SCIENTIFIC NOTE

FRUIT FLY-SPECIFIC VIRUSES IN BIOLOGICAL CONTROL: DISCOVERY AND POTENTIAL APPLICATIONS IN FRUIT FLY MANAGEMENT

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Summary
Many insects carry insect-specific viruses (ISVs) that are detrimental to host fitness and health. While some ISVs result in acute infections with clear signs of pathology, others occur as persistent covert infections without any obvious visible symptoms. Widespread uptake of next generation sequencing to study transcriptomes of diverse insect species has uncovered many hidden single and double-stranded RNA viruses that so far had been difficult to identify by conventional virus discovery protocols. Recent research has shown that tephritid fruit flies, including Australia's most significant fruit fly pests, have diverse fruit fly-specific RNA viruses that occur at very high prevalence in laboratory populations, yet at moderate to low prevalence in the field. This highlights the issue that fruit fly traits assessed in laboratory populations may be influenced by virus infections, and may not be fully representative of traits in field flies that do not have these viruses. Studies have shown that the mode of transmission of the fruit-fly specific viruses is either vertical from infected parents to offspring via the embryo, or horizontal by sharing of food and other resources that have been contaminated by infected flies. Some of these ISVs show significant host fitness effects such as reduced lifespan and irreversible paralysis when flies are exposed to high concentrations of CO_2. Therefore, these viruses have the potential to affect management strategies that involve mass-reared flies such as the sterile insect technique (SIT), or have the potential to be used as biological control agent in fruit fly control.

Keywords: Biological control, sterile insect technique, host effects, insect-specific viruses, RNA viruses, virus transmission.

Several fruit fly species (Diptera: Tephritidae) are highly invasive pests of economic significance that affect the production and global trade of many fruits and fruiting vegetables. Among these, the Queensland fruit fly, *Bactrocera tryoni*, is the most significant pest affecting horticultural crops in Australia. This species can infest nearly all cultivated fruits and many vegetable crops across eastern Australia (Sharpe et al. 2021; Morrow et al. 2023). The control of fruit fly pests includes an array of different management strategies such as surveillance, orchard hygiene, cover sprays, attract-and-kill strategies involving lures and protein baits, the sterile insect technique (SIT) and biological control. Concerns about non-target effects, including on human and environmental health, have led to the restriction and ban of many chemical insecticides, and increased the interest in alternative control measures, and in particular, biological control options such as the use of parasitoid wasps, entomopathogenic fungi and entomopathogenic nematodes. Besides these natural enemies of fruit fly that can be biological control agents, viruses may also play a role, yet the potential of viruses in biological control of fruit fly pests has not yet been widely investigated (Sharpe et al. 2021; Morrow et al. 2023).

Virus infections pose a potential threat to many insect species, and can result in a significant decline in insect populations. Understanding the viruses associated with insects and their host effects is crucially important for several reasons: a) it helps in safeguarding beneficial insects like honey bee (*Apis mellifera*) and silk moth (*Bombyx mori*) from viral infections; b) it enables the utilization of insect-specific viruses (ISVs) for the management of insect pests, including invasive species either by application of viruses as biological control agents or using the viruses to express particular proteins or affect host gene expression; c) it aids in the identification of microbial interactions that may affect vectorial capacity of insect species that transmit pathogens to humans, animals and plants, and d) apart from their role as pathogens, viruses can also establish symbiotic relationships that are beneficial to their hosts, e.g. symbiotic viruses associated with parasitoid wasps can help parasitoids overcome host defence.

Many ISVs are double or single-stranded, negative or positive sense RNA viruses. Some ISVs can be highly pathogenic, exhibiting noticeable infections with acute symptoms. Other ISVs induce infections without visible symptoms and, hence, remain covert; yet they can persistently occur and affect host fitness (Hernández-Pelegrín et al. 2022). ISVs have a strict preference for specific insect tissues and are adapted to specifically infect host species. Yet for many newly discovered ISVs there is a lack of comprehensive epidemiological information about their distribution, prevalence, transmission mode and pathology in field populations.

Recent advancements in next-generation sequencing (NGS) technologies, and their application in

transcriptome research, have unveiled a large diversity of RNA viruses in insects, including in tephritid fruit flies (Sharpe et al. 2021). Comprehensive analysis of nine transcriptome libraries of tephritid species from Australia (six species of *Bactrocera* and *Zeugodacus cucumis*), *Bactrocera dorsalis* from Asia and *Ceratitis capitata* from Europe revealed a large diversity of RNA viruses belonging to eight families (Sharpe et al. 2021). Although the detected viruses do not show obvious visible symptoms in flies, and very little information is known about their biology, the outcome of their infections may still influence fly fitness and thereby bring potential risk in mass-rearing and SIT applications. Furthermore, given that viruses were found highly prevalent in laboratory populations, and less prevalent in field-collected individuals, any biological traits of flies measured in infected laboratory populations may not be representative of these traits in field populations.

ISVs can be transmitted either vertically or horizontally, or a mixture of both (Bézier et al. 2009). Vertical transmission of the ISVs in fruit flies occurs either maternally from infected females to their offspring through their eggs, or paternally via sperm (Morrow et al. 2023). Horizontal transmission of ISVs in fruit flies can occur when flies share resources that had been contaminated by infected flies (Morrow et al. 2023). Field and laboratory populations of *B. tryoni* are commonly associated with three single-stranded RNA viruses only found in *B. tryoni* and closely related tephritid fruit fly species: cripavirus (*Dicistroviridae*) and iflavirus (*Iflaviridae*), both of the Picornavirales, and sigmavirus (*Rhabdoviridae*) of the Mononegavirales (Sharpe et al. 2021). In *B. tryoni* cripavirus has very efficient horizontal transmission, whereas iflavirus has very high levels of maternal transmission, together with very low levels of horizontal transmission (Morrow et al. 2023). Based on research on other fruit fly species it is known that sigmavirus is biparentally transmitted, with more effective maternal than paternal transmission (Longdon et al. 2017).

Vertically transmitted pathogens require their hosts to survive and reproduce in order to be transmitted to the offspring; therefore it has been hypothesised that this may lead to lower virulence, yet this means such viruses can still have substantial fitness effects. Only a few ISVs have been observed to have lethal or sublethal effects in their hosts. For instance, *Drosophila* flies infected with sigmavirus consistently exhibit reduced fitness. Furthermore, exposure of such infected flies to very high concentrations of carbon dioxide (CO_2) trigger irreversible paralysis that results in death (Longdon et al. 2017). Ceratitis capitata nora virus (CcaNV) is associated with a potential physiological cost for its host, the Mediterranean fruit fly (*Ceratitis capitata*); a higher viral load of this virus was found linked to a shorter adult lifespan, while no correlation was found between viral abundance and adult flight ability or mating behaviour (Llopis-Giménez et al. 2017). In fruit fly mass-rearing facilities, covert viral infections can directly impact fly physiology, potentially leading to negative effects on mass production and/or altering the competitiveness of males released into the field for SIT applications. Changes in insect rearing conditions could also cause covert infections to negatively affect insect colonies. Since SIT requires the mass-rearing of healthy insects, this can be jeopardised by ISVs. Conversely, due to their potential impact on fly fitness, ISVs hold significant potential for use as biological control agents when transmitted to naïve flies. Furthermore, ISVs of fruit flies have been demonstrated to affect the outcome of parasitisation with parasitoid wasps (Hernández-Pelegrín et al. 2022).

The utilization of NGS for virus discovery has enriched our understanding of the diversity of RNA viruses in fruit flies (Sharpe et al. 2021). While this development provided valuable insights to study the diversity of virus interactions of fruit flies, as well as uncovered some mechanisms by which these viruses interact with the host's immune system (Hernández-Pelegrín et al. 2022), further research is required to better understand the epidemiology of these viruses, in particular their transmission ecology and effects on the host. This additional knowledge will contribute significantly to our understanding of their potential to be used as a biological control agent in insect pest management.

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