations were determined within a strategic cherry orchard in Kent. Traps were baited with fresh fruit and were changed every two hours from sunrise to sunset for three days. The emergence of the next generation was counted to identify peaks in egg laying. The environmental conditions collected during the field oviposition trial were recreated in the laboratory and the oviposition pattern of laboratory strains recorded using the same method as in the field. Locomotion was also investigated under these environmental conditions to determine if there was a correlation between locomotion activity and oviposition.

To identify how to disrupt egg laying in *D. suzukii*, reproductive competition between *D. suzukii* and *D. melanogaster* was investigated. Each species was presented with a substrate either pre-inoculated with the opposing species' eggs or a blank substrate and the emergence of the next generation was counted.

To investigate the relationship between *D. suzukii* and UK approved plant protection products, laboratory based spray trials were performed. Sub-lethal doses of cyantraniliprole, lambda-cyhalothrin, pyrethrum and spinosad were directly applied to groups of *D. suzukii* via a bench top sprayer. Mortality, oviposition and the transgenerational effects of these doses were measured over time. In addition, the impact time of application was explored on mortality and oviposition by applying plant protection products at different time points.

Summary of the project and main conclusions

In the first year of this PhD study, it was demonstrated that the standard laboratory conditions for investigating circadian rhythms were not appropriate to predict behaviour in the field. Constant temperatures and 12:12 hour light: dark cycles produced very different locomotion patterns to fluctuating temperatures and seasonal day length. Locomotion patterns for individual flies differed from single sex and mixed sex groups. Locomotion activity was mainly driven by light and then influenced by temperature cycles. Within the second year of this PhD, a wider range of semi-field and laboratory conditions were investigated to see if laboratory strains would exhibit the same locomotion activity as those under the natural cycles. When these conditions were re-created in the laboratory, behavioural patterns of mixed sex groups and female groups correlated with those collected in the field. As this was successful, the results confirm that predictions of locomotion activity under natural conditions can be made from laboratory based assays if the correct environmental conditions are used.

Oviposition was investigated in August and October conditions and in both trials more eggs were laid in the day time than at night. In August, oviposition fell when temperatures exceeded 30°C and peak egg laying occurred in the mid-morning and evening when temperatures were between 25-29°C. In October, peak egg laying occurred at peak temperature each day. When the environmental patterns were recreated in the laboratory, laboratory strains displayed the same oviposition pattern as their wild counterparts. As with the locomotion activity, oviposition patterns of lab strains, when exposed to the recreated environmental conditions, mirrored those collected in the field. This means that oviposition rhythms can also be predicted for specific times of the year if an average light and temperature cycle is known. The locomotion of mixed sex groups, when exposed to the oviposition environmental conditions, correlated with the patterns of oviposition indicating that some of the locomotion activity during the day is the result of females searching for oviposition sites.

In the reproductive competition assay, significantly fewer *D. suzukii* emerged from the *D. melanogaster* pre-inoculated substrate than the un-inoculated substrate. This reduction is caused by female oviposition choice, with fewer eggs being laid initially. However, there may also be some egg predation with *D. melanogaster* larvae feeding on *D. suzukii* eggs and/or larvae.

In most cases, the application of sub-lethal doses of a plant protection product resulted in significant reductions in survival and overall oviposition. However for cyantraniliprole (Exirel) more eggs were laid, overall, by females that were treated with 0.3% of the field rate (FR). There was also no significant reduction in survival of the offspring. For all of the PPP doses applied, there was a significant reduction in the number of eggs laid per female within 24 hours of PPP application. There were exceptions to this but typically they were <12% of the field rate. Over time, surviving females recovered from the sub-lethal doses and continued to lay eggs. One week after application, females that survived 100% field rate of spinosad (Tracer) laid the same number of eggs as untreated females.

There were also some transgenerational effects on offspring when parents were treated with lambda-cyhalothrin (Hallmark) and spinosad. There was a significant reduction in egg to pupa, and egg to adult survival 24 hrs after application with 12, 25 and 100% of the spinosad field rate and those treated with 6% of lambda-cyhalothrin. Forty-eight hours after application there was a significant reduction in pupa to adult survival when parents were treated with 6% of lambda-cyhalothrin field rate. There was also a significant reduction in survival from egg to adult 96 and 168 hrs after application with 6 and 100% field rate of spinosad.

The results from this assay highlight the importance of following a plant protection product labelling and using the field rate stated by the producer. In many cases, using lower than the recommended field rate resulted in reduced mortality and insignificant effects on oviposition of surviving females or offspring survival. Repeatedly exposing *D. suzukii* to sub-lethal doses of topically applied plant protection products could lead to insecticide resistance.

There was no impact of time of plant protection product application in either mortality or oviposition rates of *D. suzukii* to any of the four PPPs applied. Further work on this topic is needed to cover more PPP application time points.

Conclusions

- Daily activity (locomotion) rhythms of *Drosophila suzukii* are dictated by light primarily, and then by temperature, resulting in more general activity during daylight.
- Groups of *D. suzukii* display different locomotion patterns compared to individual *D. suzukii*, housed alone, and so groups should be used to make predictions about 'wild type' behavioural rhythms.
- Locomotion and oviposition rhythms under natural conditions can be predicted in the laboratory when the correct social conditions, in addition to realistic temperature and light profiles, are used.
- Oviposition occurs in the daytime even during short daylengths and mild night conditions.
- Oviposition reduces significantly when temperatures exceed 30°C.
- *D. suzukii* emergence is reduced when in competition with *D. melanogaster* for egg laying sites.
- Female oviposition rates recover, over time, if adults recover after being treated with sub-lethal and field rate doses of pyrethrum, lambda-cyhalothrin and spinosad.
- There are some transgenerational effects of plant protection product application on offspring development and survival.
- There is no impact of time of PPP application on *D. suzukii* susceptibility, of 4 orchard plant protection products in this study.

Financial benefits

According to Defra Horticultural Statistics, the value of UK produced cherries in 2017 was £23.6 million and without the availability of effective control methods, the total losses to the pest would not be far short of this figure as D. suzukii can give rise to almost 100% loss of a cherry crop. Raspberry, blackberry, blueberry and strawberry are also extremely susceptible and losses of 50% and more are not uncommon where effective control is not implemented. With the value of UK strawberry in 2017 estimated to total £283 million and raspberry estimated to be £128 million, then losses of half of these would amount to around £141.5 million and £64 million if uncontrolled.

The research in this project is filling gaps that are not being addressed in research programmes in other projects and other countries. It is essential for maintaining the viability and profitability of the UK's important soft and stone fruit industries. *D. suzukii* seriously threatens the sustainability of production of these crops in the UK. By understanding the Chrono-physiology of the pest we can provide growers with a better understanding of how to target the control of *D. suzukii*.

Action points for growers

- Use monitoring traps to monitor flying adults but take into consideration that trap catches will be low at temperatures below 10^{o}C and above 30^{o}C.
- Do not eradicate native *Drosophila* species from a crop as the oviposition competition may be restricting population expansion in ripened and waste fruit.
- It is essential to remove waste fruit from the crop frequently to reduce oviposition and feeding sites for *D. suzukii*.
- Always follow label rate recommendations for PPPs and rotate modes of action. Reducing the dose could result in resistance build up in D. suzukii.

References

ALLADA, R. & CHUNG, B. Y. 2010. Circadian organization of behavior and physiology in *Drosophila*. *Annual Review of Physiology,* 72**,** 605-24.

BOLLINGER, T. & SCHIBLER, U. 2014. Circadian rhythms - from genes to physiology and disease. *Swiss Medical Weekly,* 144**,** w13984.

DUBOWY, C. & SEHGAL, A. 2017. Circadian Rhythms and Sleep in Drosophila melanogaster. *Genetics,* 205**,** 1373-1397.

DUBRUILLE, R. & EMERY, P. 2008. A plastic clock: how circadian rhythms respond to environmental cues in *Drosophila*. *Molecular Neurobiology,* 38**,** 129-45.

EVANS, R. K., TOEWS, M. D. & SIAL, A. A. 2017. Diel periodicity of *Drosophila suzukii* (*Diptera: Drosophilidae*) under field conditions. *PLoS One,* 12**,** e0171718.

HAMBY, K. A., KWOK, R. S., ZALOM, F. G. & CHIU, J. C. 2013. Integrating circadian activity and gene expression profiles to predict chronotoxicity of *Drosophila suzukii* Response to Insecticides. *PLoS One,* 8.

HARRIS, A. L. & SHAW, B. 2014. First record of *Drosophila suzukii* (Matsumua)(Diptera, Drosophilidae) in Great Britain. *Dipterists Digest,* 21**,** 189-192.

LIN, Q.-C., ZHAI, Y.-F., ZHOU, C.-G., LI, L.-L., ZHUANG, Q.-Y., ZHANG, X.-Y., ZALOM, F. G. & YU, Y. 2014. Behavioral rhythms of *Drosophila suzukii* and *Drosophila melanogaster Florida Entomologist,* 97**,** 1424-1433.

ROTA-STABELLI, O., BLAXTER, M. & ANFORA, G. 2013. Drosophila suzukii. *Curr Biol,* 23**,** R8-9.

XU, K., ZHENG, X. & SEHGAL, A. 2008. Regulation of feeding and metabolism by neuronal and peripheral clocks in Drosophila. *Cell Metab,* 8**,** 289-300.