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Project leader:	Dr Darren Obbard (University of Edinburgh)
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Key staff:	Dr Darren Obbard Prof Jerry Cross
Location of project:	University of Edinburgh, Edinburgh and East Malling Research, Kent
Industry Representative:	Marion Regan, Hugh Lowe Farms, Barons Place, Willow Wents, Mereworth, Maidstone, Kent, ME18 5NF
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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.

[Name] DARREN J. OBBARD
[Position] CHANCELLOR'S RESEARCH FELLOW
[Organisation] UNIVERSITY OF EDINBURGH
Signature ..... Date 21st Dec 2015

[Name]
[Position]
[Organisation]
Signature ..... Date .....

Report authorised by:

[Name]
[Position]
[Organisation]
Signature ..... Date .....

[Name]
[Position]
[Organisation]
Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headline

- New viruses discovered in Drosophila pest: the first steps on the road to a novel biopesticide.

Background and expected deliverables

Drosophila suzukii (Matsummura), also known as the spotted wing drosophila (SWD) is an invasive fly pest of soft fruits. Its recent invasion of the fruit growing regions of North America and Europe and the damage it has caused there have driven interest in finding new control solutions. Conventional chemical control methods have many drawbacks and are difficult to implement within integrated pest management (IPM) programmes, consequently the development of an alternative IPM compatible biopesticide would be beneficial for growers, consumers and pest management professionals alike. This study seeks to characterise the viral diversity of SWD with the aim of identifying a pathogen suitable for the control of this pest in UK fruit crops. A combination of approaches, both cutting edge genetic techniques and traditional lab based investigation, will be used to identify the viruses infecting SWD from large samples of wild flies, before viruses are isolated and investigated for their interactions with their *Drosophila* host or hosts in the laboratory and field.

Summary of the project and main conclusions

The viruses of *Drosophila suzukii* (spotted wing drosophila or SWD) offer good potential candidates for the development of a microbe based bioinsecticide. However, to date, the viruses of *D. suzukii* remain almost completely unstudied. Cutting edge genetic techniques have been used to sequence the genetic material of all pathogens infecting this fly. Through the lab's expertise in the study of Drosophila viruses we have identified a selection of viruses present in wild *D. suzukii*. Some viruses discovered appear to be very closely related to the viruses found in *D. melanogaster*. Others however, seem to be unique to *D. suzukii* and new to science. Through a technique called serial passage we have confirmed that an extract containing only the viruses of a large pool of wild flies has a lethal effect on *D. suzukii* in the lab. We are currently in the process of identifying which viruses cause these lethal effects and isolating pure viral isolates for further experimentation. Further sequencing is now underway for another large sample of flies caught in 2015.

Financial benefits

The impact of this pest on the European horticultural industry has already been substantial, with *D. suzukii* having caused losses of over €8 million in fruit crops in Northern Italy in 2010 and 2011 and more than €1.5 million for French strawberries in 2011 (FERA, 2015). The European and Mediterranean Plant Protection Organisation (EPPO) in a recent 'Pest Risk Analysis' deemed this organism to be a potential threat to crops in its region. Potential damage is described as "massive" and the regions ability to control it as "with much difficulty" (EPPO, 2010). In the Pacific fruit growing regions of the USA, the estimated damage due to *D. suzukii* has been calculated at over €400 million/year (Bolda et al., 2010). With damage estimates for the UK slow to emerge, it is hard to quantify the exact financial damage that this pest has done since its establishment here.

A key consideration for the damage caused by this pest is the effect of disrupting already established IPM programmes to gain control. Changes in management techniques, necessitated by the presence of this pest, often include the use of crop protection products not compatible with residue or resistance management practices. Without IPM compatible products, damage is not limited to that done by the pest itself but also extends to secondary pest damage caused by the use of broader spectrum or longer persistence products. Additionally these 'emergency use products' are often required close to harvest, endangering the low residue standards enforced by retailers.

Action points for growers

- It is too early in the life of this project to identify any action points for growers.

SCIENCE SECTION

Introduction

Drosophila suzukii is closely related to the famous lab model *Drosophila melanogaster* (Lewis et al., 2005, Kopp, 2006). Some physical characters do, however, allow *D. suzukii* to be distinguished from its well-studied relative. Amongst these, the presence of dark wing spots in the male (to which the common species name 'Spotted Wing Drosophila' refers) and a heavily sclerotized ovipositor bearing tooth-like bristles in the female are most prominent. It is this well-developed ovipositor that is considered to be the evolutionary innovation that allows *D. suzukii* to oviposit under the skin of ripening fruit still on the tree: a feature shared by few other *Drosophila* species (Atallah et al., 2014).

Once laid, the eggs of *D. suzukii* go through three larval instars inside the fruit, feeding on the mesocarp and developing from egg to adult in approximately 8 to 10 days at 25 °C, and from 21 to 25 days at 15 °C (Kanzawa, 1939). Extensive studies examining the life history traits of this species were carried out in Japan in the 1930's (Kanzawa, 1939, Kanzawa, 1935) with further information on oviposition behaviour (Mitsui et al., 2006), life stages, host range and overwintering (Walsh et al., 2011) being provided more recently. A network of monitoring traps has been established in the UK since the first detection of this pest in mainland Britain in 2012. The monitoring scheme in the UK has reported the number of *D. suzukii* adults, caught in bait traps, to peak at some point between September and November depending on weather conditions (Dr M Fountain *pers comm*). As British records of D. *suzukii* only date back three growing seasons, data on the phenology of the organism are still limited.

A very broad range of host plants makes *D. suzukii* an especially difficult pest to control. *D. suzukii* is known to oviposit in a wide variety of commercial and wild soft skinned fruit (Walsh et al., 2011, Cini et al., 2012, Mitsui et al., 2010). This allows populations to reside in wild refuges and may facilitate the reinvasion of crops after periods of intense spraying, fruit unavailability or cold weather.

Pattern of invasion

First described in Japan in 1916 (Kanzawa, 1935), *D. suzukii* was reported to be widely distributed in Japan shortly after (Kanzawa, 1939). The first records of this pest from outside Asia came from Hawaii in the 1980's (Kaneshiro, 1983). Since its detection in the southern states of the USA (Bolda, 2008) and in Spain (Calabria et al., 2012) in 2008, *D. suzukii* has been spreading northwards and was reported for the first time in the UK in 2012 (Harris and Shaw, 2014).

Economic damage

D. suzukii can cause severe damage to commercial soft fruit crops when a female fly oviposits through the skin (exocarp) of ripening fruit, leaving a puncture wound. Even if no subsequent larval feeding takes place this wound allows fungal infection to take place, thus degrading the fruit and rendering it unsalable. In cases where larval feeding occurs in the flesh (mesocarp), the fruit often collapses entirely, also leaving that fruit unmarketable. Where *D. suzukii* has established, substantial (up to 80%) crop loss has been reported on a variety of soft skinned fruit crops (Walsh et al., 2011).

Control

Despite some success developing control programmes (Beers et al., 2011, Cuthbertson et al., 2014, Van Timmeren and Isaacs, 2013) effective control of this pest has yet to be universally achieved in practice. This is in part due to the biology of the organism: a short generation time, wide host range and cryptic feeding stages in close-to-harvest fruit combine to hinder conventional control.

Most current control strategies currently include a combination of high volume, short persistence, pesticide spray programmes and attract-and-kill bait traps. These are suboptimal techniques due to high material costs, a substantial labour investment and the negative impacts associated with such spray regimes. High volume spray programmes run the risk of driving the rapid development of insecticide resistance in target and non-target pests, whilst also having a negative impact on already established integrated pest management (IPM) programmes.

IPM compatible solutions for *D. suzukii* infestation are emerging. Cultural control, in the form of crop hygiene, currently plays a large part in the control of *D. suzukii*. Collecting, neutralising and disposing of fruit waste correctly, although time consuming, has proven effective and is an important part of control recommendations disseminated to growers (ADHB, 2015). Trapping has also formed a key component of many *D. suzukii* control programmes to date, with various trap types and baits commercially available and a range of placement strategies proven to be effective (Lee et al., 2012). Trapping is generally environmentally benign and compatible with existing IPM programmes. Placement of traps does, however, pose a large investment in labour time for growers.

Studies into the biological control of *D. suzukii* using invertebrate natural enemies have given mixed results. Several studies have shown a resistance of *D .suzukii* to attack by European parasitoid wasps (Chabert et al., 2012, Kacsoh and Schlenke, 2012, Poyet et al., 2013), whilst others report the spontaneous parasitism of *D. suzukii* in the field and laboratory (Gabarra et al., 2014, Stacconi et al., 2013). Kacsoh and Schlenke (2012) and Poyet et al. (2013) report an association between resistance in *D. suzukii* to parasitoid attack and high haemocyte load in infected individuals. Potential invertebrate predators of *D. suzukii*, all belonging to the taxon Heteroptera, have been identified in lab studies (Malagnini et al., 2014, Cuthbertson et al., 2014) and in field surveys (Arnó et al., 2012), however, no effective strategy for their implementation has yet been reported.

The susceptibility of *D. suzukii* to microbial biological control agents has been tested in a number of studies with varying degrees of success. The susceptibility of *D. suzukii* to entomopathogenic fungi has been demonstrated in the lab for the pathogens: *Bauvaria bassiana, Lecanicillium muscarium* (Cuthbertson et al., 2014) and *Metarhizium brunneum* (strain EAMa 01/58-Su)(Fernández-Bravo, 2014).

The viruses of *D. suzukii* offer an interesting potential source for a microbial biological control agent. Similarly to microbial biological control agents: viruses potentially represent an environmentally benign control agent with high host specificity and low environmental persistence (Hunter-Fujita et al., 1998), making them eminently suitable for inclusion into existing IPM programmes. Although some hurdles exist in the commercialisation of insect viruses as control agents (Carter, 1984), the improvement of culturing technologies and the rationalisation of restrictive regulations may, in time, alleviate some of the current difficulties (Sun and Peng, 2007).

Characteristics of viral biological control agents

Entomopathogenic viruses are represented in many of the known virus families, with some families of virus known to occur solely in arthropods (Hunter-Fujita et al., 1998). Commercial success as a plant protection product has, however, been achieved only by a small selection of viruses. The two most notable both belonging to the family Baculoviridae. The family Baculoviridae consists of 600 described species in two genera: the nuclear polyhedrosis viruses (NPV's) and the granulosis viruses (GV's)(van Regenmortel et al., 2000). Different species of baculovirus have been isolated from many different insect orders (Hunter-Fujita et al., 1998) but their deployment as biopesticides has mainly been against Lepidopteran pests (for review see Moscardi (1999)).

Other viruses endorsed and tested for the control of insect pests belong to two other virus families: the Nudiviridae and the Parvoviridae. Oryctes nudivirus is a non-occluded dsDNA virus that was first described as *Rhabdionvirus oryctes* (Huger 1966). It was later defined as Oryctes virus and placed in a subgroup of the Baculoviridae by the International Committee on Taxonomy of Viruses (ICTV) before being incorporated into the Nudiviridae and designated as *Oryctes rhinoceros* nudivirus (OrNV) (Wang et al. 2007). This virus was introduced into Samoa in 1963, and later to other Pacific Ocean islands, to control the Coleopteran pest of cultivated Palms: *Oryctes rhinoceros.* The virus is lethal to larvae and causes feeding cessation in adults and consequently led to huge declines in pest population over the course of 1-3 years. A reduction in crop damage accompanied the reduction in population. Re-application in areas of pest resurgence has proved effective. However, after 40 years a breakdown in control in certain locations is being reported by researchers (Jackson, 2009, Huger, 2005). The virus has been studied extensively in India where successful control of *O. rhinoceros* has also been achieved (Mohan and Pillai, 1993, Gopal et al., 2001). Closely related nudiviruses have recently been discovered in *Drosophila* spp. (Unckless, 2011, Webster et al., 2015). A genus of the virus family Parvoviridae, the densoviruses or densonucleosis viruses (DNV's) are another group of viruses with potential use as viral insecticides. These single stranded DNA viruses were first discovered infecting the greater wax moth *Galleria mellonella* by Meynadier et al. (1964). Since that point they have been subsequently isolated from a range of insect taxa (see Maramorosch (2012)). No publications report their isolation from *Drosophila* spp., however, evidence of their presence has been detected in *Drosophila* transcriptome datasets (Obbard, *pers. comm.*). They have been advocated for the control of mosquitoes (Carlson et al., 2006, Ledermann et al., 2004) and cockroaches (Jiang et al., 2008) although field studies into their application are yet to be published.

Drosophila virus diversity

Considering its status as a model organism the full diversity of viruses infecting *D. melanogaster* has only recently been explored (Webster et al., 2015) and studies examining the viruses of other members of the genus remain relatively rare. Prior to wide scale metagenomic viral discovery only 11 viruses were known in *D. melanogaster* (Brun and Plus, 1980) with only five of these isolated, sequenced and available for experimental study: *Drosophila melanogaster* sigma virus (DmelSV), *Drosophila* C virus (DCV), *Drosophila* A virus (DAV), *Drosophila* Nora Virus and *Drosophila* X virus (DXV).

Few studies have focused on the diversity of viruses in wild *Drosophila* populations. Recently, however, the development of metagenomic techniques has facilitated a new approach to viral

discovery and has expanded our knowledge of insect virus diversity immensely (Liu et al., 2011). Webster et al. (2015) used next generation sequencing technology to identify more than 20 previously undescribed RNA and DNA viruses associated with *D. melanogaster*. Their survey of over 2,000 individual wild flies showed 30% of flies to carry at least one virus and 6% of flies to carry multiple viruses. This study also involved the analysis of publically available RNA-seq datasets to estimate viral prevalence in laboratory stocks.

Very little is known about the viruses of wild populations of the vast majority of *Drosophila* species, with *D. melanogaster* being by far the best studied. A study by Unckless (2011) has, however, identified a DNA nudivirus infecting wild *Drosophila innubila*. This viruses is closely related to the OrNV discussed above for its use as a biological control agent of Coleopteran palm pests. Also closely related to OrNV, a nudivirus of *D. melanogaster* was discovered by Webster et al. (2015). Named Kallithea virus, this virus was found to be relatively common in wild *D. melanogaster* (4.6% prevalence globally) and was shown to be interacting with antiviral immune pathways in its host.

Antiviral immunity in Drosophila

To fight invading pathogens, insects rely solely on an innate immune response, as opposed to the familiar, adaptive, immune response found solely in vertebrates.

The most important antiviral system in insects is thought to be that of RNA interference (RNAi). Three RNAi pathways have been identified in *Drosophila*: the small-interfering (si)RNA pathway, the micro (mi)RNA pathway and the PIWI interacting (pi)RNA pathway (reviewed by Kim et al. (2009)). The siRNA pathway is most often associated with the antiviral response in insects. On infection by a virus 'Dicer' proteins in the cytoplasm recognise and bind to viral dsRNA, cleaving it into siRNA fragments and initiating the pathway. These siRNAs are then loaded in to the RNA induced silencing complex (RISC) which guides the slicing enzyme Argonaut to complementary viral RNA sequences which are in turn cleaved, preventing viral replication.

Materials and methods

Specimen collection

To collect adequate numbers of flies for this project, field work with preliminary experiments assessing trapping methods started in August 2014. Small preliminary assessments of the commercial *D. suzukii* trap (Droso-tract, Biobest, Westerlo, Belgium) were conducted at East Malling Research (EMR) in a quarantine facility designed for notifiable crop pests. Small groups of adult flies were placed into mesh insect rearing cages (Bugdorm®, Watkins and Doncaster, Leominster, UK) along with one commercial fly trap. Traps were baited and flies were released in three different combinations to assess trap retentive quality. The results suggested that the most effective method for catching live flies would be the commercial trap (Figure 1) baited with cotton wool balls soaked in liquid *D. suzukii* bait similar in composition to 'Droskidrink' (Grassi et al., 2014). This was the trap set-up used for initial field collections. A further type of trap was constructed and used in subsequent samplings that consisted of a clear lidded bucket, sprayed partially red with entrance holes constructed from Eppendorf tubes (Figure 1).

Collections of flies have been mainly seasonal with peaks in fly catches being in late summer and early autumn. Wild flies were collected in the autumns of 2014 and 2015. All collections undertaken in 2015 occurred at the EMR site in Kent.

Trapping took place at several locations around Kent during the autumn of 2014. Although *D. suzukii* is reported from many different regions of the UK (M. Fountain *pers. comm*.), the first record and potentially the largest populations of *D. suzukii* are established in the South East of England (Harris and Shaw, 2014). Information about the distribution of *D. suzukii* is still difficult to obtain due to the commercial sensitivities of fruit growers. This may change in the coming years as damage becomes more widespread, however, for the purposes of this project the exact locations of farms where *D. suzukii* has been successfully collected are to remain confidential at the request of the respective farm managers. Trapping was attempted at a Scottish fruit farm during October 2014 but no individuals were collected. This is reflected by low catches in the national monitoring scheme in Scotland (A. Doland *pers. comm.*) and may be a result of unfavourable climatic conditions or low starting populations.

Effort has been made to obtain flies collected from outside the UK. A small number of male flies caught in southern France were received from Dr S. Fellous at the Centre for Biological Management of Populations (CBGP) at the French National Institute for Agricultural Research (INRA). Contact has also been established with researchers at the University of Hawaii. Dr Donald Price has offered to collect and send *D. suzukii* specimens from field locations on Hawaii, however, no flies have yet been received and lines of contact need to be re-established.

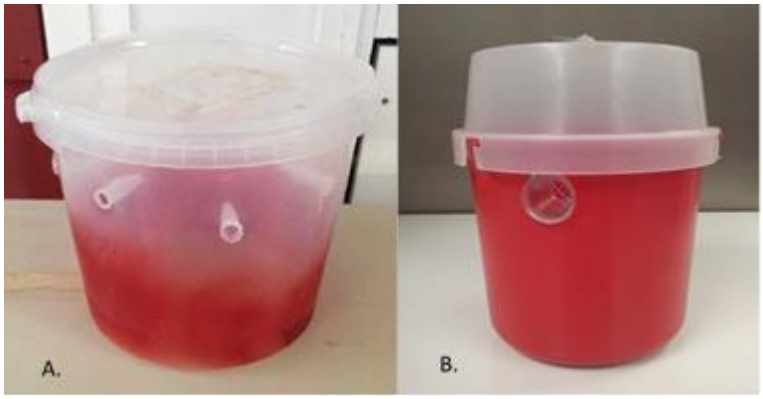

Figure 1. Trap types used for specimen collection: A.) Lab made bucket style trap B.) Commercial trap type, Droso-trap®

Culturing and iso-female lines

An effort has been made, from the outset of this project, to optimise culturing methods for *D. suzukii* in order to provide a steady supply of specimens for experimentation. Advice from collaborators, experience with other species of *Drosophila* and casual experimentation with different media combinations has led to a successful and stable culturing method. All *D. suzukii* are now maintained on Lewis standard medium (Lewis, 1960) with the addition of yeast granules, as protein source, and a folded piece of lab tissue (Kimwipe®, Kimberly-Clark Worldwide, Inc.) as an eclosion site.

Quantifiable egg collection was also achieved in a small proof-of-concept experiment in which adult *D. suzukii* were allowed to oviposit into a hard grape agar layer for 24hrs in a 30cm x 30cm x 50 cm Perspex mating cage. Using this method a known number of *D. suzukii* eggs can be recovered and counted onto another media types, allowing for larval survival assays to be performed.

Iso-female lines of *D. suzukii* were established for use later in the project when flies of a uniform genetic background will be needed to test host-virus interactions. Nine separate lines originating from single females caught in Kent in autumn 2014 are being maintained and expanded. Given time these lines may be of some use to the *D. suzukii* research community at large. Full details of trap location and time of capture for all these lines has been recorded. A useful fly culture was received and stabilised lines from researcher N. Gompel, who provided us with the 'Davis' line used in recent genomic work on the organism (Chiu et al., 2013).

Serial passages

Serial passage technique (Brun and Plus, 1980) is being used to screen large pools of flies for lethal viruses present in wild populations. This constitutes the cyclic homogenisation and injection of infected flies. Wild flies were macerated in typically 0.5ml of sterile ringer's saline (an aqueous solution of sodium, potassium and calcium salts with an approximate pH of 7.3). Half of this suspension was then returned to -80°C storage. The remaining suspension was then diluted by the addition of ringer's saline until a fly-to-ringers concentration of 50µl per fly was reached. This suspension was then passed through a 0.22µm filter (Millex®*) before use in the microinjection assays described below. The 0.22 µm pore size is smaller than almost all known bacteria and larger than almost all known (non-occulated) viruses.

An additional method of virus purification was employed in later passage experiments. This involved repeatedly centrifuging the sample and aimed to sediment bacterial cells whilst maintaining any viruses occluded into large protein bodies, such as those in the baculoviridae (see above), in solution. Samples were centrifuged at 4°C and 4000 x g for 15 minutes, the supernatant removed and centrifuged at 15,000 x g for 5 minutes. The final supernatant, free from bacterial contamination (Vale *pers comms.*) was then taken forward for injection. This method needs to be refined in future experiments in light of further research (Eberle et al., 2012, Hunter-Fujita et al., 1998). Suspensions potentially containing virus were used immediately or returned to storage at -80°C until needed and were stored for no longer than 12 hours.

The control treatment for the first stages of the serial passage process consisted of flies given a sham injection with sterile ringer's solution. Subsequent rounds of the experiment, passages, featured two control groups: the initial control group macerated and filtered in an identical manner to the suspected viral treatment groups and a group injected with fresh sterilised ringers (Figure 2).

Anaesthetised flies were infected via micro-injection (Drummond™, NanoJect®) into the upper half of the ventral abdominal surface, laterally to sternites 1 or 2. A volume of 69.0nl was transferred per injection for all treatments. Flies were transferred to Lewis media (Lewis, 1960) or yeast provisioned hard agar vials and incubated at 24°C for a minimum of one week before the next passage was initiated. Mortality was monitored at regular intervals during this period, with mortality during the first 24hrs after injection being attributed to injury from injection. All experiments consisted of at least four rounds passage.

The *D. suzukii* line 'Davis' (see above) was used for all serial passage experiments, as adequate numbers of individuals were reliably reared in the lab. It also offers a known genetic background being the line of *D. suzukii* that was used in a recent whole genome project (Chiu et al., 2013). Alongside this *D. suzukii* line an immunocompromised *D. melanogaster*, DCR2 knockout (Lee et al., 2004), was also passaged in the same way. This *D. melanogaster* mutant has an essential part of its RNAi antiviral pathway knocked out (see Antiviral Immunity section

above) and was selected because of its resultant susceptibility to viral infection (Zambon et al., 2006). All pooled samples of *D. suzukii* (as described above) were included in at least one full passage experiment. Initially, individual pools of flies were homogenised and passaged through both species lines, however, no significant mortality effects were observed after testing a number of arbitrarily selected pools. To increase the probability of encountering virus a combined homogenate sample was created from all pools of *D. suzukii*.

The volume of homogenate each pooled sample contributed to this combined mix was proportional to the number of flies in the pool. Although pooling samples in this way has the effect of diluting viral titre, the aim of this technique is to identify a highly virulent pathogen and is not a comprehensive survey of all possible viruses.

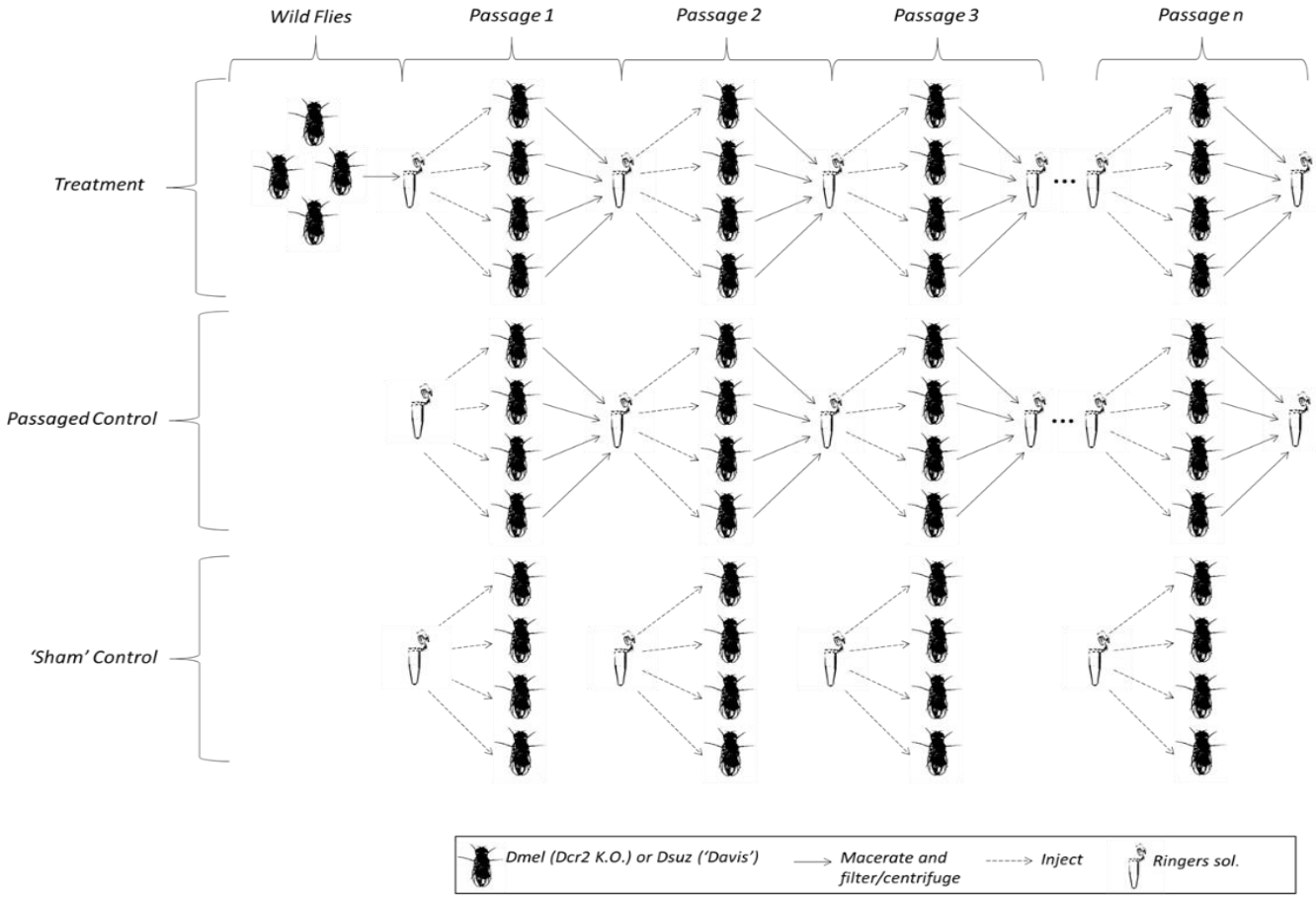

Figure 2. Experimental design of serial passage experiment.

Metagenomic viral discovery

For metagenomic RNA sequencing, RNA was extracted from all individual pooled samples of wild caught *D. suzukii* using Trizol® (Life Technologies) and DNAse treated (Life Technologies). Quality checks were applied to extracted RNA using Qubit® and Nanodrop® appliances as well as running all samples on 1% agarose electrophoresis gels to check for RNA degradation. Aliquots of all wild caught fly samples were mixed into one pooled sample.

The volume that each individual sample contributed to the larger pool was proportional to the quantity of RNA present in that sample, a measure that should be roughly equivalent to the number of individual flies in the original sample pool. RNA-seq was performed by Edinburgh Genomics (Edinburgh) on an Illumina next generation sequencing platform. Ribosome depletion was conducted on the samples using RiboZero™.

Results

In total 849 individual *D. suzukii* have been caught and identified to date, along with equal or greater numbers of each *D. melanogaster* and *D. immigrans*. Identification of these species, along with several other species of British drosophilid, required familiarisation with the defining characters of the group and the relevant identification keys (D'Assis Fonseca, 1965, Bächli et al., 2004). Flies were shipped, alive, in hard agar vials to the University of Edinburgh. All flies caught were grouped into pools of between 3 and 40 flies based on their species, geographic location and habitat in which they were caught. 76 pooled samples (36 of *D. suzukii*) were frozen at -80°C immediately after identification (Appendix 1.).

Table 1. The most common viruses identified by metagenomic sequencing survey in *D. suzukii*. Viral reads here are quantified and normalised by the number of reads for *D. suzukii* CO1 gene. *D. suzukii* (FRA) and *D. melanogaster* (UK) samples predate this project and were collected as part of an ongoing project funded by the Welcome Trust, they are included in this table for comparison only.

		Sample species and location		
Virus	***Closest virus family***	***D. suzukii* (UK)**	***D. suzukii* (FRA)**	***D. melanogaster* (UK)**
Motts Mill virus	Luteoviridae	4935.9	5.2	0.0
La Jolla virus	Iflaviridae	605.0	0.0	107.2
Ngewotan virus	Negeviridae	94.7	3.7	0.0
Galbut virus	Unknown	11.1	0.0	489.6
Bloomfield virus	Reoviridae	7.1	0.0	14.6
Chaq_virus	Unknown	5.8	0.0	520.4
Carmovirus	Carminoviridae	2.3	0.0	0.1
Ixodes scapularis associated virus 2	Unknown	0.5	0.0	0.0

Treatment mortality was observed in immunocompromised *D. melanogaster* inoculated with this mixture when either filtered or centrifuged after two passages (Figure 3). Increased mortality was also observed in inoculated *D. suzukii,* although higher mortality was recorded in flies injected with centrifuged homogenate than in those injected with filtered homogenate (Figure 3). Data is newly generated and has not been yet analysed in the appropriate manner. For analysis of this survival data a Cox's proportional hazards mixed effect model will be employed.

Metagenomic data showed a selection of RNA viruses present in 2014 D. suzukii samples. The number of viral reads was normalized by the number of reads that mapped to the CO1 gene of the fly in which the viruses were identified, giving a rough quantitative measure of viral prevalence (see Table 1).

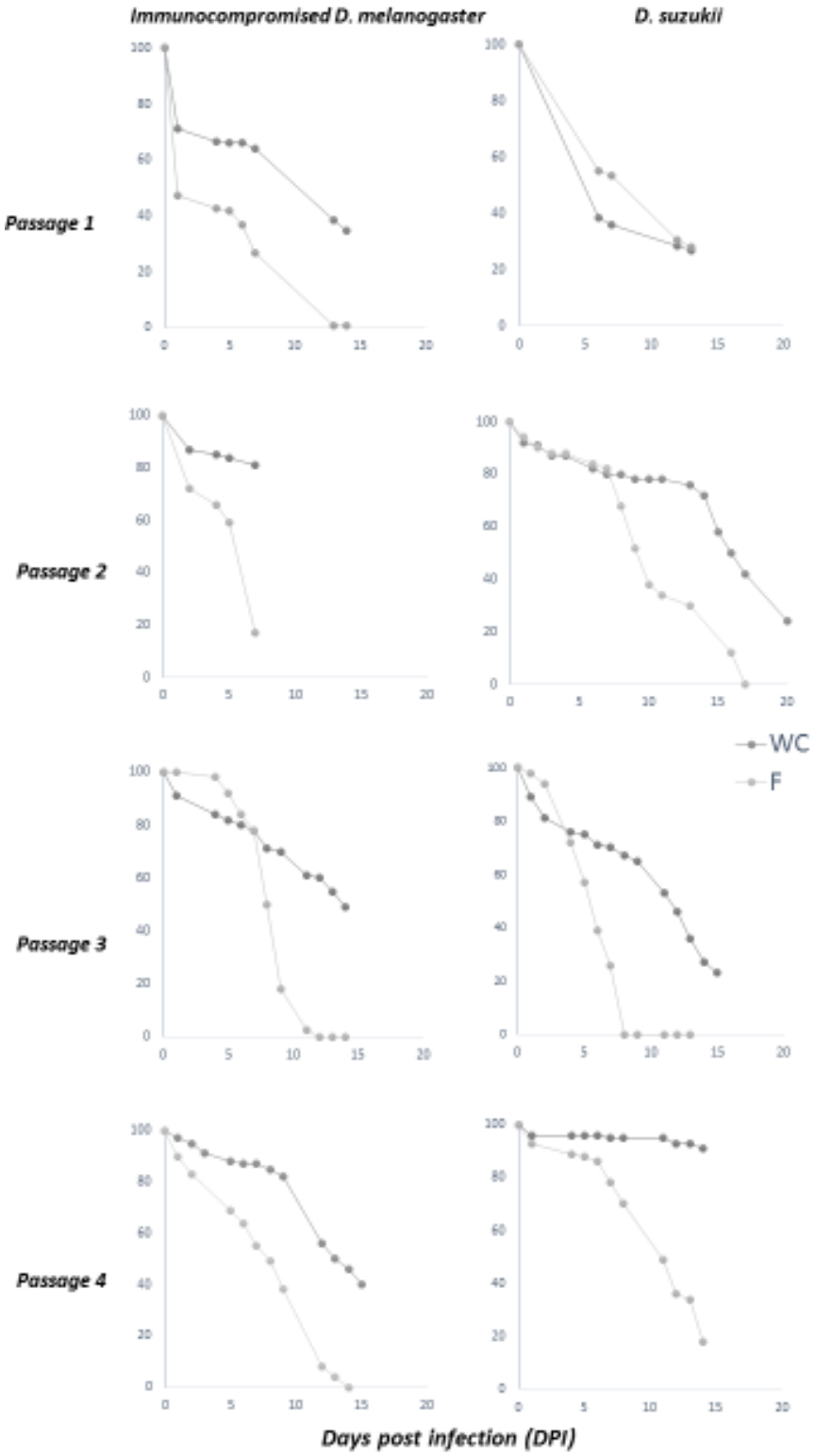

Figure 3. Survival of flies during serial passage. WC= weekly or sham control with filtered ringers sol., F= wild fly treatment of large pool of 2014 *D. suzukii* passed through 0.22µm filter

[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.]

Discussion

The two large collections of wild flies now made have provided an interesting first insight into the viral diversity of this pest species. With a second round of RNA extraction now completed and sequencing underway more data will soon be available for analysis. The optimisation of the bioinformatic techniques used to identify viruses from sequence data is continuous and reliability of this method is ever increasing. Known *D. melanogaster* viruses identified in the first sample of wild flies have been identified in particular wild fly pools by RT PCR and now primers for newly discovered viruses are being designed.

Serial passage experiments suggest the presence of a lethal virus in large pools of wild flies. Flies that died in these experiments were immediately stored at -80°C in order to preserve their viruses. We are in the process of extracting RNA from these individuals in order to identify the virus/viruses responsible for this increase in mortality. Once virus is confirmed in samples, isolation of viruses can begin. The process of virus isolation has been attempted for the Nudivirus of *D. melanogaster*, Kallithea Virus, with some success. Not only could Nudivirus itself represent an interesting prospect for future work in *Drosophila suzukii*, the techniques used will be applicable to other viruses of interest.

Conclusions

- Large numbers of wild caught *Drosophila suzukii* have been successfully sampled and their viruses surveyed metagenomically.
- Some of the Viruses found in *D. suzukii* are very similar to those found in the closely related *Drosophila melanogaster*. Other viruses appear to be new to science and possible unique to *D. suzukii*.
- When an extract consisting of wild flies, crushed and filtered through a filter specific for viruses, is injected into lab reared flies, increased mortality is seen in those flies. The lethality of this extract increases as this process is repeated in a serial passage experiment, suggesting the presence of lethal viruses in wild fly extracts.
- Assays confirming virus presence by PCR have identified particular viruses to particular samples from known trap locations and dates.
- Survey work continues with the metagenomic sequencing of further large pooled samples of flies and the design of novel PCR primers for new and interesting viruses.
- Virus isolation protocols are being tested with the DNA Nudivirus of *D. melanogaster*, Kallithea Virus.

Knowledge and Technology Transfer

None to date

Glossary

DNA virus: A virus in which genomic sequence is made up of DNA (Deoxyribonucleic acid).

Metagenomics: A method for sequencing all genetic material present in an environmental or whole-organism sample. Results in the identification of all species present in that sample.

PCR: Polymerase Chain Reaction. A molecular method used to amplify particular segments of DNA.

Primers: a short sequence of DNA used during a PCR reaction to amplify a particular piece of target DNA.

RNA virus: A virus in which genomic sequence is made up of RNA (Ribonucleic acid).

RT-PCR: Reverse Transcriptase PCR (see above). During the RT reaction RNA is transcribed into a complimentary DNA which can be taken forward into a conventional PCR protocol.

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Appendix

Virus	Length	Sdef	Dtri	Dsuz_Natl	Dsuz_Sim	Dsus	Dsub	Dobs	Dmel_UK	MIX_NonUK	Dimm_RZ	Dimm_DS	Tbrom
MultiFly_089_len3037-Motts_Mill_virus	3037	0.00	0.00	3109.84	1.11	0.00	0.02	0.00	0.03	0.07	0.00	0.00	0.00
MultiFly_188_len1644-Motts_Mill_virus	1637	0.00	0.00	1826.09	4.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dsuz_COI_1504nt	1502	3.01	0.00	622.74	627.17	0.00	0.00	0.00	73.39	126.34	0.39	0.08	1.08
MultiFly_020_len10340-La_Jolla_virus	10309	0.00	0.01	582.67	0.02	0.05	109.06	10.43	97.51	2453.98	2.59	5.81	0.00
MultiFly_016_len11667-Ngewotan_virus	11665	0.00	0.00	94.69	3.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MultiFly_021_len10316-La_Jolla_virus	10298	0.00	0.00	22.37	0.00	0.00	6.73	0.85	9.67	739.48	0.64	1.63	0.00
Dsub_COI_1504nt	1502	2.77	0.01	21.56	18.44	0.36	650.74	2.13	0.03	20.32	0.00	0.00	0.93
MultiFly_302_len944-Lymantria_dispar_multiple_nucleopolyhedrovirus--Alphabaculovirus	940	0.01	0.00	14.94	34.52	0.00	0.01	0.00	1.04	0.63	0.00	0.08	0.00
MultiFly_400_len570-Acanthocystis_turfacea_Chlorella_virus_1--Chlorovirus	570	0.00	0.00	13.07	0.84	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00
Dsus_COI_1504nt	1502	0.48	0.08	11.64	3.95	665.33	0.53	0.07	0.03	25.06	0.00	0.00	0.26
MultiFly_207_len1513-Galbut_virus	1514	0.00	0.00	6.10	0.00	0.00	0.00	0.00	231.07	62.92	0.66	2.64	0.00
MultiFly_204_len1529-Chaq_virus	1525	0.00	0.00	5.80	0.00	0.00	0.00	0.00	520.38	144.95	0.38	1.79	0.00
MultiFly_124_len2230-Galbut_virus	2231	0.00	0.00	5.01	0.00	0.00	0.00	0.00	258.57	150.35	0.97	2.34	0.00
Dimm_COI_1504nt	1502	2.13	0.00	3.23	1.83	0.01	0.02	0.01	1.80	87.91	664.80	665.12	7.35
DmelORE_COI_1504nt	1502	3.05	0.00	3.17	3.31	0.00	0.00	0.01	590.41	334.28	0.59	0.58	6.31
MultiFly_058_len3959-Melon_necrotic_spot_virus--Carmovirus	3958	0.01	0.00	2.31	0.00	0.00	2.55	0.64	0.08	0.10	0.03	0.30	0.00
MultiFly_008_len12510-Drosophila_immigrans_sigmavirus--Sigmavirus	12503	0.00	0.00	2.05	0.00	0.00	0.00	0.00	0.12	0.00	287.17	728.94	0.00
Dtri_COI_1504nt	1502	1.38	665.59	1.77	3.24	0.02	14.47	0.36	0.05	23.49	0.00	0.00	1.94
MultiFly_047_len4741-Kallithea_virus	4743	0.14	0.31	1.40	1.14	0.44	1.11	0.46	2.29	8.62	0.00	0.02	0.00
MultiFly_384_len596-Bloomfield_virus	598	0.00	0.00	1.15	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00
MultiFly_117_len2378-Bloomfield_virus	2368	0.00	0.00	1.01	0.00	0.00	0.00	0.00	2.12	0.12	0.00	0.00	0.00
MultiFly_274_len1168-Bloomfield_virus	1169	0.00	0.00	1.00	0.00	0.00	0.00	0.00	3.87	0.03	0.00	0.00	0.00
MultiFly_055_len4189-Bloomfield_virus	4189	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.12	0.00	0.00	0.00	0.00
Dobs_COI_1504nt	1502	0.30	0.09	0.92	0.18	0.05	0.03	663.17	0.02	23.94	0.00	0.00	0.11
MultiFly_091_len3005-Bloomfield_virus	2993	0.00	0.00	0.91	0.00	0.00	0.00	0.00	1.89	0.02	0.00	0.00	0.00
MultiFly_162_len1844-Bloomfield_virus	1855	0.00	0.00	0.72	0.00	0.00	0.00	0.00	3.21	0.03	0.00	0.00	0.00
MultiFly_059_len3955-Bloomfield_virus	3917	0.00	0.00	0.66	0.00	0.00	0.00	0.00	2.04	0.02	0.00	0.00	0.00
MultiFly_085_len3099-Bloomfield_virus	3101	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00
MultiFly_044_len4829-Kallithea_virus_note_fragment_4	4831	0.13	0.96	0.57	0.31	0.91	0.88	1.00	0.65	7.08	0.02	0.16	0.00
MultiFly_107_len2633-Ixodes_scapularis_associated_virus_2	2634	0.00	0.00	0.48	0.00	0.02	0.00	0.03	0.00	0.00	0.00	0.00	0.00