Surveying and testing locally occurring insect viruses for use in FAW management

Final Technical Report

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Queensland Government

Plant Health AUSTRALIA

Australian Government
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Surveying and testing locally occurring insect viruses for use in Fall Armyworm management

For: Plant Health Australia Ltd.

PHA REF: P533.

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Executive Summary

Fall Armyworm (FAW) has recently invaded Australia and is a major pest of sweetcorn, maize sorghum and other crops. It is resistant to most of the older chemical pesticides and is difficult to manage. Newer chemicals are expensive and only work well with good spray coverage and timing. Furthermore, there is a high risk that FAW will also develop resistance to these limited newer chemicals. In order to manage FAW in a sustainable way, alternative non-chemical management options are required. Endemic virus-based biopesticides have been used for managing other similar Australian pests (e.g. *Helicoverpa armigera*). Nuclear Polyhedrosis Viruses (NPVs) can be highly effective at killing lepidopteran larvae and highly specific (usually only affecting the target insect), therefore preserving beneficial arthropods.

Viruses have previously been found in Australia on related armyworms (other *Spodoptera* species) and more recently, local NPVs have been found in the newly established FAW populations. These previous and more recently found virus samples have been collected and stored by DAF researchers.

In this project we surveyed FAW populations for new endemic virus strains and conducted laboratory bioassays to determine the efficacy of the endemic viruses (including isolates from the historical DAF collection) against FAW, and compared these with an imported commercial NPV biopesticide (Fawligen™). From the seven sites and 645 FAW larvae that were collected from north Queensland, most mortality was from wasp and fly parasitoid insects (22%), unknown and other factors (9%) and a *Metarhizium rileyi* fungi (3%); with only 0.3% (2) dying from virus symptoms. However, other DAF staff have collected another 11 virus affected FAW specimens from other projects and locations across Queensland, that have been assessed in these bioassays. The data and pathogen isolates collected from this project will contribute to other simultaneous or ongoing projects.

For the purpose of a developing a positive control, feeding bioassays were undertaken using the commercial biopesticide NPV product Fawligen™ on cultured second instar FAW larvae. The bioassays demonstrated an LD50 (lethal dose at which 50% mortality occurs) of approximately 0.1 to 1ml/L of Fawligen (equivalent of 10 to 100ml/Ha applied at 100L/Ha). However, we decided to use 1ml/L (equivalent of 100ml/Ha at 100L/Ha, or $3x10^6$ PIBs per ml, or $6x10^3$ PIBs per larvae) of Fawligen for our subsequent positive controls in the bioassays, as it is equivalent to the lower label rate, and produced an estimated lethal dose of approximately 60% (approximate LD60).

From the historic collection we ran bioassays on 28 endemic NPV virus isolates originally isolated from various other similar armyworm hosts (*Spodoptera litura*, *S. mauritia*, and others). We also assayed 13 endemic NPV isolates collected from wild field collected FAW. Each isolate was tested on small second instar FAW larvae, against negative (water/dye) and positive controls (Fawligen™). The experiments were repeated on larger larvae (3^{rd} to 4^{th} Instar) for some virus isolates. For the small larvae bioassays, the endemic viruses showed less efficacy than Fawligen. However, when comparing the endemic viruses against Fawligen for the larger larvae, many of the endemic viruses were comparable or better than the positive control. These include many of the endemic isolates that were recently found in FAW within Australia as well as some isolates from the historic collection from other Spodoptera hosts.

If resources allow, more research should investigate the suitability of the endemic viruses, by replicating and expanding the bioassays of some of the more promising isolates. This may be of particular value if a virus isolate is found that has greater field efficacy against larger size FAW larvae.

Background

Fall Armyworm (FAW) has recently invaded Australia and has swiftly spread across northern Australia and as far south as Victoria. It is a major pest of sweetcorn, maize sorghum and other crops. It is resistant to most of the older chemical pesticides and is proving very difficult to manage resulting in crop failures. Newer chemicals are expensive and only work well with good spray coverage and timing. Furthermore, there is a high risk that FAW will also develop resistance to these limited newer chemicals. In order to manage FAW in a sustainable way, alternative non-chemical management options are also required.

DAF is working with researchers across Australia on Fall Armyworm (FAW) research through a multipronged approach in numerous areas. These include chemical control, chemical resistance management, semiochemicals, monitoring, thresholds, cultural and biological control.

So far, a number of naturally occurring endemic natural enemies have been found that attack FAW; these include generalist predators (e.g. predatory shield bugs and spiders), parasitic wasps and flies (attacking eggs and larvae), pathogenic fungi and viruses. A short-term Hort Innovation project (MT19015, involving DAF, NT and WA Governments) recently surveyed FAW populations for parasitoids and predators across QLD, NT and WA.

A potential biological control option for FAW is the use of Entomopathogens (e.g. fungi and viruses) as a biopesticide. A DAF-led ACIAR funded project (HORT/2018/194) is collecting and isolating endemic entomopathogenic fungi for use against FAW. *Metarhizium rileyi* has been commonly found across Queensland infecting FAW in epizootics. Fungal isolates are being tested for efficacy in laboratory bioassays and small-scale production is being investigated.

Endemic virus-based biopesticides have been used for managing other similar Australian pests (e.g. *Helicoverpa armigera*) and may offer some options for managing FAW. Nuclear Polyhedrosis Viruses (NPVs) can be highly effective at killing lepidopteran larvae and highly specific (usually only affecting the target insect), therefore preserving beneficial arthropods. In Australia, endemic NPV products (e.g. VivusMax™) have been developed and used to suppress *Helicoverpa armigera* populations, resulting in a reduced reliance on chemical controls. Such products have a perfect IPM fit, because they have no side effects on predators, parasitoids and pollinators; no environmental, health or residue issues; and no resistance issues.

Insect viruses are also known to kill FAW. Imported virus based NPV biopesticides have been developed overseas as commercial biopesticides for FAW (e.g. Fawligen™ and Spodovir Plus™), however overall, there is very little efficacy data on these products and they may not work on strains of FAW in Australia. They also may not work well on larger (older) larvae. The field efficacy of these products are being assessed in other projects.

Viruses have previously been found in Australia on related armyworms (other *Spodoptera* species) and DAF has recently completed molecular characterisation on virus isolates from the DAF collection. More recently, local NPV viruses have been found in the newly established FAW populations in Queensland. More endemic virus isolates are likely to be found and these virus isolates will clearly be causing FAW mortality under Australian conditions. These previous and more recently found virus samples have been collected and stored by DAF researchers. It was thought that there was some probability that these local virus strains could prove to be more effective than the imported NPVs.

Commercial-scale production of NPVs is well established, therefore providing that an effective NVP isolate is found (i.e. a product that works in Australian field conditions), there is a high chance of success that an effective FAW product can be delivered to industry in the short-medium term.

This project assessed if the locally found NPVs have efficacy against FAW compared to an imported commercial NPV biopesticide. In this project, we surveyed wild FAW populations for new endemic virus strains and conducted laboratory bioassays to determine the efficacy of the endemic viruses (including isolates from the historical DAF collection) against FAW, and compared these with the imported commercial biopesticide virus strains.

Methodology

Field Collections

We collected FAW from seven different sites, on the Atherton Tablelands (Atherton, Kairi, Walkamin, Mareeba) and Innisfail (coastal). Multiple collections have been made from Kairi and Walkamin. They were collected from maize and sweet corn. Collections consisted of 50-70 larvae each site/time point, and all available sizes were collected if present. Some 645 larvae have been collected. All larvae were individually reared (in individual cells) on artificial diet (General Diet for Lepidoptera, Product #F9772: Soy-wheat Germ Diet with Vitamins & Agar, Frontier Scientific Services USA) at 26 ^{o}C and monitored until moth development or death. Cadavers with virus like symptoms (typically black/brown and liquidised) were removed and stored in sealed tubes at -20 ^{o}C., for later assessment. Fungal pathogens and parasitoid insects were collected, curated and stored for use in other projects.

Historic Collections

DAF maintain a historic collection of insect virus isolates, which contains over 30 viruses isolated from other *Spodoptera*/armyworms including *Spodoptera litura*, *S. Mauritia*, unknown *Spodoptera* species and other Lepidoptera hosts. These virus isolates go back to collections from the 1970s and have been stored at -20 ^{o}C since that time. It was not known if all of these isolates were still viable. This collection was shipped (frozen in dry ice) to Mareeba for the project.

Recent FAW Virus Collections

More recently another 11 virus isolates have been found in wild FAW since its arrival in Australia. These samples have been collected from researchers and others across Queensland. These virus samples were also shipped to Mareeba with the historic virus collection.

Determining Positive Control Rates

Feeding bioassays were undertaken using the commercial biopesticide NPV product Fawligen™ (for the purpose of a positive control) on second instar FAW larvae. The bioassays were untaken on a wide range of rates (doses), ranging from 0.001 to 100 ml/L and 1% blue food dye (Queen™ Classic Blue). The virus was also quantified by counting the virus polyhedral inclusion bodies (PIBs) on a haemocytometer under differential phase contrast compound microscope. Negative controls consisted of water and blue food dye only. 2µl of each suspension was placed onto a 1mm cube of artificial diet for each insect. Each second instar FAW larva was placed in an individual cell with the diet cube (diet described above in 'Field Collections') and allowed 48 hours to consume the food. The blue dye could be seen in the frass, indicating they have consumed the virus. The larva was then placed into a new cell with clean diet and allowed to develop.

Each treatment sample consisted of 16 individual insects. The bioassays were repeated six times, to narrow down the rate range and due to problems with negative control mortality, which caused by asphyxiation (overcome by modifying containers and diet moisture content).

The bioassays demonstrated an LD50 (lethal dose at which 50% mortality occurs) of approximately 0.1 to 1ml/L of Fawligen (equivalent of 10 to 100ml/Ha applied at 100L/Ha) (Figure 1). However, the response was fairly flat, meaning doubling to 2ml/L (equivalent 200ml/Ha) made minimal difference and very large doses 100ml/L (equivalent of 10L/Ha) did not always reach 100% mortality.

We decided to use 1ml/L (equivalent of 100ml/Ha at 100L/Ha, or $3x10^6$ PIBs per ml, or $6x10^3$ PIBs per larvae) of Fawligen for our subsequent positive controls, as it is equivalent to the lower label rate in many cases, and produced an estimated lethal dose of approximately 60% (≈LD60).

Screening Bioassays of Endemic Virus Isolates

Bioassays were completed on endemic virus samples collected from FAW and related hosts collected in Queensland. Initially screening the samples used a high-dose method (described below). This was done to determine if the virus was still active or had any virulence against FAW. From this initial high-dose screening, non-responding virus isolates were culled from the virus isolates that do respond (i.e. the most promising candidates), inoculum was collected from cadavers for further bioassays, if required.

From the historic collection we assayed 28 isolates originally isolated from various other similar armyworm hosts (*Spodoptera litura*, *Spodoptera mauritia*, and others). We also assayed 13 virus isolates collected from wild field collected FAW (as described above). All of the virus samples tested (including host insects where known) and collection details are listed in Appendix 1. The samples were prepared as described similar to above (for the positive control bioassays).

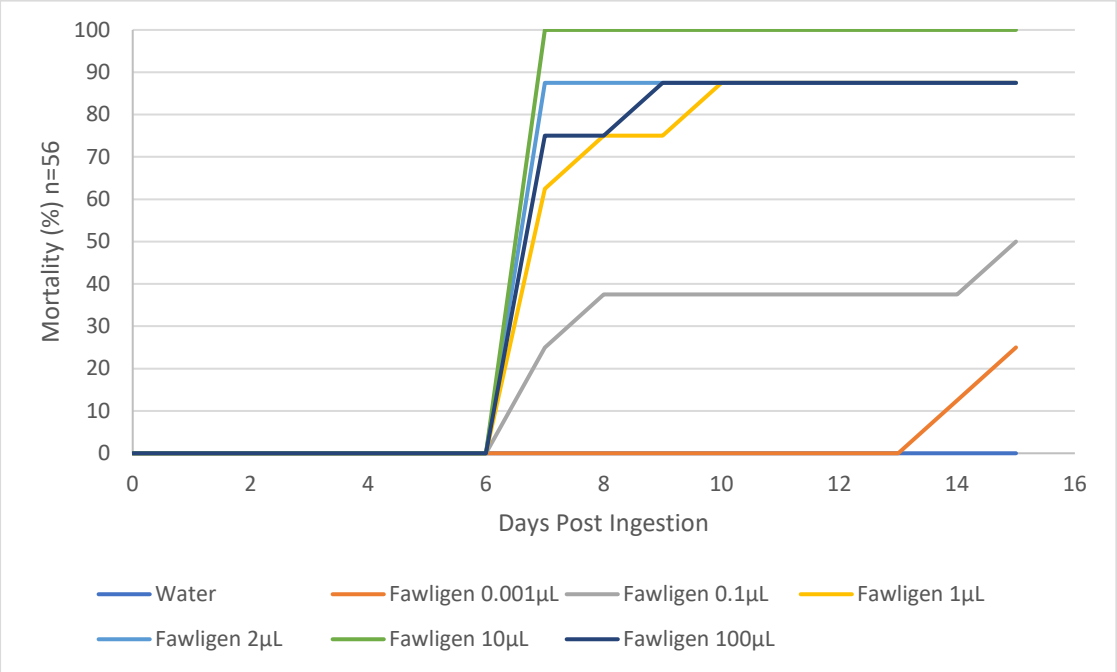

Figure 1. Example one of the Positive control bioassays. Note 1µL (1µl/ml) is the equivalent of 1ml/L and 100ml/Ha applied at a rate of 100L water/Ha.

A small section (approximately 1mm^3) was taken from each frozen insect/virus sample and diluted with 100µl of water and 1% blue food dye and shaken and vortexed to create a suspension and 2µl of the suspension was placed onto a 1mm cube of artificial diet for each insect. Each isolate was tested on 16 individual second instar FAW larvae, against 16 water plus dye negative controls, 16 water only negative controls and 16 positive controls (Fawligen at 1ml/L, plus dye). The experiments were repeated on larger larvae (3rd to 4th Instar) for some virus isolates. The virus treatments were quantified by counting the PIBs on a haemocytometer (as described above). For the purpose of rapid assessments, the treatments were not diluted as the concentrations were often considerable higher than that of the positive control. However, the samples yielding lower rates would need to be passaged through a susceptible host to bulk up inoculum (provided the virus is still even viable). This could not be done within the timeframe of the project, so the lower rates were initially used (PIBs counts provided in results).

Results

Field Collections

From the seven sites and 645 FAW larvae that were collected from north Queensland, approximately 66% of larvae survived and emerged to adults (Table 1). Most mortality was from wasp and fly parasitoid insects (22%), unknown and other factors (9%) and *Metarhizium rileyi* fungi (3%); with only 0.3% (2) dying from virus symptoms. However, other DAF staff have collected another 11 virus affected FAW specimens from other projects and locations across Queensland, that have been assessed (below). The data and pathogen isolates collected from this project will contribute to other simultaneous or ongoing projects in entomopathogenic fungi (ACIAR project HORT/2018/194) and parasitoid surveys (Hort Innovation project MT19015).

Table 1. Mortality from field collections of FAW from north Queensland.

Location	Larvae Collected	Mortality by Parasitoids (%)	Mortality by Fungi (%)	Mortality by Virus (%)	Mortality by Other Factors (%)	Emerged Adults (%)
Walkamin Research Station	190	26.8	3.16	1.05	14.2	54.7
Lockwood Road	78	43.6	2.56	0	7.69	46.1
Kairi Research Station	159	12.6	1.89	0	5.66	79.9
Koci Road	50	6	6	0	2	86
Gallo Road	53	5.66	1.89	0	15.1	77.3
Boogan Road	51	18	0	0	4	78
McIllrath Road	65	36.9	3.07	0	9.23	50.7
TOTAL	**645**	**22.3**	**2.63**	**0.31**	**9.15**	**65.6**

Screening Bioassays of Endemic Virus Isolates

From the historic collection, none of the isolates performed better than the positive control (Fawligen) in second instar larvae, with most producing mortality rates of less than half of Fawligen (see Table 2 and example below, Figure 2). Some isolates initially looked promising, but due to high mortality in the negative controls, need to be repeated, and were no better than the positive control (Fawligen). Unfortunately, negative control mortality was high and variable throughout the bioassays. We are uncertain as to what was causing control mortality.

In most of the second instar assays the positive control (Fawligen) produced 50 -94% mortality. Whereas the endemic virus strains usually produced less than 50% mortality (Table 2, Figure 2). Some of the virus strains did produce mortality of 62-81%. However, the positive control mortality was higher 87-94% in these particular bioasssays. The higher mortality was probably caused by other factors as negative control mortality was high (>50%, not shown) in these bioassays. Unfortunately, problems with FAW culture mortality and limited time did not allow these assays to be repeated. From the more recent endemic virus isolates collected from wild field collected FAW, mortality has also been less than that of the positive controls, ranging from about 6 to 56% compared to 50-94% mortality for the positive controls. It should be noted with some caution that the results from this rapid analysis are not statistically significant as it wasn't replicated. However, this preliminary analysis pointed to some isolates that should be investigated further. These include: FAWNPV1, FAWNPV2, FAWNPV4, FAWNPV5, FAWNPV9 (all from FAW in Australia), E8-1 (*Spodoptera litura*) F42 (*Spodoptera sp*.).

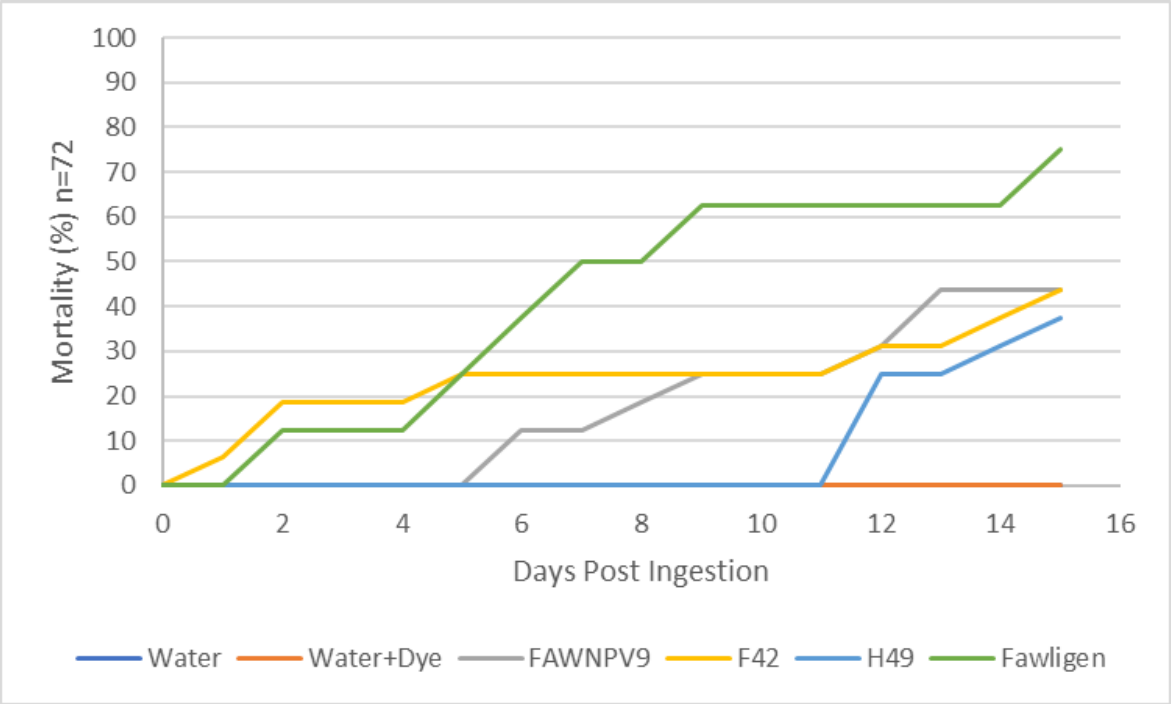

Figure 2. Example of a bioassay, with most endemic virus isolates producing approximately about half the mortality of the positive control (Fawligen).

Table 2. Virus Screening Bioassays on 2nd instar larvae

Bioassay Number	Virus Isolate	Mortality Rate of Isolate	Positive Control Mortality (%) at $3x10^3$ PIBs/µL	Estimated Viral Concentration (PIB/µL) (x2 gives PIBs/larva)
1	E8-1	43.75	50	119625
1	L23	0	50	55075
1	T23D [SF]	12.5	50	3625
2	FAWNPV1 [SF]	56.25	75	570
2	FAWNPV3 [SF]	12.5	75	430
2	FAWNPV4 [SF]	31.25	75	3750
3	FAWNPV5 [SF]	50	87.5	2145
3	FAWNPV7 [SF]	31.25	87.5	265
3	FAWNPV8 [SF]	31.25	87.5	545
4	FAWNPV9 [SF]	43.75	75	330
4	F42	43.75	75	82525
4	H49	37.5	75	175000
5	M146	31.25	69	525
5	MON1 [SF]	18.75	69	1015
5	FAW44 [SF]	6.25	69	150
6	FAWNPV2 [SF]	43.75	75	1325
6	FAWNPV6 [SF]	37.5	75	500
6	MON2 [SF]	37.5	75	195
7	E8-1	81.25*	94	163975
7	FAWNPV1	50	94	120
7	FAWNPV4	50	94	4950
8	E11	0	69	37925
8	H102	0	69	9400
8	W72	12.5	69	39825
9	H49/2	0	12.5	16825
9	L23/2	0	12.5	315
9	E8/2	12.5	12.5	230
10	H48/JA1	75*	87.5	65750
10	H49-2/JC4	68.75*	87.5	27300
10	267	62.5*	87.5	12475

SF: Denotes *Spodoptera frugiperda* (Fall Armyworm) was host. All other isolates from other hosts

*****Mortality rates were high for these isolates, however the positive controls (Fawligen) was higher 87-94%. The higher mortality was probably caused by other factors as negative control mortality was high, >50%.

For the bioassays on the larger larvae (3^{rd}-4^{th} Instar) the results were also highly variable. Mortality ranged from zero (0) to 75% for the endemic viral isolates (Table 3). However, the mortality of the positive control (Fawligen) was also highly variable, and also ranged from zero (0) to 75%. Negative control mortality was also variable from zero (0) to 38% (not shown), but was usually below 25%. A number of the endemic virus isolates appeared to be at least as virulent as the positive control. These included E8-1, F42, H49, FAWNPV1, FAWNPV2, FAWNPV4, FAWNPV5, FAWNPV6 and FAWNPV9. However, these assays were not repeated, so are not statistically significant.

Table 3. Bioassays on 3rd and 4th instar larvae

Bioassay Number	Virus Isolate	Mortality Rate (%)	Positive Control Mortality (%) at $3x10^3$ PIBs/µL	Estimated Viral Concentration (PIB/µL) (x2 gives PIBs/larva)
11	E8-1	75	37.5	285
11	FAWNPV1 SF	62.5	37.5	85
11	FAWNPV4 SF	50	37.5	4850
12	FAWNPV5 SF	62.5	75	3650
12	FAWNPV9 SF	75	75	85
12	F42	75	75	10025
13	FAWNPV6 SF	75	37.5	1600
13	FAWNPV2 SF	50	37.5	415
13	H49	56.25	37.5	28750
14	N209	12.5	25	80875
14	H100	6.25	25	11375
14	M198	25	25	255
15	Z18	0	0	7300
15	M146	12.5	0	160
15	M198	12.5	0	14000
16	267	0	12.5	3100
16	W72	0	12.5	15650
16	H102/M32	0	12.5	22450
17	E8	0	12.5	765
17	E11	0	12.5	16650
17	M146	0	12.5	160
17	UNKNOWN1	12.5	12.5	205
17	UNKNOWN2	37.5	12.5	2850

Discussion

For the small larvae (2nd instar) bioassays, the endemic viruses showed less efficacy than the positive control (Fawligen). However, some caution must be taken with these results as they were largely single replications and therefore are not statistically significant. Furthermore, there was considerable variability in the positive and negative controls. For more certainty, and to reduce the variability, these bioassays should be repeated a number of times. However, due to the number of isolates, the limitations of the FAW culture at the time, and a limited time frame, more replication was not possible. Some of the endemic virus isolates also had low amounts of viral inoculum (low PIB counts), often due to size of the host and condition of the material.

The rapid assessment used in this study was a reasonably preliminary pilot study method and did indicate that some isolates may have some efficacy and should be investigated further. These include many of the endemic isolates that were recently found in FAW within Australia (FAWNPV1, FAWNPV2, FAWNPV4, FAWNPV5, FAWNPV9) as well as some isolates from the historic collection from other Spodoptera hosts, being F42 (*Spodoptera sp.*) and E8-1 (*Spodoptera litura*).

Interestingly, isolate E8-1 also demonstrated perhaps one of the greatest efficacies amongst the endemic virus isolates against the larger (3rd /4th Instar) FAW larvae, which was at least as comparable to the positive control (Fawligen). F42 and H49 also showed promise against larger larvae. Again, some of the endemic isolates that were recently found in FAW within Australia (FAWNPV1, FAWNPV2, FAWNPV4, FAWNPV5, FAWNPV6 and FAWNPV9) were promising against larger larvae and were comparable or better than the positive control. As with the other bioassays these results should be interpreted with some caution (for the same reasons). They should also be replicated for greater certainty and to reduce variability.

Recommendations

Although no single endemic virus isolate in this study was proven to be any better than the positive control (Fawligen) against small instar FAW larvae, many isolates show promise and may warrant further investigation. However, when comparing the endemic viruses against Fawligen for larger larvae, many of the endemic viruses were comparable or better than the positive control.

This was a preliminary study across a large number of isolates with little or no replication. Due to limitations and problems with the FAW culture at the time, more replication was not possible within the time frame of the project. If resources allow, more research should investigate the suitability of the endemic viruses, by replicating and expanding the bioassays of some of the more promising isolates. This may be of particular value if a virus isolate is found that has greater field efficacy against larger size FAW larvae.

Appendices

Appendix 1.

List of Virus isolates tested with known host and collection details

Accession no.	Path ID	Other ID no.	Date Collected	Host	Notes	Collection Method	Collector	Location	Location details
1	FAWNPV		5/02/2021	Spodoptera frugiperda				McVeigh, Dalby	Maize
2	FAWNPV		5/02/2021	Spodoptera frugiperda				McVeigh, Dalby	Maize
3	FAWNPV		5/02/2021	Spodoptera frugiperda				Johns, Bowenville	Maize
4	FAWNPV		15/10/2020	Spodoptera frugiperda				Bowen	Sweet Corn
5	FAWNPV		30/11/2020	Spodoptera frugiperda				Ayr	Maize
6	FAWNPV		14/10/2020	Spodoptera frugiperda				Ayr	Maize
7	FAWNPV		1/07/2020	Spodoptera frugiperda				Mackay	Maize
8	FAWNPV		1/03/2020	Spodoptera frugiperda				Mareeba	Maize
9	FAWNPV		12/02/2021	Spodoptera frugiperda				Kalbar	Maize
		H49	23/03/1976	Spodoptera sp	NPV, ex grass, Mareeba				
		E8-1	1/02/1970	S. litura	NPV, cadaver	dead wild insect			
		E11	27/02/1970	S. litura	NPV, Ex E8-1				
		F42	24/02/1971	Spodoptera	NPV, Indooroopilly (probably S litura)				
		H102	5/10/1973	S. litura	NPV				
		W72	23/07/1986	S. litura	NPV, Ex lab culture at Biocontrol				
		L23	11/03/1976	S. mauritia	NPV, Passaged L25				
		H49/2	2017	Spodoptera sp	NPV, Passaged by AgBitech from H49				
		L23/2	2017	S. mauritia	NPV, Passaged by AgBitech from L23				
		E8/2	2017	S. litura	NPV, Passaged by AgBitech from E8				
1		MON	31/08/2020	Spodoptera frugiperda	Sample provided to MMiles Dec 2020. Not constantly frozen, in solution.	dead wild insect	Chris Monsour	Bowen, DAF RS	Sweet corn
2		MON	27/07/2020	Spodoptera frugiperda	Sample provided to MMiles Dec 2020. Not constantly frozen, in solution.	dead wild insect	Chris Monsour	Bowen, DAF RS	Sweet corn

		H48/JA1	22/03/1973	Catopsila pomono pomono	NPV, R.H. Broodley				
		H49-2/JC4	22/03/1973	Spodoptera sp.	NPV, R.H. Broodley, ex grass			Mareeba	
		267	10/05/1989		NPV				
		N209	6/10/1978		NPV				
			30/05/1969	Anthera varia	NPV				
		H100	3/10/1973	Spodoptera litura	Dosed with H32 N polyhedrosis at 6 days				
		M146	20/09/1977		NPV				
			20/01/1970	Anthera varia					
		M198	8/07/1978		NPV				
		Z18	31/01/1989	Hesperiidae, skipper	NPV, ex rice grass, parnara sp.			Ayr	
		FAW44	3/03/2021	S. frugiperda	NPV, ex pacific seed farm			Gatton	
		M146	20/09/1977		NPV				
		M198	30/12/1977		NPV, ex M176				
		267							
		W72			Ex Ab				
		H102/M32	15/03/1977	Spodoptera litura	NPV Passaged				
		E8	3/02/1970	Spodoptera litura	NPV				
		E11	5/03/1970	Spodoptera litura	NPV				
		M146	20/09/1977		NP Virus, passaged M92 Virus				
		T23D	5/08/2021	Spodoptera frugiperda	Possible virus	collected live, died in culture	Charlee MacDonald	Walkamin Research Station, Walkamin, Qld	Maize
	Unknown 1								
	Unknown 2								

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