Hort Innovation

Final Report

Trapping efficacy in table grape vineyards for area wide management of Queensland fruit fly

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Project code:

TG19001

Project:

Trapping efficacy in table grape vineyards for area wide management of Queensland fruit fly (TG19001)

Disclaimer:

Funding statement:

This project has been funded by Hort Innovation, using the table grape research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Publishing details:

ISBN 978-0-7341-4816-2

Published and distributed by: Hort Innovation

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Public summary

Managing Qfly in table grape vineyards has presented a major challenge in recent years, particularly in Greater Sunraysia (Victoria) where around 70% of Australian table grapes are grown. With the withdrawal of several pesticides that previously controlled this pest, an urgent need has arisen for a toolkit of technologies and practices to effectively manage Qfly both on-farm and through an area-wide approach.

This project conducted four case studies in commercial vineyards, together with additional experimental work, to better understand how mass trapping might be employed in the management of Queensland fruit fly (Qfly) in table grapes. Three case studies focused specifically on whether protein baited traps *(Biotrap*) and / or a new trap that uses a synthetic fruit-odour lure (Agriculture Victoria Research's "*AVR trap*") might be used; building on recent research that demonstrated the *AVR trap* was the most effective trap in pome and stone fruit orchards. The case studies also explored trap placement within and around vineyards. The results of this research led to several important findings: i) the new *AVR trap* was not as effective as the protein-baited *Biotrap*; being a sticky trap, it was susceptible to dust and debris due to frequent dust storms in the area; (ii) trapping around the perimeter of the block was significantly more effective in catching flies compared to placing traps on interior vines, and (iii) nearby fruit trees harboured fly populations ("hot spots") and growers should consider the increased risk this poses to Qfly infestations in vineyards.

Vineyards differ markedly from fruit orchards in the shelter they provide for adult fruit flies, and the increased catches of flies around the perimeter may be due to flies moving in and out of the crop on a daily basis. A fourth case study using *RapidAIM* traps (which collect real-time data on male fly catches) revealed diurnal variation in the activity of male flies and provided additional evidence that perimeter traps outperform interior traps. A laboratory study explored whether *RapidAIM* traps could be modified to target female flies. The study showed that the use of protein bait within the *RapidAIM* traps instead of cue-lure would catch low numbers of females, and the addition of a red visual cue increased *RapidAIM* trap captures in the laboratory, though trapping in the field was still low.

In the absence of workshops, due to COVID-19 restrictions in 2020-2021, a meeting with pest scouts and industry representatives was used to discuss mass trapping and communicate the research in this project, and an anonymous online survey was conducted to better understand table grape grower perceptions regarding Qfly management. These engagements led to several important findings regarding grower perceptions about Qfly management in table grapes, including stigma related to an infestation and the cost of mass trapping. A laboratory experiment explored a question raised on susceptibility of different grape varieties to Qfly, and while results demonstrated that some grape varieties are more attractive to female flies than others, field trials would be essential to validate this. The project has provided new knowledge to help inform table grape growers on the potential for mass trapping Qfly in their vineyards. Trapping around the perimeter of the vineyard and in nearby host fruit trees might be the most cost-effective approach for both male and female mass trapping strategies, though more research is needed to fully evaluate this. Sticky traps are not recommended in and around Mildura, and protein traps are currently the best option. Further research could develop a synthetic fruit-lure trap that instead uses an insecticide or biopesticide as the killing agent.

Technical summary

This project utilised four case studies in commercial vineyards together with additional experimental work to better understand how mass trapping might be employed in the management of Queensland fruit fly (Qfly) in table grapes. In March 2020, a preliminary trapping study was conducted in eight table grape vineyards in and around Mildura (Sunraysia, Victoria) to establish relationships with table grape growers and select suitable vineyards for the case studies. Cue-lure baited *Biotrap* (male), protein-baited *Biotrap* (female) traps, and a new mated female *AVR trap* designed by Agriculture Victoria (see below for more details on all trap types) were deployed both within the vineyard and at potential fruit fly host "hot-spots"—nearby fruit trees such as stone fruit and citrus trees. Monitoring traps were deployed for eight weeks and Qfly captures were counted. Additionally, in-person interviews were conducted with growers to gain knowledge of their general experiences with Qfly, including whether they used trapping, which types of traps (if any) they had trialled, and what their experience with trapping had been.

Based on the preliminary study and interviews, three aspects of mass trapping were deemed of high relevance to Qfly management in table grapes: (i) the type of trap to be used, (ii) the optimal placement of traps within and around the crop, and (iii) the presence of nearby fruit trees as potential sources of Qfly ("hot spots"). The four case studies conducted between 2021 and 2022 explored these aspects further. These selected vineyards were identified in 2020 as having Qfly populations (from Qfly monitoring traps) and differing in their proximity to nearby fruit tree hot spots. The identity and /or location of the case study sites has been kept confidential and any identifying information removed, due to sensitivities relating to Qfly presence in the region, and as part of the agreement with the growers involved.

Case studies 1-3. Trap type and placement.

Evaluating effectiveness of different traps at trapping Qfly in table grapes. At three of the case study sites we conducted trials comparing Qfly trap catches using three different types of trap: (i) protein-baited traps (*Biotrap*, using *Biotrap* protein gel), (ii) a new trap developed by Agriculture Victoria Research ("*AVR trap*"), which utilises a synthetic fruit-volatile lure and a visual cue attractive to mated females, and (iii) cue-lure traps (*Biotrap* with cue-lure). Our aim here was primarily to evaluate the effectiveness of these traps in table grapes, as a previous study comparing the effectiveness of the two female-targeted traps found that the *AVR trap* was the most effective at catching female flies (both mated and unmated). Mating status of captured females was determined by dissection. In contrast to our previous studies in pome and stone fruits, the *AVR trap* (synthetic fruit lure) performed poorly in table grapes. Being a sticky trap, the *AVR trap* quickly became coated in dust and debris as a result of frequent dust storms, reducing its efficacy. However, as with the previous studies, the *AVR trap* captured the highest proportion of mated females, while the protein-baited traps captured a higher proportion of virgin females. Male flies were captured in both protein baited and cue-lure baited *Biotrap*. The difference in female mating status targeted by the two traps suggests that a mixed trapping strategy (i.e., deploying both traps) could be beneficial to growers. The *AVR trap* in its present form is, however, unsuitable for trapping where dust storms compromise its efficacy.

Trap placement (perimeter vs within-block) and importance of hot spots. Case studies 1-3 were also used to collect data on the placement of each trap, as being either around the perimeter (placed on a boundary vine or at the end of a row) or within the block (placed on an interior vine). Perimeter protein-baited and cue-lure *Biotrap* consistently captured more Qfly than interior traps (*AVR trap* caught too few flies). A strategy of perimeter-only trapping may therefore be a more cost-effective mass trapping strategy for table grape vineyards, as it would significantly reduce the number of traps needed, as well as the time spent servicing and checking on traps. Further work is needed to evaluate this. Our trapping data revealed that vineyards with nearby fruit tree hot spots had higher populations of Qfly than those without. These hot spots of Qfly hosts may ultimately be sources of Qfly that disperse into table grape vineyards. The architecture of grape vines may provide insufficient shelter for flies, particularly from sunlight, and thus flies rely on refuge sites such as host trees from which to move to and from the vines. Fruit trees within or surrounding vineyard properties, particularly if poorly managed, will also serve as Qfly breeding sites throughout the year, as well as a potential source of late season flies that may overwinter around the vineyard and infest fruit trees the following spring. Our findings provide data that might help inform table growers as to the benefits of managing (or removing) trees to reduce infestations, which could include the use of traps.

Case study 4. Activity of flies in and around the vineyard: This case study focussed on further improving our understanding of Qfly activity in and around vineyards. The study utilised *RapidAIM* smart traps to collect real-time data on male Qfly captures. *RapidAIM* traps were placed along boundary vines or within the block, and also in nearby host fruit trees within 25 m of the vineyard ("hot spots"). We also trialled protein bait as a substituted lure within the traps (plus the addition of a red visual cue, see below) to explore whether these traps could potentially be used to monitor the activity of virgin/immature females—noting that the *RapidAIM* algorithm has not been validated for female fly activity. Trap treatments (*RapidAIM* trap with cue lure or protein bait) were alternated along each transect and equally distributed between each location. Male Qfly activity was found to be highest between 8am and 12pm. We were unable to draw strong conclusions as to whether male Qfly are moving directly from hot spots into the vineyard. Nevertheless, consistent with our findings from case studies 1 to 3, male trap captures were significantly higher in traps placed on perimeter vines compared to interior vines. Significantly, more males were also captured in traps placed in hot spots compared to interior vines. Few females were captured using protein-baited *RapidAIM* traps. Information on Qfly activity in table grape vineyards could be used by growers to inform the timing of pesticide or protein bait spraying.

Modifications to the AVR trap to avoid the use of an adherent: Sticky traps have compromised efficacy in table grape vineyards in and around Mildura, due to frequent dust storms. A laboratory study was carried out to explore modifications to the trap, utilising a localised insecticide instead of a sticky glue to kill alighting insects. Mated female

Qfly (20 per replicate) were placed in a mesh cage together with a modified *AVR trap* that contained two DDVP cubes placed inside the red sphere. No adhesive glue was applied to the trap surface. Alighting behaviour of female flies was observed until 10 individuals had landed on the trap (n = 5 replicates). The length of time they remained on the trap (and which parts of the trap they contacted) was recorded. Flies that did not die while in contact with the trap were captured and placed in individual plastic tubs with sugar and water for up to 24 hours. The traps successfully killed insects that landed on the sphere, but did not kill insects that landed on other parts of the trap (the yellow rectangle). Further modifications might be needed to ensure the insecticide is delivered across the entire trap surface.

Modifications to the RapidAIM trap to improve female Qfly capture. A laboratory study explored whether *RapidAIM* traps that contained a protein lure could potentially monitor the movement of female flies. For each assay, we modified the *RapidAIM* trap using different odour lures and in some treatments an additional red visual cue. Odour lures were (i) the *AVR trap* lure (synthetic fruit odour lure), (ii) a protein bait lure (*Biotrap* gel) and (iii) a synthetic nectarine lure developed by AVR. The additional visual cue, a red bottle cap (34 mm diameter) was placed inside the trap so that it was visible to flies alighting at the entrance. Modified *RapidAIM* traps did not contain cue-lure. An additional trial used male Qfly and the original *RapidAIM* trap (cue-lure) as a positive control. 100 mated Qfly were placed in a large cage for each trial. *RapidAIM* traps modified by using a protein attractant and red visual cue captured the most females. However, only 18% of the females that entered this trap were detected by the *RapidAIM* sensors. In the positive control assay (cue-lure with male flies), 39% were detected by the app. The algorithms used to decode activity picked up by *RapidAIM* sensors may thus require modification to better detect females as they enter the trap using a feeding (protein) bait. The low detection rate in male flies entering cue-lure baited traps (as standards) may imply the sensors have difficulty detecting the behavioural fingerprint of flies under laboratory conditions.

Investigating host suitability of different grape varieties: During grower interviews conducted in March 2020, and in the online anonymous survey participants expressed that they felt some grape varieties were more prone to Qfly infestations than others. No-choice cage assays were conducted using five grape varieties: Adora Seedless, Cotton Candy, Midnight Beauty, Red Globe and Thompson Seedless. For each assay, 10 mated female Qfly were placed in a mesh cage with a single grape (n = 10 replicates per variety). Adults were observed for one hour and the number of females observed ovipositing into (stinging) fruits was recorded, together with the number of times females alighted (landed) on the fruit but did not sting it. Larval survival trials were also conducted, infesting individual grapes with five Qfly eggs (n = 20 grapes per variety). Results of no-choice trials demonstrated that female Qfly were more likely to alight on Adora Seedless, Midnight Beauty and Thompson Seedless compared to Cotton Candy and Red Globe grape varieties, and significantly more likely to oviposit in Thompson Seedless compared Cotton Candy grapes. It is important to clarify with scouts, growers and industry that further fieldwork is essential to provide context as to how the lab results might relate to varietal susceptibility in a vineyard setting.

Keywords

Table grapes; Queensland fruit fly; Integrated pest management; Mass trapping; Female traps Introduction

Introduction

Since the loss of pest free areas in Greater Sunraysia in 2016, this major fruit growing region has experienced increasingly problematic Queensland fruit fly (Qfly) populations, with a significant impact on productivity and trade. Around 70% of all Australian table grapes are grown in Victoria and consequently the table grape industry faces a major challenge to manage fruit flies now and in the future. The Australian Table Grape Association (ATGA) has recently invested in levy-funded research to develop a toolbox of control strategies to manage Qfly on-farm and operate within an area-wide IPM framework (Hort Innovation, TG18001). This ongoing program (TG18001) aims to improve grower understanding and adoption of on-farm Qfly integrated pest management (IPM) practices, such as those that target adult male flies (male annihilation technique, MAT), female flies (protein bait spraying) flies, and larval stages (hygiene) and includes the delivery of workshops and training materials.

Targeting female flies in Qfly IPM is particularly important as these insects cause direct damage through stinging fruits and egg-laying (oviposition). Whilst protein bait spraying can easily be incorporated into existing IPM strategies and has shown success in controlling Qfly in table grapes (Oag, 2011), there are considerable downsides to this strategy in terms of practicality, damage to produce, efficacy at targeting mated female flies, and cost (Dominiak 2006, Balagawi et al. 2012). A mass trapping program for female Qfly offers a possible alternative to protein bait spraying. Mass trapping programs have been developed for tephritid fruit fly pests worldwide (e.g., Broumas et al., 2002; Stupp et al., 2021) including Qfly (Dominiak et al., 2016) and may focus on killing male or female flies. There is a wealth of scientific evidence demonstrating that mass trapping strategies can be an effective means of decreasing insect pest populations when incorporated into IPM systems (see review by El-Sayed et al., 2009). Traps typically consist of a combination of visual and olfactory stimuli specifically designed to attract the target species and contain a killing agent such as a localised insecticide, liquid to drown the captured insects, or a sticky surface. When combined with other IPM strategies, mass trapping has been shown to successfully reduce damage to the crop and/or the size of pest populations in lepidopteran pests, thrips, and beetles (e.g., Goda et al., 2015, Sampson and Kirk, 2013, Schlyter et al., 2001); and drosophilid fruit flies such as spotted wing drosophila (Baroffio et al., 2017), which has a similar ecology to Qfly. Several international studies on tephritid fruit flies have demonstrated that mass trapping targeting female flies can be effective in reducing damage by key fruit fly pests including, olive fruit fly (Broumas et al., 2002), Mediterranean fruit fly (Leza et al., 2008), and Oriental fruit fly (Pinero et al., 2010).

Mass trapping of male Qfly utilises the male-specific parapheromone, cue-lure, commonly employed in monitoring programs (Cowley et al., 1990). The trap may also be dispensed, such that the lure plus insecticide is distributed throughout the crop as a Male Annihilation Technique (MAT) (Manovkis et al., 2019). To be successful, however, male mass trapping must be able to reduce the local population of male flies to such an extent that females cannot find a mate (Barclay and Hendrichs 2014), and this may not be possible for Qfly as females mate multiple times (Shadmany et al., 2021). Female flies are known to be attracted to protein as a requirement for egg development (Meats and Leighton; 2004), and protein-based food traps and bait sprays have shown success at attracting and killing female and male insects in the field (e.g., Dominiak et al., 2016, Oag 2011). Area-wide mass trapping programs using protein traps have been trialled in urban areas in Sunraysia and Swan Hill (Greater Sunraysia Pest Free Area Industry Development Committee (IDC)) with some reported evidence of success, but in-field mass trapping using protein-based traps is still largely an unknown area. Research has shown that protein odours attract predominantly unmated (virgin) flies and questions remain as to whether protein traps and bait sprays will effectively target mated females or even protein-sated virgin females in the field (Balagawi et al., 2012, 2014).

Traps that effectively attract mated female tephritid fruit flies, including Qfly, have been slower to emerge. Mated female flies are known to be attracted to ripening fruit volatiles and visual cues (Cunningham et al., 2016, Schutze et al. 2016), and this can be exploited in trap design: the commercially available *Fruition* trap comprises a synthetic blend of fruit volatiles and a fruit mimic trap, and Agriculture Victoria Research have recently developed a new "AVR" trap (based on a combination of fruit and fungal volatiles) that has proven very effective at attracting large numbers of mated and virgin females in pome fruit, stone fruit, and citrus orchards under repeated trials in 2018/19 (Henneken et al., in review). As protein- and fruit-lure based traps preferentially attract different development stages of the adult female fly population, a mixed trapping strategy using both traps may be a more effective means of controlling on-farm fruit fly populations (Henneken et al., in review, see also our recent article in Australian Tree Crop, Oct 2019). This finding is supported by the ecology of tephritid fruit flies: virgin females require protein for oogenesis (Meats and Leighton, 2004), and mated females prefer to oviposit in ripening fruit (Clarke et al., 2011).

The aim of this project was to conduct research to enable the design of a mass trapping strategy targeting female Qfly, suitable for implementation in table grape vineyards. Research involved evaluating the effectiveness of protein and synthetic fruit odour-based traps in vineyards, exploring the optimal (and cost-effective) placement of traps in and around the crop, and examining the importance of nearby host trees that might be attractive to Qfly.

Methodology

Preliminary trials. To refine our research questions, establish relationships with growers, and select suitable vineyards as case studies (see below) we set out a series of monitoring traps in March 2020 in and around eight table grape vineyards. At each site, we set out three types of traps (described in more detail below). Traps were deployed both within the vineyard and at potential host “hot-spots”; i.e., nearby host fruit trees that might be attractive to Qfly (e.g. stone fruit and citrus trees). Traps were deployed for eight weeks and Qfly captures were counted.

Case studies 1-3. Trap type and placement. Three case studies on mass trapping were carried out in table grape vineyards in the 2020/1 and 2021/2 seasons. The case study vineyards were selected from eight candidate sites identified in 2020 as having Qfly populations (from Qfly monitoring traps) and differing in their proximity to nearby fruit trees (“hot spots”), such as citrus and stone fruits. Hot spots at each vineyard were: within 300 m of the vineyard (case study 3), within 25 m of the vineyard (case study 2), or were not present within 500 m of the vineyard (case study 1). The identity and /or location of the case study sites has been kept confidential and any identifying information removed, due to sensitivities relating to Qfly presence in the region, and as part of the agreement with the growers involved. At three of the case study sites we conducted trials comparing trap catches using three different types of trap: (i) protein-baited traps (*Biotrap*, using *Biotrap* gel), (ii) a new trap developed by Agriculture Victoria Research (“*AVR trap*”), which utilises a synthetic fruit-volatile lure and a visual cue that is attractive to mated females, and (iii) male cue-lure traps (*Biotrap*). Our aim here was primarily to evaluate the effectiveness of traps that targeted female Qfly (protein-baited *Biotrap* and *AVR trap*), using the cue-lure (male targeted) trap as a widely utilised trap to compare against. A previous study conducted by Agriculture Victoria Research (Henneken et al. in review) compared the effectiveness of the two female-targeted traps, and a third trap (*Fruition*), which also uses a synthetic fruit odour. The study found that the *AVR trap* was the most effective at catching female flies (both mated and unmated flies), and that the *Fruition* trap had very poor efficacy. As mass trapping can be expensive and time-consuming, we collected data on the placement of each trap as being either around the perimeter (placed on a boundary vine or at the end of a row) or within the block (placed on an interior vine). Trap numbers varied among vineyards (case studies 1 and 2: n = 24 per trap treatment and case study 3: n = 9 per trap treatment). Traps were deployed for either 10 (case study 2) or 12 weeks (case studies 1 and 3). Approximately every two weeks the traps deployed at each mass trapping case study were checked and the captured insects collected. Trapped insects were sexed and the mating status of captured females was determined based on the presence of sperm in their spermathecae) following Avanesyan et al., 2017. For more detailed information please refer to the technical report (**Appendix 1**).

Case study 4. Activity of flies in and around the vineyard: This case study focussed on further improving our understanding of Qfly activity in and around vineyards. The study was conducted between December 2021 and March 2022 and utilised *RapidAIM* smart traps to collect real-time data on male Qfly captures. *RapidAIM* traps were placed along boundary vines (perimeter) or within the block (interior), and also in nearby host fruit trees within 25 m of the vineyard (“hot spots”). Data collection corresponded to the time the insect was detected by the trap’s sensors and the total number of insects collected from each trap. As *RapidAIM* smart traps are baited with cue-lure and thus only attract males, we also trialled protein bait as a substituted lure within the traps to explore whether these traps could potentially be used to monitor the activity of virgin/immature females—noting that the *RapidAIM* algorithm has not been validated for female fly activity and/or activity related to protein odours. Further modifications of this latter trap included a red visual cue (red bottle cap), placed inside of the lid of traps, as this was shown to increase female captures in a laboratory trial (see below, also Appendix 3). Trap treatments (*RapidAIM* trap with cue-lure or protein bait) were alternated along each transect and equally distributed between each location.

Modifications to the AVR trap to avoid the use of an adherent: Mass trapping case studies revealed that sticky traps have compromised efficacy in table grapes vineyards in and around Mildura, due to frequent dust storms. As this trap has shown superior efficacy to protein-baited *Biotraps* in stone and pome fruit orchards (Henneken et al. in review), a laboratory study was carried out to explore modifications to the trap, utilising a localised insecticide instead of a sticky glue to kill alighting insects. Mated female Qfly (20 per replicate) were placed in a mesh cage (47.5 x 47.5 x 47.5 cm) together with a modified *AVR trap* that contained two DDVP cubes placed inside the red sphere. No adhesive glue was applied to the trap surface, and the trap was attached to the top of the cage. Alighting behaviour of female flies was observed until 10 individuals had landed on the trap, after which the trial was terminated, and 20 new flies were placed into the cage (n = 5 replicates). For each Qfly that alighted on the trap, we recorded the length of time they remained on the trap and which part of the trap they contacted (red sphere, yellow rectangle, or both). Flies that did not die while in contact with the trap were captured and placed in individual plastic tubs with sugar and water for up to 24 hours. We then recorded whether the collected individuals died and the time until death. See **Appendix 2** for full details.

Modifications to the RapidAIM trap to improve female Qfly capture: A laboratory study explored whether *RapidAIM* traps that contained a protein lure could potentially monitor movement of female flies, which might differ from that of male flies (again, noting that the algorithms used to detect female flies entering the trap have not yet been validated). For each assay, we modified the *RapidAIM* trap using different odour lures and an additional red visual cue. Odour lures were (i) a synthetic lure developed by Agriculture Victoria Research and used in the *AVR trap* (AVR lure), (ii) a protein bait lure (*Biotrap* gel) and (iii) a synthetic nectarine lure developed by AVR. The visual cue, a red bottle cap (34 mm diameter) was placed inside the trap so that it was visible to flies alighting at the trap's entrance. The modified *RapidAIM* traps trialled did not contain cue-lure, and instead contained i) *AVR lure* ii) *AVR lure + red visual cue* iii) *AVR lure + protein attractant* iv) *protein attractant* v) *protein attractant + red visual cue* vi) *synthetic nectarine lure* vii) *red cue only*. An additional trial used male Qfly and the original *RapidAIM* trap (cue-lure) as a positive control. 100 mated Qfly were placed in a large cage (47.5 x 47.5 x 93 cm) with sugar, water, and hydrolysed yeast. The cage was housed in a greenhouse, which allowed the *RapidAIM* trap to connect to satellite data and enabled Qfly behaviour to be observed under natural light. Traps were individually introduced into each cage in the morning, and the number of Qfly captured in the trap monitored every hour from 09:00 until 16:00. The number of flies captured in the trap was also compared to the number of flies detected by the *RapidAIM* mobile app. See Appendix 3**.**

Investigating host suitability of different grape varieties: During grower interviews conducted in March 2020 and in the online anonymous survey participants expressed that they felt some grape varieties were more prone to Qfly infestations than others. In this experiment, we therefore aimed to provide growers with some initial data on host suitability of table grape varieties. *Adult fly preferences:* No-choice cage assays were conducted using five grape varieties: Adora Seedless, Cotton Candy, Midnight Beauty, Red Globe and Thompson Seedless. For each assay, 10 mated female Qfly were placed in a mesh cage with a single grape (n = 10 replicates per variety). Adults were observed for one hour and the number of females observed ovipositing into (stinging) fruits was recorded, together with the number of times females alighted (landed) on the fruit but did not sting it. *Larval survival trials*: Individual grapes were infested with five Qfly eggs (n = 20 grapes per variety) by making a small incision into the fruit and gently transferring the eggs with forceps. Infested grapes were then placed on vermiculite and checked daily for the presence of adult Qfly emerging from pupae. See Appendix 4 for details.

Workshop and online confidential grower survey: In the absence of field walks and in person workshops, due to COVID-19 travel and gathering restrictions in 2020-2021, an online workshop with pest scouts and industry representatives was used to discuss mass trapping and communicate the research in this project. Additionally, an anonymous online survey was conducted to better understand table grape grower perceptions regarding Qfly management. The online workshop was held on the 16th of September 2021. Representatives from the Australian Table Grape Association (ATGA) attended, and the meeting was moderated by Alison MacGregor (ATGA) who is familiar with the scouts. The online survey was conducted using the web-based platform *Survey Monkey*. A Privacy Assessment Index (PIA) was completed and approved by an Agriculture Victoria privacy officer, prior to the survey's release to ensure grower anonymity. The survey consisted of 16 questions and was advertised in grower targeted newsletters and social media pages. See Appendix 7 and Appendix 8 for more details on the workshop and survey respectively.

Results and discussion

Preliminary trials. A total of 15 Qfly were captured in traps during preliminary trials across all vineyards. Seven Qfly were captured by traps placed in host trees and the remaining eight were captured by traps placed on table grape vines. Of interest, we noted that the *AVR trap*s quickly became coated in dust and leaves. Our key research questions as a result of these trials were: i) How important are "hot-spots" as sources of Qfly in table grape vineyards? ii) Which traps or combination of traps are best suited to table grape vineyard? and iii) Where should traps be placed to maximise their effectiveness?

Case studies 1-3. Trap type and placement. The results of the three case studies led to several important findings regarding the design of a mass trapping strategy specific to table grapes.

Type of trap used: In contrast to our previous studies which evaluated traps with protein and synthetic-fruit lures in pome and stone fruits, the *AVR trap* (synthetic fruit lure) performed poorly in table grapes. Being a sticky trap, the *AVR trap* quickly becomes coated in dust and debris as a result of frequent dust storms, reducing its efficacy in trapping alighting insects, and possibly in attracting insects (as the dust also obscured the trap's bright colours). Consistent with our studies in pome and stone fruit, however, we found that the protein-baited *Biotrap* and *AVR trap* (synthetic fruit lure) differed in their attractiveness to the reproductive stage of adult female Qfly. Despite the reduced efficacy of the *AVR trap*, it captured the highest proportion of *mated* females; while the protein baited *Biotrap* captured a higher proportion of *virgin* females. Mated female flies are attracted to fruit odours as egg-laying sites, whereas virgin females seek protein for egg development. Male flies were captured in all traps, but most were captured in protein baited and cue-lure baited *Biotrap* (trap catches varied among case studies). The difference in female mating status targeted by the two traps suggest that a mixed trapping strategy (i.e., deploying both traps) could be beneficial to growers. The *AVR trap* in its present form is, however, unsuitable for trapping in Mildura and other areas where dust storms compromise the efficacy of the traps. Modifications could be made to this trap such that an adhesive (sticky surface) is not used as a killing agent (see experimental work below).

Placement of trap: Perimeter protein-baited and cue-lure-bait *Biotraps* (placed on boundary vines or at the end of the row) consistently captured more Qfly than interior traps. Trap catches with the *AVR trap* did not differ significantly between perimeter and interior locations, but these traps caught very few flies in total and were badly contaminated with dust around the perimeter (traps on interior vines had some protection from the dust). A strategy of perimeter-only trapping may therefore be a more cost-effective mass trapping strategy for table grape vineyards. Perimeter traps may be intercepting flies before they can disperse further into the vineyard and/or fruit flies may be spending more at the perimeter of the vineyard. A strategy for perimeter trapping would significantly reduce the number of traps needed, as well as the time spent servicing and checking on traps.

Management of nearby fruit trees, as host "hot spots". Our trapping data revealed that vineyards with nearby fruit tree hot spots had higher populations of Qfly than those without. These hot spots of Qfly hosts may ultimately be sources of Qfly that disperse into table grape vineyards. The architecture of grape vines may provide insufficient shelter for flies, particularly from sunlight, and thus flies rely on refuge sites such as host trees from which to move to and from the vines. Fruit trees within or surrounding vineyard properties, particularly if poorly managed, will also serve as Qfly breeding sites throughout the year, as well as a potential source of late season flies that may overwinter around the vineyard and infest fruit trees the following spring. Our findings provide data that might help inform table growers as to the benefits of managing (or removing) trees to reduce infestations, which could include the use of traps. Future work could evaluate this further.

Case study 4. Activity of flies in and around the vineyard: Male Qfly activity monitored by *RapidAIM* traps placed on interior vines was highest between 8am and 12pm. Traps placed on perimeter vines and in "hot spots" (i.e., nearby host trees that are attractive to Qfly) also experienced a peak (albeit not significantly) at this time. We were unable to draw strong conclusions as to whether male Qfly are moving directly from "hot spots" into the vineyard. Nevertheless, consistent with our findings from case studies 1 to 3, and our hypothesis that flies are moving from hot spots into vines on a daily basis, male trap captures were significantly higher in traps placed on perimeter compared to interior vines, and significantly more males were captured in traps placed in hot spots compared to traps placed on interior vines. Few females were captured using protein-baited *RapidAIM* traps (and noting the *RapidAIM* sensors have not yet been validated for trapping females), which was unfortunate as we were unable to collect data on female fly activity to compare with males. Male mass trapping strategies that use cue-lure products (e.g. *Amulet*, cue-lure baited traps, or *SPLAT*) might also consider perimeter and hot-spot trapping as a more cost-effective strategy than placing male traps throughout the entire crop, though further work is needed to evaluate the efficacy of this strategy. Information on Qfly activity in table grape vineyards could be used by growers to inform the timing of pesticide or protein bait spraying. Application of these products could be timed to coincide with the highest Qfly activity to ensure individuals are more likely to contact a lethal dose (Swoboda-Bhattrai et al., 2020).

Modifications to the AVR trap to avoid the use of an adherent: DDVP insecticide cubes placed within the red sphere of the *AVR trap* successfully killed insects that landed on the sphere, but did not kill insects that landed on other parts of the trap (the yellow rectangle). Fifty-six percent of females that landed on the trap contacted the red sphere, either by directly alighting on the sphere, or after alighting first on the yellow rectangle and then walking onto the red sphere and died within minutes of making contact with the red sphere. Females that contacted just the yellow rectangle were still alive 24 hours following the assay. Further modifications might be needed to ensure the insecticide is delivered across the entire trap surface. Observing insect behaviour on and around traps in a laboratory setting, such as achieved here (and in the experiment with *RapidAIM* traps below) may more generally assist with designing more effective traps for use in mass trapping and monitoring Qfly, prior to field evaluation. Low trap efficacy may even increase damage to surrounding fruit by increasing Qfly activity within the crop (Hampton et al., 2014). See Appendix 2 for more details.

Modifications to the *RapidAIM* trap to improve female Qfly capture. Behavioural assays revealed that *RapidAIM* traps modified by using a protein attractant and red visual cue captured the most females. However, only 18% of the females that entered this trap were detected by the *RapidAIM* sensors (this was also the highest percentage of females detected out of all the modified traps). All other modification treatments captured low numbers of females (between 4 and 15 females) and accurate detection of females by the sensors was also low for these treatments (between 0 and 6.66 %). In the positive control assay (cue-lure with male flies), the trap captured 34 flies, 38.23% of which were detected by the app. The algorithms used to decode activity picked up by *RapidAIM* sensors may thus require modification to better detect females as they enter the trap using a feeding (protein) bait. The low detection rate in male flies entering cue-lure baited traps (as standards) may imply the sensors have difficulty detecting the behavioural fingerprint of laboratory-reared flies, or that fly behaviour entering traps in laboratory cages is different from in the field. Indeed, during a field study in a table grape vineyard (technical report: Appendix 1) false negative detections of flies were very rare.

We also observed diurnal variation in the number of individuals captured, in trials using *Protein attractant + red cue* and *cue-lure* (positive control with male flies). Male Qfly are reported to call and then mate with females at dusk (Inkeep et al., 2021) and are presumed to forage for raspberry ketone (a natural pre-cursor to cue-lure that increases their attractiveness to females; Khan et al., 2019) during the day. Our results suggest that males may increase their foraging for raspberry ketone at two times of the day, whereas females exhibited protein foraging behaviour just once a day (at around 14:00). See Appendix 3 for more details.

Investigating host suitability of different grape varieties: This study was conducted as a result of anecdotal reports by growers during this project, that grape varieties might differ in susceptibly to Qfly. Our results of no-choice trials demonstrated that female Qfly were more likely to alight on Adora Seedless, Midnight Beauty and Thompson Seedless compared to Cotton Candy and Red Globe grape varieties, and significantly more likely to oviposit in Thompson Seedless compared Cotton Candy grapes. There was no difference in larval survival among the grape varieties examined in this study. More comprehensive field-based studies should, however, be conducted to validate these results in a field (vineyard) context. Refer to Appendix 4 for more details.

Workshop and online confidential grower survey: There were six key findings from the online workshop with pest scouts and industry representatives: i) Growers obtain most of their information about management strategies directly from pest scouts; ii) Pest scouts use their experience to inform growers and do not get much (if any) information from researchers or industry reports; iii) Pest scouts have observed more instances of Qfly in vineyards closer to urban areas than vineyards in regional areas; iv) Cost is the biggest barrier for growers implementing new management strategies (including mass trapping); v) Grower privacy was highlighted as a key challenge in managing Qfly in table grapes; and vi) there was interest as to whether grape varieties might differ in susceptibility to Qfly infestations. These findings were used to inform the confidential grower survey and should be considered when designing outreach activities (for example pest scout representatives should be included in future workshops). See Appendix 7 for more details.

The majority of the growers (57.14%) who responded to the survey reported encountering Qfly in their vineyards every season. When asked why Qfly was a concern, responses ranged from damage to fruit to stigma surrounding Qfly infestations. Time spent managing Qfly was the most common challenge reported by growers and was also the most common barrier reported to the use of mass trapping. Mass trapping strategies that reduce the number of traps such as perimeter trapping could therefore be beneficial to growers. Growers also reported most commonly encountering Qfly as larvae in grapes. Easy to use keys could be made available to growers for distinguishing Qfly (a tephritid fruit fly) from drosophila flies. For more accurate identification, molecular technologies such as the "LAMP" assays could be supplied to industry and / or regional fruit fly coordinators. See Appendix 8 for full details of the survey.

Outputs

Communication and extension. Communication activities originally envisioned for this project (field walks and workshops) were modified as a result of the COVID-19 pandemic to align with travel and gathering restrictions. In hindsight, the new activities were more suitable for the table grape industry due to perceived stigma and privacy concerns expressed by growers in confidential interviews. Activities included an online workshop with pest scouts who work closely with table grape growers in Mildura and surrounding areas; an online survey where growers could share their experiences with us openly and anonymously; two articles published on outcomes of the mass trapping research in the grower accessible magazine, Vines; and a factsheet sharing key findings and recommendations from this research. Research on perimeter trapping is being prepared for publication in a peer-reviewed journal.

Table 1. Output summary

Output	Description	Detail
An article on mass trapping published in a grower accessible source	Two articles were published in the grower-accessible magazine, *Vines.*	*Grand designs: Designing a female mass trapping strategy for managing fruit flies in table grapes*. https://issuu.com/vine-magazine/docs/2_vinemag_vol16iss2_fin_digital2 *Mass trapping female fruit flies in vineyards*. https://issuu.com/vine-magazine/docs/vine_magazine_august_2021/s/13072678 Further details are available in Appendix 5.
Two industry factsheets on female fruit fly mass trapping	One factsheet and one industry article (see extra article above) were produced. The factsheet was double-sided and we believe covered all the essential findings of the project.	The factsheet, *Trapping efficacy in table grape vineyards for area-wide management of Queensland fruit fly* is attached as Appendix 6. For the additional industry article, please see above.
A technical report on development of mass trapping protocols and evaluation programs for Qfly in table	A technical report proving detailed information on the experimental protocols and the results of all mass trapping case studies and additional field work conducted in table grape vineyards as part of this	The technical report has not yet been made publicly available but is included as Appendix 1.

grapes trialled in this project.	project has been submitted in conjunction with this report.	
Three workshops and field walks on fruit fly mass trapping delivered to growers and industry	Workshops and field walks could not go ahead due to travel and gathering restrictions in place due to the COVID-19 pandemic. A variation was approved with Hort Innovation, and in place of these face to face events, we conducted an online confidential grower survey and delivered an online workshop to pest scouts who work closely with table grape growers. Additional lab work addressing a frequent question by growers (do grape varieties differ in susceptibility to Qfly) was also carried out.	The details and findings from the online pest scout workshop are outlined in Appendix 7. Attendees included, Alison McGregor, Jeff Scott, Jenny Treeby, several scouts, and the project team. The results of the online confidential grower survey are available in Appendix 8. There were 7 participants. The experimental protocols and results of the host suitability study are detailed in Appendix 4.

Outcomes

Table 2. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Protocols developed and evaluated for mass trapping of Qfly in vineyards.	Intermediate outcome (M&E plan)	New protocols have been developed that include advice on trap selection, perimeter trapping, and management of nearby fruit trees.	Technical report, Appendix 1, and supplementary material (Appendices 2-8)
Commercial and new prototype female traps screened for effectiveness in table grapes.	Intermediate outcome (M&E plan)	Commercial and prototype traps were screened in line with project aims.	Technical report, Appendix 1.
Growers and advisors acquire new knowledge in female Qfly trapping through workshops and articles.	Intermediate outcome (M&E plan)	Articles and factsheets have been delivered to inform growers (see Outputs). Face to face workshops did not take place due to COVID restrictions (approved in	Pest scout workshop Two articles in *Vines* Factsheet delivering project findings. See supplementary material in Appendices 5-

		project variation)	8
Improved grower and industry knowledge of mass trapping strategies for Qfly management in table grapes	End of project outcome	All outcomes align with this SIP outcome (in M&E plan, SIP Outcome 3), and with Table Grape SIPs (2022-2026) addressing Outcome 2, Strategy 1.	Delivered through all intermediate outcomes above.

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 restrictions throughout the project)	
How relevant was the project to the needs of intended beneficiaries?	The project is very relevant to the needs of beneficiaries (data driven knowledge on the potential for mass trapping in table grapes). Recommendations provided in the technical report and factsheet should directly benefit growers considering the use of Qfly traps as part of their Qfly management toolkit	Additional research could further evaluate perimeter trapping as a cost-effective Qfly management strategy—a key finding of this project.
How well have intended beneficiaries been engaged in the project?	Given the considerable challenges met during two years of COVID restrictions, particularly in Victoria, beneficiaries have been engaged well in the project.	Improved grower engagement in online surveys could be nurtured through connecting with scouts to help encourage participation.
To what extent were engagement processes appropriate to the target audience/s of the project?	The process was very appropriate, especially given grower concerns about confidentiality, and stigma, that were identified through this project.	We identified early in the project that in person workshops were unlikely to engage growers due to stigma surrounding Qfly. We changed our engagement processes to align with this, and also major travel and gathering restrictions due to the COVID-19 pandemic. Speaking to industry representatives prior to committing to engagement processes arose as a key learning opportunity to ensure the engagement activities align with the

		industry culture.
What efforts did the project make to improve efficiency?	The project made considerable efforts to improve efficiency, given two years of COVID restrictions.	

Recommendations

Mass trapping may have value as a strategy for managing Qfly in table grapes, if considered as part of an IPM toolkit. However, placing traps in a regular pattern throughout the crop, as recommended in many mass-trapping programs, may not be cost effective for growers. Instead, a trapping strategy could be developed in line with the findings of this project; one that focuses in particular on trapping around the perimeter and improved management of host plants (especially fruit trees) in the near vicinity. Based on the findings from our case studies, the following recommendations are provided for table grape growers.

- **Which type of trap to use?** Despite some exciting progress in the development of a trap that targets mated female Qfly (*AVR trap*), which was shown to be effective in pome and stone fruit orchards, sticky traps are not recommended for use in table grapes due to contamination with dust (especially where dust storms are likely). Protein-baited and cue-lure baited traps (e.g. *Biotrap*) performed well at capturing females (though mostly unmated) and males, and could be placed strategically to optimise their effectiveness (see below).
- **Should growers consider a strategy that focuses on trapping around the perimeter of the vineyard?** The evidence from the case studies suggests this should certainly be considered. We consistently found that more male and female Qfly were captured in perimeter traps. These traps may well be intercepting Qfly as they travel into and out of the table grape block on a daily basis. There might be particular benefits to trapping along boundary vines adjacent to host "hot-spots" (e.g., nearby fruit trees)—if aiming to minimise traps used. We found that these traps typically captured the most Qfly; but additional research is recommended to evaluate this.
- **To what extent are nearby host trees such as fruit trees an issue?** The results from the case studies suggest that vineyards with host trees ("hot spots") close to their vines have higher populations of Qfly in their vineyard. These trees may provide Qfly populations with resting sites, shelter, mating sites, and hosts for ovipositing females. Host trees may increase the risk of populations establishing if infested fruit is not managed. Nearby trees that are in fruit outside of the table grape growing season should also be managed as these may serve to increase local Qfly populations. Growers are advised to consider the trade-off in maintaining host trees if they are not of commercial value and invest in managing these trees as potential sources of Qfly.
- **Improved knowledge of on-farm Qfly behaviour.** Understanding the location, dispersal, and reproductive habits of Qfly in and around the table grape farm could lead to more effective on-farm management strategies. We recommend future trapping research in this area.
- **Invest in the design and evaluation of mated female traps that retain captured insects without relying on a sticky surface.** This will allow growers to utilise a mated female trap alongside protein-baited traps (that target predominantly unmated females) in a mixed-trapping strategy, and also monitor for female Qfly activity to address any "hot-spots" if female Qfly captures spike.
- **Address grower concerns regarding Qfly in table grapes.** The survey and grower interviews revealed elements of doubt regarding the ability of Qfly to infest table grapes, and also the feelings of stigma surrounding perceived infestations at their vineyards. Further efforts should be made to address this, including drawing on the findings of this project.

Referred Scientific Publications

No publications have been produced to date, but we are preparing the following manuscript for submission in a peer-reviewed journal:

Journal article

Henneken, J., Cunningham, P.J., (in prep). Perimeter trapping as a promising strategy for mass trapping Queensland fruit fly in table grape vineyards

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Intellectual property

No project IP or commercialisation to report

Acknowledgements

Thank you to the many people in the table grape industry and staff at Agriculture Victoria who contributed their knowledge and time to this project. We are especially grateful to the table grape growers who provided access to their vineyards for mass trapping trials, participated in and shared their experiences with us during grower interviews and took the time to complete the grower survey. We are also grateful to the pest scouts who participated in the workshop and the representatives from the Australian Table Grape Association who helped us organise and facilitate the event. We also wish to thank the Regional Fruit Fly Co-ordinators for assisting in monitoring trials and providing their feedback on drafts of the grower survey. The grower survey was distributed to growers by both the Regional Fruit Fly Co-ordinators and the Australian Table Grape Association.

We are grateful to Jakson Bachelor, Lauren Daw, Cait Selleck, Stephen Tobin and Fazila Yousuf from Agriculture Victoria for assisting in the mass trapping trials. We also wish to acknowledge the knowledge and insight Jakson Bachelor lent to this project from his experience working in table grape vineyards.

Appendices

Appendix 1: Technical report

Appendix 2: Modifications to the *AVR trap*

Appendix 3: Modifications to the *RapidAIM* trap

Appendix 4: Variation in host suitability of different grape varieties

Appendix 5: Articles in grower accessible magazine

Appendix 6: Factsheet

Appendix 7: Online pest scout workshop

Appendix 8: Confidential grower survey