Hort Innovation

Final Report

Parasitoids for the management of fruit flies in Australia

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Delivery partner:

Victorian Department of Jobs, Precincts and Regions

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Project:

Parasitoids for the management of fruit flies in Australia (MT19003)

Disclaimer:

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Contents

Public summary

Whilst biocontrol has been recognised as a core component of fruit fly management programs around the world, this has yet to be the case in Australia. This project conducted essential studies that will form the backbone of a parasitoid biocontrol program for Australian pest fruit flies. The project focused on improving knowledge of parasitoid distribution in Australia, developing new protocols for mass rearing releases of fruit flies using parasitoids, and conducting trial parasitoid releases in fruit orchards and urban/peri-urban sites across Victoria.

New information on the distribution of fruit fly parasitoids in Australia was delivered through a comprehensive data mining exercise of both published and unpublished data. This information was presented in a scientific publication paper that also comprehensively reviewed and updated what is known about Australian fruit fly infesting parasitoids, paving the way for a new generation of Australian fruit fly parasitoid research. Fruit fly populations in Victoria have increased significantly over the last decade, and yet fruit fly parasitoids had not yet been identified in the state at the beginning of this project. We conducted additional survey work, collecting fruit fly infected fruits across the state, and discovered the first record of a tephritid fruit fly parasitoid in Victoria (*Diachasmimorpha kraussii*), providing evidence that this insect may be beginning to establish in the state; but as its rate of establishment may be slow, fast-tracking and bolstering this process would be a very good investment for horticultural industries, as evidenced by a benefit-cost analysis.

Having selected two parasitoid species, *Fopius arisanus* and *Diachasmimorpha kraussii*, as the most suitable candidates for fruit fly biocontrol, wild parasitoids of both species were collected from host plants in Queensland. Protocols to mass-rear these insects were developed, and pilot releases took place across Victoria in two consecutive growing seasons. *Fopius arisanus* cultures were ramped up to 40,000 individuals for the first pilot releases in 2020/21 season, and weekly releases of up to 10,000 *F. arisanus* and 6,000 *D. kraussii* took place in the 2021/22 season. In total, 95,000 parasitoids were released at three orchard and nine urban sites across Victoria in nineteen individual releases over the two growing seasons. The results demonstrated that both species of parasitoids have potential to become established in Victoria, persisting seasonally in the environment, reproducing, and dispersing from release sites. Of the two parasitoids, *D. kraussii* is currently the best candidate for augmentative releases, due to ease of mass rearing, greater establishment, and indications of small populations in NE Victoria. Further research into the persistence of either species between seasons (overwintering) would be fundamental to the application of mass releases of parasitoids as biocontrol agents for fruit fly in Victoria. Research is also required to ascertain whether inundative releases of parasitoids could be used as an on-farm control strategy to manage flies. This component of the project aligned with the DAWE project 4-EKSH327 *A National Biocontrol Program to Manage Pest Fruit Flies in Australia*, which co-funded the release, evaluation work and conducted additional studies.

The research was delivered despite 2020/21 COVID-19 lockdowns restricting travel and attendance at the workplace. An additional field season (2021/22) was utilised to perform parasitoid releases, and webinars and online meetings replaced face-to-face workshops. The use of parasitoids for fruit fly biocontrol is showing considerable promise, particularly for area-wide management of fruit fly in urban towns and cities.

Technical summary

This project conducted the essential studies that will form the backbone of a national parasitoid biocontrol program for Australian pest fruit flies. Specific aims were (i) improving knowledge on parasitoid distribution in Australia, (ii) developing protocols for augmentative biocontrol (mass releases of natural enemies) using the two parasitoid species (*Fopius arisanus* and *Diachasmimorpha kraussii*), and (iii) conducting trial parasitoid releases in fruit orchards and urban/peri-urban sites in Victoria, including evaluation of within-season establishment. The project aligned with DAWE project (4-EKSH327), which extended the scope of this work to enable additional releases, off-target studies, a conservation biology component, and a benefit-cost analysis.

Improving knowledge on parasitoid distribution in Australia. Data mining of a QUT Masters thesis, supplemented by additional records from published literature on Australian fruit fly parasitoids, and unpublished QDAF data, was used to develop new distribution maps for five fruit fly parasitoid species present in Australia that may be suitable as biocontrol agents. The distribution maps have been published in a new paper that also comprehensively reviews and updates what is known about Australian fruit fly infesting parasitoids (Clarke et al., 2022). This output will be an invaluable resource, paving the way for a new generation of Australian fruit fly parasitoid research. The outcomes of this study, together with additional survey work (below) were also used to select the second species of parasitoid, *Diachasmimorpha kraussii*,

which (in addition to *Fopius arisanus*) was mass reared and released through this project. Fruit fly populations in Victoria have increased significantly over the last decade, and yet fruit fly parasitoids had not yet been identified in the state at the beginning of this project. Our examination of parasitoids from the 2017-18 fruit collection survey in Victoria looked at 391 wasp specimens from 23 sites across the state. None were found to be fruit fly parasitoids. An additional survey of 2638 individual pieces of fruit collected from across Victoria (19 different Qfly hosts) identified five *D. kraussii*. This is the first recorded evidence of any tephritid fruit fly parasitoid in Victoria, providing evidence that *D. kraussii* may be beginning to establish in the state. As its rate of establishment may be slow, fast-tracking and bolstering this process would be a very good investment for horticultural industries, as evidenced by a recent benefit-cost analysis (aligned DAWE project 4_EKSH327).

Mass-rearing protocols for *Fopius arisanus* and *Diachasmimorpha kraussii* developed. A literature review was first completed to establish practices used in mass-rearing of fruit fly parasitoids around the world. *Fopius arisanus* and *Diachasmimorpha kraussii* collected from wild hosts in Queensland were brought into culture and a mass-rearing program was successfully developed at Tatura Smartfarm, including the use of specialized rearing equipment to enable large numbers of these two parasitoid species to be mass-reared, and a ramping schedule for seasonal releases. In the initial rearing of wild *F. arisanus*, sexes were separated to allow males to mature before being exposed to females. Experimental work demonstrated that this was no longer required once *F. arisanus* were established in large cultures. *Fopius arisanus* cultures were ramped up to approximately 40,000 individuals before the first pilot releases in February and March 2021; and in the second season, weekly releases of up to 10,000 *F. arisanus* and 6,000 *D. kraussii* were achieved. A technical report detailing mass-rearing protocols has been produced. This will be a useful resource that will guide practices on fruit fly parasitoid mass-rearing for augmentative biocontrol releases.

Augmentative biocontrol (mass-releases) of parasitoid wasps successfully piloted. Ninety-five thousand parasitoids cultured at Tatura SmartFarm were released at three orchard and nine urban sites across Victoria in nineteen individual releases over the 2020/21 and 2021/22 growing seasons. First season releases of *Fopius arisanus* comprised approximately 34,000 adults. Monitoring studies demonstrated mean parasitism rates immediately post-release as high as 58% in a pome fruit orchard. Parasitism rate averaged across all fruit collections was more moderate at 9%, though differences across fruit types should be considered here, as well as the small (inoculative) release size. Parasitoid dispersal was recorded at least 20m from release site within two days of release and *F. arisanus* populations persisted in the environment for at least 10 weeks post-release. Second season releases comprised approximately 43,000 *F. arisanus* as well as 18,000 *Diachasmimorpha kraussii* at two orchard sites and eight regional towns / cities. *F. arisanus* established during the season at two out of the five sites monitored by fruit collection and was found in four fruit varieties. Average parasitism was 9%, with specific collections demonstrating parasitism as high as 32%. *D. kraussii* releases showed particularly promising results, with parasitism recorded at all four sites monitored by fruit collection and an average parasitism rate of 16% across seven fruit varieties. Parasitism was recorded at five- and seven-weeks post-release at two sites (and is ongoing) indicating seasonal establishment, with dispersal of parasitoids identified 200m and 1.5km from release sites. The results demonstrate that both species of parasitoids have potential to become established in Victoria, persisting seasonally in the environment and dispersing from release sites. Of the two parasitoids, *D. kraussii* is currently the best candidate for augmentative releases, due to ease of mass rearing, greater establishment, and indications of small populations in NE Victoria. Further research into the persistence of either species between seasons (overwintering) would be fundamental to the application of mass releases of parasitoids as biocontrol agents for fruit fly in Victoria. Research is also required to ascertain whether inundative releases of parasitoids could be used as an on-farm control strategy to manage flies. Full details are provided in a technical report. This component of the project aligned with the DAWE project 4-EKSH327 *A National Biocontrol Program to Manage Pest Fruit Flies in Australia*, which co-funded the release and evaluation work.

During the project, a variation was made to milestones to enable the research to be delivered despite 2020/21 COVID-19 lockdowns. This was particularly challenging in Victoria, where lockdowns and restrictions severely compromised travel within the state and attendance at the workplace. An additional field season (2021/22) was utilised to perform parasitoid releases, and webinars and online meetings replaced face-to-face workshops. Despite these severe restrictions, the key outputs were still delivered, and project outcomes realised. The use of parasitoids for fruit fly biocontrol is showing considerable promise, particularly for area-wide management of fruit fly in urban towns and cities.

Keywords

Queensland fruit fly, *Bactrocera tryoni*, parasitoids, *Fopius arisanus*, *Diachasmimorpha kraussii*, area-wide management, Integrated Pest Management, biocontrol, biological control.

Introduction

One of the primary reasons for an escalating fruit fly problem in Australia is the lack of a standalone replacement for organophosphate cover sprays that once dominated grower control programs prior to their withdrawal. There is now increased reliance on a multi-component fruit fly management toolbox operating within an area-wide IPM framework. The tools in this toolbox target predominantly adult stages of fruit fly (bait spraying, mass trapping, potentially sterile insects). To date, egg and larval stages have escaped control but continue to seed future pest generations. Importantly, these egg and larval stages could be targeted using a suitable biocontrol strategy (Clarke, 2019, Clarke et al., 2011), promising a major step towards solving the fruit fly problem.

The use of natural enemies (predators, parasitoids, pathogens) as biocontrol agents against insect pests is generally regarded as a core plank of sustainable pest control (New, 2002). These biocontrol strategies can involve the creation of suitable environments for natural enemies (conservation biocontrol), culture and mass release of biocontrol agents on a local or regional scale (augmentative biocontrol), or importation of new agents if the pest is of exotic origin (classical biocontrol). When applied effectively, biocontrol can drastically reduce pest populations by targeting not only the target crop, but also non-crop refuges and other breeding areas, thus becoming an important tool in area-wide management (Koul et al., 2008).

In northern states of Australia where fruit flies are native, the loss of organophosphates has required ongoing development of alternative controls, while in southern states, area freedom status has been lost from designated fruit fly exclusion zones in Victoria and New South Wales in 2013. Since then, the Queensland fruit fly (Qfly) problem in Victoria has become particularly severe, especially in the Goulburn Valley (the state's stone- and pome- fruit growing centre), across Sunraysia (where fruit flies threaten citrus, table grapes, and stone fruit production), and in urban and peri-urban areas across the state (where unmanaged fruit trees act as breeding sites for populations). Across the border in South Australia, the Riverland has experienced Qfly outbreaks in the last few years. Escalating Qfly populations in Victoria may be occurring due to an ecological phenomenon called "enemy free space" (Barrios-O'Neill et al., 2015, Jeffries and Lawton, 1984), where a newly arrived invasive species thrives without key predators, either because it has adapted to the environment (e.g. climate) and its predators have yet to catch up, or simply because it has escaped them geographically. Enemy free space pest population escalations can be ideal candidates for applying biocontrol solutions.

Whilst biocontrol has been recognised as a core component of fruit fly management programs, this has yet to be the case in Australia (Clarke et al., 2011, Zamek et al., 2012). Australian pest fruit flies (e.g. Queensland fruit fly, Jarvis's fruit fly, and Medfly) are attacked by a specialist group of wasp parasitoids, most belonging to the subfamily Opiinae of the family Braconidae (Carmichael et al., 2005, Wharton and Gilstrap, 1983, Wharton and Yoder, 2010). Eleven fruit fly attacking opiine braconids are known from Australia, of which eight have been recorded from Qfly (*Bactrocera tryoni*) and three (based on offshore records) from Medfly (*Ceratitis capitata*) (Carmichael et al., 2005, Spinner et al., 2011). Most of these species are tropically/sub-tropically distributed, with a limited number of species (*Fopius arisanus*, *F. schlingeri*, *Diachasmimorpha kraussii*, *D. tryoni*) known to extend as far south as central NSW (Carmichael et al., 2005, Spinner et al., 2011). An initial look at state records shows, as yet, no records of any of these parasitoids in Victoria (Australian Plant Pest Database and Victorian Agricultural Insects Collection). *Fopius arisanus* has been the central focus of parasitoid-based biocontrol control programs against fruit flies around the world for well over half a century. This insect was released from Hawaii into Australia as a biocontrol agent in the 1950's, and there are reports of parasitism levels on *B. tryoni* of 50 to 80% (Merkel et al., 2019, Snowball, 1966). Being an egg-pupal parasitoid, this wasp searches for, and oviposits into, fruit fly eggs and adult wasps emerge from the fruit fly pupae. Native parasitoid species in the genus *Diachasmimorpha* might also prove to be complementary agents for release with *F. arisanus*, or perhaps even superior candidates for augmentative release: these wasps will attack second and third instar fruit fly larvae, including infested fallen fruit (A. Masry, pers obs.), and this genus may be more resilient to the climate of the southern states, and capable of overwintering (Zamek et al., 2012).

This project conducted the essential studies that will form the backbone of a national parasitoid biocontrol program for fruit flies. Specific aims were (i) improving knowledge on parasitoid distribution in Australia; this was achieved by utilising a wealth of existing data on parasitoid ecology and distribution that has already been collected, supplementing this data with additional survey work (Victoria); (ii) developing protocols for augmentative biocontrol using the two parasitoid species (*F. arisanus* and *D. kraussii*); achieved by collecting and culturing wild insects, and developing new protocols for mass-rearing, and (iii) conducting trial parasitoid releases in fruit orchards and urban/peri-urban sites in Victoria; including evaluation of within-season establishment. The project engaged with individual growers and grower networks, community groups, industry development officers and comms personnel, and regional fruit fly coordinators to enable the

project to be delivered and ensure an outcome of improved awareness of the potential benefits of fruit fly biocontrol to both area-wide and on-farm management. The project aligned with DAWE project (4-EKSH327), which extended the scope of this work to enable additional releases, off-target studies, a conservation biology component, and a benefit-cost analysis. Variations were made to milestones to enable the project to be delivered throughout the 2020/21 COVID-19 lockdowns. This was particularly challenging in Victoria, where lockdowns placed severe restrictions on travel and attendance at the workplace. An additional field season (2021/2) was utilised to carry out releases, and webinars and online meetings replaced face to face workshops. Despite this considerable challenge, key outputs and outcomes of the project were still delivered.

Methodology

Improving knowledge on fruit fly parasitoid distribution in Australia.

*Parasitoid distribution mapping***.** Data mining of a QUT Masters thesis (Carmichael, 2009) was transcribed into an Excel spread sheet. The thesis included spatial data, host fruit fly affiliations and host fruit affiliations. The spatial data was then supplemented by additional records from published literature on Australian fruit fly parasitoids, and unpublished QDAF data. New distribution maps were developed, focusing on five fruit fly parasitoid species present in Australia that may be suitable as biocontrol agents. A comprehensive review updating knowledge on Australian fruit fly parasitoids and their potential for use in biocontrol programs was written by the project team.

Additional surveys. Fruit fly populations in Victoria have increased significantly over the last decade, and yet fruit fly parasitoids had not yet been identified in the state at the beginning of this project. As there were no records of any recent survey data looking specifically for parasitoids, we conducted a survey as part of this project. Agriculture Victoria has preserved insect specimens from a 2017-18 study that collected insects emerging from crop and non-crop infested fruits around Victoria. Whilst this "rear-out" study focused only on identifying endemic and pest fruit fly species, all insects emerging from fruits were collected and preserved: non-tephritid species were not examined due to time constraints. Here, we examined wasp specimens preserved from the 2017-18 study for *Fopius arisanus* and *Diachasmimorpha spp*. To supplement this survey data, fruits were collected from key horticultural production regions across Victoria including Sunraysia, Goulburn Valley and Rutherglen. Collections in Gippsland were not possible due to COVID-19 travel restrictions. To assist in targeting seasonal fruit hosts, a database was developed to document known Qfly host plants and regional Victorian fruit ripening succession timing. Fruits were also collected opportunistically from other localities including the Central Goldfields, Yarra Valley and Melbourne. Only ripe fruit and fruit with signs of Qfly infestation such as sting marks, rot or the presence of Qfly larvae were collected. Collected fruits were transported to the AVR facility at Agribio to rear-out larvae to adult insects. The date of first emergence of Qfly/parasitoids was recorded for each sample and all Qfly/parasitoids had their identification confirmed.

Developing mass rearing protocols.

A literature review was first conducted to establish protocols used in mass-rearing of fruit fly parasitoids around the world, including laboratory tests to quality-control rearing methods. *Fopius arisanus* and *Diachasmimorpha kraussii* were collected as parasitised Queensland fruit fly larvae from wild hosts in Queensland (by the QUT project team) and cultured in the laboratory. Parasitised pupae were then shipped to Agriculture Victoria's mass-rearing facility at Tatura Smartfarm (*F. arisanus* in 2020 and *D. kraussii* in 2021). Rearing protocols were further developed to enable large numbers of parasitoids to be produced. Problems were reported in the initial rearing of wild *F. arisanus*, which led to sexes being separated, such that males were allowed to mature before being exposed to females. Separating individuals was a time-consuming practice, and a laboratory experiment investigating the need for continued sex separation in established cultures was performed.

Parasitoid releases in Victoria.

In the first season of releases in 2021, cages of adult *F. arisanus* that were mass reared at Tatura Smartfarm were transported to two orchard and four urban sites in the Goulburn Valley and Sunraysia that had been identified as having Qfly present, either through trapping or observation of infested fruit. Methodology for releases was confirmed through observation of parasitoid activity prior to and behaviour post release. Parasitoid persistence in the environment and parasitism rates were monitored at three sites (two orchard and one urban) using sentinel fruits and collection and rear-out of infested fruit in the laboratory. Second season releases (2021/2022) of adults of both *F. arisanus* and *D. kraussii* were undertaken at two orchards and eight regional towns across Sunraysia, Goulburn Valley and metropolitan Melbourne identified as having Qfly present. Dispersal of parasitoids from urban release sites to nearby properties was monitored using a citizen science approach, with a leaflet delivered to properties surrounding urban release sites asking residents to fill in a questionnaire and donate infested fruit to AVR. Parasitism rates and parasitoid establishment in the field was monitored by the collection of infested fruit from seven of the release sites and any impact on Qfly populations is being demonstrated through the comparison of monthly Qfly catches at traps at the release site with "control traps" (with similar historical fruit fly trap numbers) identified from Victoria's fruit fly surveillance program using BioWeb/TrapBase software.

Communication and engagement activities.

Due to COVID-19 restrictions in 2020 and 2021, in person meetings (particularly in Victoria) were not possible. These

communication and engagement activities switched to online webinars, talks and online workshops (as approved by a project variation). All other comms activities were produced in line with (and exceeding) outputs stated in the contract.

Photos/images/other audio-visual material

All photos and other images are available in the Appendices. Larger files with higher resolution can be submitted to Hort Innovation on request.

Results and discussion

Improved knowledge on fruit fly parasitoids in Australia. We have published a paper that comprehensively reviews and updates what is known about the Australian fruit fly infesting parasitoids (Clarke et al., 2022). The history of fruit fly biological control in Australia is comprehensively reviewed, and the potential for inoculative or inundative biocontrol using parasitoids (mass-rearing and release) discussed, with consideration to how conservation biological control may help increase the impacts of parasitoids in areas where they are already established. New distribution maps for six parasitoid species that attack pest fruit flies are presented, using the results of the data mining and survey work. The review synthesises knowledge using published and unpublished records on fruit parasitoids, identifying knowledge gaps and providing recommendations for future work. This will be an invaluable resource, paving the way for a new generation of Australian fruit fly parasitoid research. The outcomes of this study, together with additional survey work (below) were also used to select the second species of parasitoid, *Diachasmimorpha kraussii*, which (in addition to *Fopius arisanus*) would be mass-reared and released through this project. The review is attached as Appendix A1.

First records of fruit fly parasitoids in Victoria. Our examination of parasitoids from the 2017-18 fruit collection survey in Victoria looked at 391 wasp specimens from 23 sites across Sunraysia, the Goulburn Valley and the Yarra Valley. None were found to be fruit fly parasitoids. In the additional survey as part of this project, a total of 2638 individual pieces of fruit were collected from across Victoria, representing 19 different Qfly hosts. Fruits were collected from trees in residential properties, roadsides or parks and orchards. Five fruit fly parasitoid wasps emerged from fruits collected in NE Victoria (Rutherglen) and were identified, using DNA barcoding, as *Diachasmimorpha kraussii*. *D. kraussii* has been recorded in southern NSW (see Clarke et al., 2022), but this is the first recorded evidence of this fruit fly parasitoid in Victoria. Additionally, a further five *D. kraussii* eclosed from fruit collected just over the NSW border in Corowa. Our results provide evidence that this species may be beginning to establish in the state. While this is good news in terms of the potential survival of this key native natural enemy of Queensland fruit fly in the Victorian environment, its rate of establishment across the state may be slow due to low populations, seasonal fluctuations in fruit fly prevalence, and long dispersal distances required. Fast-tracking and bolstering this establishment process would be a very good investment for horticultural industries, as evidenced by a recent benefit-cost analysis (aligned DAWE project 4_EKSH327). For more information on the survey work, refer to Appendix A4.

Mass rearing protocols for *Fopius arisanus* and *Diachasmimorpha kraussii* developed. A literature review was completed to establish practices used in mass-rearing of fruit fly parasitoids around the world. *Diachasmimorpha kraussii* and *Fopius arisanus* collected from wild hosts in Queensland were brought into culture and a mass-rearing program was successfully developed at Tatura Smartfarm, including the use of specialized rearing equipment to enable large numbers of these two parasitoid species to be mass-reared, and a ramping schedule for seasonal releases. Experimental work demonstrated that separation of sexes to allow males to mature was no longer required once large cultures of *F. arisanus* were established in the lab (this was not necessary for *D. kraussii*). *Fopius arisanus* cultures were ramped up to approximately 40,000 individuals before the first pilot releases in February and March 2021 and in the second season of releases mass-rearing allowed for weekly releases of up to 10,000 *F. arisanus* and 6,000 *D. kraussii*. A technical report (Appendix A2) detailing mass-rearing protocols has been produced. This will be a useful resource that will guide practices on fruit fly parasitoid mass rearing for augmentative biocontrol releases.

Fruit fly parasitoids released across Victoria in a pilot augmentative biocontrol program. This component of the project aligned with the DAWE project 4-EKSH327 *A National Biocontrol Program to Manage Pest Fruit Flies in Australia*, which co-funded the release and evaluation work. Ninety-five thousand parasitoids were released at three orchard and nine urban sites in nineteen individual releases. First season releases of *Fopius arisanus* in February to March 2021 in Sunraysia and the Goulburn Valley comprised approximately 34,000 adults and demonstrated very promising results, with female parasitoids observed actively searching on and ovipositing into sentinel fruits within the first two hours after release. Monitoring studies demonstrated that parasitoids can successfully locate and parasitise infested fruits, with mean parasitism rates immediately post-release as high as 58% in a pome fruit orchard. Parasitism rate averaged across all fruit collections was more moderate at 9%; differences across fruit types should be considered here, as well as the small (inoculative) release sizes. Parasitoid dispersal was recorded at least 20m from release site within two days of release. Monitoring at an urban site in Sunraysia demonstrated that *F. arisanus* can persist in the environment for at least 10 weeks post-release, indicating breeding is occurring in the field.

Second season releases comprised approximately 43,000 *F. arisanus* as well as 18,000 *Diachasmimorpha kraussii* at two

orchard sites and eight regional towns across Sunraysia, Goulburn Valley and metropolitan Melbourne. Second season results show that *F. arisanus* is parasitising fruits at two out of the five sites monitored by fruit collection, and found in four fruit varieties. Average parasitism levels at sites with demonstrated presence of parasitoids is 9%, with specific collections demonstrating parasitism as high as 32%. The results of parasitism, establishment and dispersal of *D. kraussii* is particularly promising, with parasitism recorded at all four sites monitored by fruit collection and an average parasitism rate of 16% across seven fruit varieties. Parasitism was recorded at five and seven weeks post-release at two sites (and is ongoing) indicating seasonal establishment at these sites with dispersal of parasitoids 200m from release at Swan Hill and 1.5km from release site at Irymple.

The results of the two seasons of mass releases demonstrate that both species of parasitoids have potential to become established in Victoria, persisting seasonally in the environment and dispersing from release sites. Of the two parasitoids, *D. kraussii* is currently the best candidate for augmentative releases, due to ease of mass-rearing, greater establishment, and indications of small populations in NE Victoria. Further research into the persistence of either species between seasons (overwintering) would be fundamental to the application of mass releases of parasitoids as biocontrol agents for fruit fly in Victoria. More research is required to explore whether inundative releases of parasitoids could be used as an on-farm control strategy to manage flies. Full details are provided in the technical report attached as Appendix A3.

Communication and engagement activities

Several online webinars, talks and online workshops were delivered during the project, including a 90-minute webinar on the fruit fly biocontrol program (both Hort Innovation and DAWE projects), including project updates from team members, delivered on 23/02/21 as part of the National Fruit Fly Council Webinar series, coordinated by Plant Health Australia. There were 99 attendees, including scientists, industry representatives, regional fruit fly coordinators (VIC), growers and members of the general public. For all other comms and engagement activities, see Outputs.

Key findings:

- Fruit fly parasitoids *Fopius arisanus* and *Diachasmimorpha kraussii* were successfully mass reared and released across Victoria.
- Both species showed evidence for within-season establishment of local populations.
- Parasitism rate varied across host plants; and given the relatively small release sizes results were very promising
- *Diachasmimorpha kraussii* was found at sites 200m and 1.5km from the release site.
- Of the two parasitoids, *D. kraussii* is currently the best candidate for augmentative releases, due to ease of mass -rearing, greater establishment, and indications of small populations in NE Victoria.

Photos/images/other audio-visual material

See photographs and images in attached Appendices.

Outputs

Table 1. Output summary

Output	Description	Detail
Technical report on mass rearing protocols	A technical report providing detailed information on fruit fly parasitoid rearing methods. For use by researchers, commercial biocontrol suppliers, or industry, to culture these insects, particularly for	This output has not yet been made publicly available. Attached as Appendix A2

	augmentative releases to establish or bolster fruit fly parasitoid populations.	
Technical report on parasitoid releases in Victoria.	A technical report covering releases of *Fopius arisanus* and *Diachasmimorpha kraussii* in Victoria in 2020/21 and 2021/22 growing seasons. Also includes details of the survey work. To be published in industry and scientific publications, and presented in talks, webinars, conferences, to industry and the scientific community.	This output has not yet been made publicly available. Attached as Appendix A3. See published articles and communications listed below, for communication of project aims and findings related to releases.
Published paper on the use of parasitoids for the augmentative and conservation biological control of Dacini fruit flies	Scientific paper that comprehensively reviews and updates what is known about the Australian fruit fly parasitoids. Intended for research community (key fields; fruit fly, parasitoids, biocontrol)	Clarke, A. R., Harris, C., Kay, B.J., Mainali, B.P, McLay, L.K., Strutt, F. and Cunningham, J.P. (2022) Opiine parasitoids (Hymenoptera: Braconidae) and biological control of fruit flies (Diptera: Tephritidae) in Australia: Past, present and future. Annals of Applied Biology 180 (1), 44-72. https://doi.org/10.1111/aab.12724 (Attached as Appendix A1)
Additional scientific publications	Two additional publications on fruit fly parasitoids are aligned to this work, for which HI has been acknowledged.	Mahat K. & Clarke A.R. (2021) Fruit fly parasitoids (Hymenoptera: Braconidae) in South-east Queensland, Australia. Austral Entomology 60,738–745. https://doi.org/10.1111/aen.12565 Mahat K. & Clarke A.R. (2021) Competition between Diachasmimorpha kraussii and Fopius arisanus in Bactrocera tryoni: does a native parasitoid-host association matter? *BioControl* volume 66, pages297–306. doi.org/10.1007/s10526-020-10068-1
Industry publications on fruit fly parasitoids	5 articles published in grower-accessible sources. Target audience; fruit and vegetable producers, crop consultants and pest scouts, biocontrol commercial businesses, hort industry representatives.	AUSVEG Website: https://ausveg.com.au/articles/parasitoid-wasps-the-focus-of-a-new-biocontrol-program-for-queensland-fruit-fly/ Fresh Plaza (via AusVeg): *Parasitoid wasps: The focus of a new biocontrol program for Queensland fruit fly*. https://www.freshplaza.com/article/9323920/parasitoid-wasps-the-focus-of-a-new-biocontrol-program-for-queensland-fruit-fly/ Greater Sunraysia Pest free Area, Queensland fruit fly Newsletter, Issue #11 – September 2021. *Using natural enemies to control fruit fly.* https://mailchi.mp/greatersunraysiapfa/qflynewsletter-september2021 Citrus News: *Natural predators another link in biocontrol chain*. https://citrusaustralia.com.au/news/latest-news/natural-predators-another-link-in-biocontrol-chain McKinnon, A & Cunningham, JP (2021). "Could biopesticides help tackle Qfly populations?" *Australian Tree Crop* Oct/Nov 2021.

		https://www.treecrop.com.au/news/could-biopesticides-help-tackle-qfly-populations/ Cunningham, JP & Clarke, AR (2021). "Using biocontrol to tackle fruit fly populations in orchards and urban areas." *Australian Berry Journal*. December edition. https://berries.net.au/home/news/abjournal/
Factsheet relating to fruit fly biocontrol / research conducted in the project.	One factsheet about the project (as a brochure / leaflet) was produced and made available through community workshops, a letterbox drop, and to growers and community interested in knowing more about the project / participating in the research. A second factsheet summarising the project work and key findings has been submitted with this final report.	Community available Parasitoid Information Flyer attached as Appendix A5 Project work and findings factsheet attached as Appendix A7
Webinars, talks, online grower meetings.	A 90-minute webinar on the fruit fly biocontrol program, including project updates from team members (23/2/21) was delivered as part of the National Fruit Fly Council Webinar series. Presentation at cherry growers meeting 24/6/2022. Presentation to Apple and Pear Community of Practice meeting 1/7/2021.	There were 99 attendees to the webinar, including scientists, industry representatives, regional fruit fly coordinators (VIC), growers and members of the general public. Workshop talk delivered to Cherry Growers Australia, Appendix A6.
Project M&E plan	Developed for M102	Attached as Appendix A8

Outcome

Table 2. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Gaps in knowledge / datasets on Qfly parasitoid distribution and seasonal abundance identified.	Intermediate outcome (M&E Plan). KPI 1 (Effectiveness, (ii) researcher knowledge)	This outcome was fully realized by the publication of the comprehensive review (Appendix 1).	Annals of Applied Biology is an international journal with a strong impact factor (2.8), and a wide reach to the scientific community.
Growers acquire new knowledge in biocontrol through workshops, articles & factsheets	Intermediate outcome (M&E Plan).	This outcome was realized through industry publications, webinars, online grower meetings, and factsheets (see outputs)	Industry publications have a wide reach for Australian fruit growers (*Aus Tree Crop, Fresh Plaza*), or targeted industries (*Citrus News, Aus. Berry Journal*). See NFFC webinar stats in Outputs). Releases in Sunraysia reported on Greater Sunraysia Pest Free Area's Facebook.
Australian knowledge and capability improved in mass rearing and releasing parasitoid wasps	Intermediate outcome (M&E Plan).	Technical report on mass rearing produced (Appendix 2)	The report will be made available for scientists and / or biocontrol companies, and methods incorporated into a scientific publication on augmentative releases.
Local growers and regional fruit fly coordinators in Victoria gain knowledge through participation in orchard and urban releases of parasitoid wasps.	Intermediate outcome (M&E Plan).	11 growers at release sites engaged in releases/monitoring with input to timing of releases. Meetings held with Regional Fruit Fly Coordinators throughout the project. Leaflet about the project distributed to approximately 270 homes.	Positive verbal and written (email) feedback.
Growers, consultants and industry stakeholders are better informed on fruit fly biocontrol and its integration into IPM and AWM strategies through workshops and on-farm trials	End-of-project outcome	Achieved wholistically through intermediate outcomes above. Face to face meetings were not possible due to COVID-19 restrictions 2020-2022.	See all intermediate outcomes above.
Protocols for mass rearing and release of selected	End-of-project outcome	Achieved. Technical Report produced, Appendix 2.	See all intermediate outcomes above.

parasitoid species have been developed and trialled			
SIP outcome (as stated in M&E plan, Summerfruit 2017-2021) Costs have reduced at every level of the supply chain to grow industry profitability. Strategy: *continue with a prioritised R&D program to manage pest and disease challenges and threats.*	Relevant SIP outcome. *OUTCOME 2: Industry supply, productivity and sustainability.* Specific strategy statements for Summerfruit, Apple & Pear, and Berry industries are listed below the table.	All outcomes align with this SIP outcome (in M&E plan), and with industry SIPs (2022-2026) addressing Outcome 2.	This is a long-term and continuing outcome, and has been addressed in this project.

SIP alignment (2022-2026):

Summerfruit: Outcome 2, Strategy 1: "Improve industry preparedness and resilience to biosecurity threats and support a national approach to fruit fly management, including improving access to existing and new markets.

Apple & Pear, Outcome 2, Strategy 2: Enhance industry biosecurity preparedness and resilience to ensure business continuity, and market access and trade, both domestically and internationally

Berry Industry, Outcome 2, Strategy 2: Develop and optimise fit-for-purpose, sustainable pest and disease management strategies for berry production systems.

Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
To what extent has the project achieved its expected outcomes?	All outcomes have been achieved, with the exception of face-to-face meetings and workshops (due to COVID-19 restrictions throughout the project)	Face to face meetings and workshops could now be resumed, if this project progresses to a stage 2.
How relevant was the project to the needs of intended beneficiaries?	*Has the project contributed to the development and evaluation of biocontrol as tool in fruit fly management?* Yes, details in outputs clearly demonstrate this (especially Appendices 2 and 3).	The project delivered well in terms of developing and evaluating biocontrol, but to fully realise an effective biocontrol program for fruit flies in Australia, further research and extension activities are essential.
How well have intended beneficiaries been engaged in the project?	*Have sufficient numbers of growers/consultants been engaged in the workshops / extension activities aimed at improving knowledge of biocontrol?* Yes, see details in outputs and outcomes. *Have the agreed levels of communication (articles, fact sheets, workshops) been delivered? Were industry extension officers and communication programmes used (e.g. Regional fruit Fly coordinators / teams)?* Yes. Engagement of Regional fruit Fly coordinators in Sunraysia to identify release sites and residents to facilitate release monitoring. Communications (publications, webinars, online meetings) above agreed levels. *Were growers engaged to field test pilot releases, and how was their feedback incorporated into further development of the techniques?* Yes. 11 growers at release sites engaged in releases/monitoring with input to timing of releases and	Engagement and extension activities could be reinvigorated in a subsequent (much needed) phase of work to engage further with all beneficiaries (growers, industry, community, biosecurity, fruit fly coordinators).

	infested fruit collection	
To what extent were engagement processes appropriate to the target audience/s of the project?	*Were engagement events undertaken across the state of Victoria, and at appropriate times to expose growers to project information?* Yes, as described, but online only due to COVID-19 restrictions. *How well were the extension materials (fact sheets, articles, workshops) utilised by participants and was the information considered appropriate by the growers / consultants?* Parasitoid factsheet distributed via post office in one regional town. Where hand distributed, recipients were very interested, however few questionnaire responses were received by AVR.	Further engagement with community organisations recommended, such as community farms and growers clubs
What efforts did the project make to improve efficiency?	*Did mass releases take place as planned?* Yes. *Did collaboration with partners (QUT & IPM technologies) go as planned?* Collaborations with QUT went as planned, including establishment of parasitoid culture, optimization of cultures and review writing. Collaboration with IPM Technologies in delivering face to face workshops did not take place due to COVID-19 restrictions. *Were efficiency improvements evident in aligning the project with DAWE Roadmap activities?* Yes, the two projects aligned very well, enabling an extra field season and additional sites for releases, and additional research (off target effects, conservation biology research, benefit-cost analysis) through the DAWE project.	Rearing methods used fresh papaya as a substrate for Qfly and parasitoid oviposition and larval development. Development of artificial fruit media for parasitoid rearing would be beneficial in terms of cost and practicality.

Recommendations

- This initial-phase project has presented evidence that fruit fly parasitoids could be used in biocontrol programs to assist with the area-wide management of Qfly.
- Of the two parasitoids, *D. kraussii* is currently the best candidate for augmentative releases, due to ease of mass -rearing, greater evidence for establishment, and indications that small populations of this species have now reached as far south as NE Victoria.
- Continuation of this research is highly recommended to increase the potential for rapid establishment of one or both of these key Qfly predators in states and/or localities where populations are rare or absent.
- Releases could be particularly beneficial in urban localities where fruit fly populations are becoming increasingly problematic.
- A recent benefit-cost analysis has demonstrated this would be a worthy investment to see reduced damage, particularly in farms surrounding urban areas (aligned DAWE project 4-EKSH327 *A National Biocontrol Program to Manage Pest Fruit Flies in Australia*)
- The parasitoid release program should be extended to include Medfly biocontrol (WA, SA), as well as augmentative releases in NSW, SA, and WA to control Qfly.
- Further research into the persistence of these parasitoid species between seasons (overwintering) is fundamental to understanding the establishment of fruit fly biocontrol agents in Victoria
- New sourcing of populations of *F. arisanus* from its native Asian range could facilitate better climate matching with southern Australian environments.
- Additional research is required to explore whether inundative on-farm releases of parasitoids could be used as a management strategy as part of an IPM approach.

Refereed scientific publications

Journal articles

Clarke, A. R., Harris, C., Kay, B.J., Mainali, B.P, McLay, L.K., Strutt, F. and Cunningham, J.P. (2022) Opiine parasitoids (Hymenoptera: Braconidae) and biological control of fruit flies (Diptera: Tephritidae) in Australia: Past, present and future. Annals of Applied Biology 180 (1), 44-72. https://doi.org/10.1111/aab.12724

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Mahat K. & Clarke A.R. (2021) Competition between Diachasmimorpha kraussii and Fopius arisanus in Bactrocera tryoni: does a native parasitoid-host association matter? *BioControl* volume 66, pages297–306. https://doi.org/10.1007/s10526-020-10068-1

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Intellectual property

No project IP or commercialisation to report

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Appendices

Appendix A1 – 2022 Clarke et al, Annals Appl Biol

Appendix A2 – Mass Rearing Protocols for Fruit Fly Parasitoids (technical report)

Appendix A3 – Parasitoid releases for Fruit Fly BioControl (technical report)

Appendix A4a – Fruit fly parasitoid surveys in Victoria

Appendix A4b – Fruit collection hosts lists database

Appendix A5 – Parasitoid Information Flyer

Appendix A6 – Cherry Industry Workshop Slides

Appendix A7 – Factsheet on Parasitoid Releases

Appendix A8 – M & E plan MT19003