Hort Innovation

Final Report

Implementing brown sugar flotation for assuring freedom from fruit fly

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Applied Horticultural Research

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Implementing brown sugar flotation for assuring freedom from fruit fly CY16011

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Content

Summary

While cherries are readily infested by fruit flies in the laboratory, it is rare to find them naturally infested in the field. Despite this, cherries must be subjected to severe quarantine treatments in order to access fruit fly sensitive markets. Previous research (CY14009) demonstrated that brown sugar flotation (BSF) offers a quick and easy way to check for the presence of eggs and larvae in cherry fruit. It can therefore be used to generate data on rates of infestation in cherry fruit. Used as part of a systems approach, it could also be used to verify that batches of cherries are fruit fly free.

The aim of this project was to further refine BSF for cherries and implement it commercially. Trials conducted by NSW DPI demonstrated that the brown sugar solution needed to be at least 16°Brix for eggs to float. It was also shown that the solution could be made several weeks in advance and cold stored, that the time between adding solution and examining for eggs and larvae was not critical and that ensuring cherries were thoroughly crushed maximised recovery of eggs and larvae.

A standardised procedure was developed then adapted following implementation on farm. During the project NSW DPI developed a protocol for application of BSF as part of a systems approach to access South Australia. The protocol mandated examination of a 600 fruit sample from each registered block, to be conducted twice during the season. The process was streamlined accordingly. Over 17,000 NSW cherry fruit from 15 businesses and over 3,000 cherry fruit from Victorian businesses were examined for fruit fly using BSF during the 2018-19 season. While a number of other insects were found, including a single Island fly egg (*Dirioxa pornia*), no Queensland fruit fly (Qfly) eggs or larvae were detected.

The revised procedure has been documented, and provided to the cherry industry along with identification guides for use during the process. In addition, a short, instructional video has been made to demonstrate the process and further assist growers and packers to identify fruit fly larvae.

Keywords

Cherry; Fruit fly; Bactrocera; Brown sugar; Market access; Surveillance

Introduction

Market access for cherries

Sweet cherries are a host of Queensland fruit fly (*Bactrocera tryoni*)[1] and Medfly (*Ceratitis capitata*)[2]. Cherries are readily infested in the laboratory, being extremely attractive to mature flies. Moreover, larvae reared on cherries are able to pupate and emerge as normal, reproductive adult flies.

Although trapping programs show fruit flies do occasionally enter orchards, it is extremely rare to find cherry fruit naturally infested in the field. The reasons for this are unclear. Cherries tend to be grown in areas with cold winters, which make these regions marginal for endemic fruit fly survival[3]. Cherries are also one of the first fruits of summer, which means they are harvested before the major summer build up in fruit fly populations[4]. Another factor may be that cherries are non-climacteric fruit. This means they do not ripen with the burst of volatiles, ethylene release and heightened respiration that occurs for fruit such as peaches, mangoes and tomatoes. This, combined with the thick foliage of cherry trees, may help conceal the fruit from female flies searching for hosts.

Figure 1. Infested cherry fruit showing oviposition hole (left), and second instar Qfly larvae inside a cherry

Despite this, protocols for cherries accessing fruit fly sensitive markets are based on extremely high levels of field infestation. Stringent treatments are mandated to reduce risk to negligible levels. Cold storage, fumigation with methyl bromide, and irradiation are all used as “kill steps” to guarantee cherries are free of fruit fly before export. These treatments significantly impact fruit quality, add cost and reduce efficiency in supply chains. They act as a barrier to trade when, in reality, it is extremely unlikely that any fruit fly larvae were present in the fruit anyway.

Even where a “kill step” is not mandated, such as for sale interstate, requirements often include inspection of packed fruit. For example ICA 21 requires pre-harvest applications of insecticide (Trichlorfon / Maldison or Clothianidin) every 7-10 days, inspection of reject fruit at harvest and postharvest inspection of 2% of packed fruit. Unnecessary insecticide applications disrupt IPM programs and harm non-target insects, while the inspections required are expensive and time-consuming.

Brown sugar flotation

While there is much observational evidence that fruit flies rarely infest cherries, there has been little supporting data for this conclusion. It is clearly difficult to cut open and inspect large volumes of fruit for larvae. Alternatively, fruit may be incubated to allow any larvae present to pupate and emerge as adult flies – a lengthy, messy and smelly process requiring significant labour and storage space.

Brown sugar flotation (BSF) allows rapid separation of larvae from fruit. The process is fast, so can be done in

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[1] Ekman JH, Pristijono P. 2010. Combining radio frequency heating and cool storage to disinfest cherries against Queensland fruit fly. Acta Hort. 877:1441-1448.

[2] Papadopoulos NT, Katsoyannos BI, Nestel D. 2003. Spatial autocorrelation analysis of a Ceratitis capitata (Dipetera: Tephritidae) adult population in a mixed deciduous fruit orchard in northern Greece. Environ. Entomol. 32:319-326.

[3] Dominiak BC, Mavi HS, Nicol HI. 2006. Effect of town microclimate on the Queensland fruit fly Bactrocera tryoni. Aust. J. Exp. Ag. 46:1239-1249.

[4] Yonow T, Zalucki MP, Sutherst RW et al. 2004. Modelling the population dynamics of the Queensland fruit fly, Bactrocera (Dacus) tryoni: a cohort based approach incorporating the effects of weather. Ecol Model. 173:9-30.

real time, allowing inspection and segregation of batches of fruit from different blocks or orchards. It can also be used to gather data on the probability of field infestation of cherry fruit, especially when linked with trapping data; even if male flies are found in traps, this may not parallel infestation in nearby fruit.

Moreover, BSF has been accepted by China, Taiwan and the USA as part of a systems approach. The process is used to verify that cherries from Canada are free of cherry fruit fly. BSF is also widely used to detect spotted wing drosophila in blueberries. It is most likely to be useful when infestation rates are low, as it effectively verifies that other control measures are working correctly.

Recent work in Australia (CY14009) demonstrated that this method can also be used to detect Qfly and Medfly in cherries. There are therefore two main objectives for using BSF in Australia:

- Determine actual levels of fruit fly infestation in cherries, before and/or after packing, as well as in fruit rejected at the packing shed.
- Enhance market access through adoption of BSF within a systems approach

This project was designed to further refine the method and develop a standardised protocol for use of BSF with cherry fruit. This would then be communicated widely, conducting workshops with cherry growers and generating training materials for industry use. The overall objective was to encourage use of the method, allowing collation of data on fruit fly infestation rates in cherries.

Methodology

Developing a standardised protocol for BSF of cherries

Work conducted by NSW DPI for project CY14009 had already developed the basic procedure for BSF of cherries. Key findings from this project were:

- Breaking cherries open using a mechanical crusher instead of by hand improved detection of eggs
- So long as there were >5 eggs inside an infested cherry (Qflies usually deposit 6 eggs in a single oviposition), the probability of detecting at least one egg was 87–100%
- There was 100% probability of detecting at least one larvae from an infested cherry fruit, even if less than 5 were present
- Including 80 or more cherries within a crush slightly reduced detections of larvae

Trials were conducted to further optimise the method. These examined:

- The effect of different sugar concentrations (brix) on detection of eggs
- Whether making the solution several weeks in advance altered its effectiveness
- Whether increasing the settling time improved detection of floating eggs
- The effect of changing the gap settings inside the cherry crusher on the percentage of eggs floating free

NSW DPI conducted these tests at the Ourimbah laboratory using a mechanical cherry crusher. Each trial was replicated three times. A unit consisted of a single, infested cherry fruit added to 34 non-infested cherries. Eggs were used instead of larvae as these are the most difficult to detect. The full methodology is included as **Appendix 1** to this report.

Conduct workshops and develop training materials on use of BSF

Training materials were prepared, tested, and re-drafted based on comments from workshop participants as well as experience implementing the procedure on-farm. The final draft of these materials is included as **Appendix 2** to this report. Workshops were organised through the cherry industry, and the project promoted through the cherry industry newsletter and presentations at

In addition, a short video was made to demonstrate the process.

Implementation of BSF as part of a systems approach for market access

During the project South Australia agreed to trial a systems approach for cherries produced in fruit fly endemic areas. This approach combined in-field monitoring using a trapping grid and postharvest inspection using BSF. The procedure involved sampling 600 reject fruit twice during the harvest season from each of the blocks listed for access to this market.

In December 2018 and January 2019 the BSF procedure was conducted on fruit from orchards in Orange and Young that had nominated to send to this market. The tests were conducted by the project team in collaboration with Lloyd Kingham from NSW DPI Plant Biosecurity and Kate Fielder from CSIRO under the Hort Innovation Asian Markets Frontiers Fund systems approach project. Fifteen businesses submitted samples for testing. Any suspect items from the crushed fruit were extracted using a pipette, labelled and sent to the NSW DPI Collections unit for proper identification.

Outputs

- A standardised procedure has been developed and distributed to members of the cherry industry. It will be made available through the CGA website as a downloadable document and is included as **Appendix 2** to this report.
- The procedure includes some "ID Sheets" to assist with identifying fruit fly eggs and larvae, and distinguishing them from vinegar fly (*Drosophila* sp.)
- A video has also been made to demonstrate the standard procedure. This will be available through the cherry industry website and on YouTube.
- It can currently be viewed at https://vimeo.com/user5670026/review/320689720/1432c92f64
- A draft paper has been prepared for publication based on the process verification trials conducted by NSW DPI.
- Results were presented to members of the cherry industry in June and September 2018.
- An article on the project was provided to the cherry industry newsletter in November 2017.

Outcomes

Developing a standardised protocol for BSF of cherries

The results demonstrated that Brix levels less than approximately 16.5° were ineffective at floating eggs. It is therefore recommended that the brown sugar solution should be at least 17° Brix to ensure effectiveness.

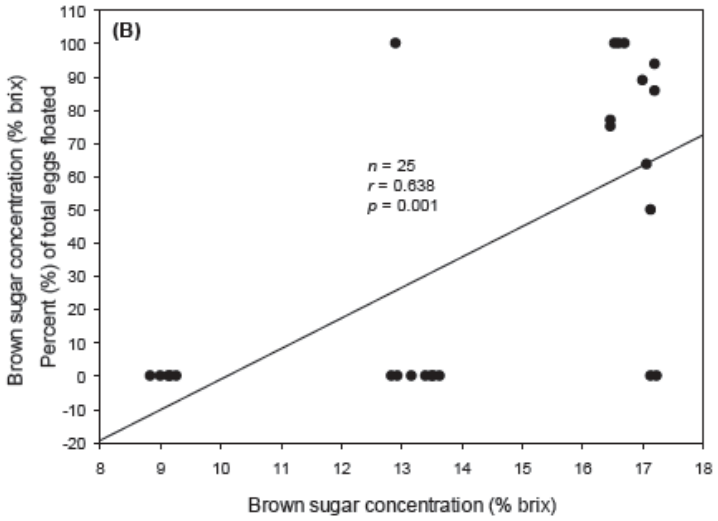

Figure 2. Effect of Brix on the percentage of Qfly eggs floating on the solution.

Brix of brown sugar solutions did not change during up to 19 days storage at 4°C. The percentage of eggs that floated was the same for fresh solutions and solutions that were up to 8 days old. It is concluded that brown sugar solutions can be made up in advance and stored for at least 3 weeks before use.

Settling times of 10 minutes, 1 or 2 hours similarly made no difference to the percentage of eggs that could be seen floating on the solution surface. This demonstrates that the time taken between adding the solution to crushed fruit and examining for signs of eggs and larvae is not critical.

There was no significant difference in recovery rates when cherries were crushed between a 5mm or 3mm gap. However, there was a trend to dislodging more eggs as the cherries were more finely crushed. It seems reasonable to suggest that cherries should be crushed as thoroughly as possible during the BSF procedure, in order to ensure any eggs or larvae are dislodged from the fruit.

Conduct workshops and develop training materials on use of BSF

Workshops were conducted in Young and Orange, NSW, during November 2017 and in Yarck, Victoria, in August 2018. NSW training was conducted away from production areas, which meant it was possible to bring along some (securely packed) eggs and larvae. In Victoria, the training was conducted during winter in order to avoid concerns over this issue. It was important to be able to bring actual eggs and larvae to the sessions, as many participants had not previously seen Qfly larvae. Seeing and understanding what you are looking for is critical to the success of this process.

Figure 3. Dr Solomon Balagawi demonstrating BSF to cherry industry members in Yarck, Victoria. Three packhouses and the CGA were represented at this session.

The results from the project were presented to and discussed with members of the cherry industry at the cherry market access working group meeting in June 2018 in Canberra.

The aim of the project and results were also presented at the National Fruit Fly Council symposium in Melbourne in September 2018. A number of members of the cherry industry were also present at this event.

Implementation of BSF as part of a systems approach for market access

During the 2018-2019 harvest season 17,135 cherries were examined from 15 accredited businesses in Orange and Young, as well as three control blocks in Mudgee. Thirty samples of suspect objects recovered form the containers were submitted to the NSW DPI Collections unit for further identification. These were identified as beetles, thrips, a louse and general debris. One fruit fly egg was recovered from the crushed fruit on January 11. This appeared to be slightly smaller than a Qfly egg, being less than 1mm long.

The DNA of the egg was extracted and successfully multiplied using PCR. It was formally identified as being Island fly (*Dirioxa pornia*). These flies are commonly found in orchards, where they lay eggs in damaged fruit. While the use of reject fruit likely led to this outcome, it also confirms that this fruit is the most likely to be infested, that the procedure is being done correctly, and that it BSF can detect fruit fly eggs in cherries.

At least three NSW businesses used the protocol to export to SA during the 2018-19 season.

In Victoria, four packhouses also submitted samples for BSF testing. In total approximately 3,060 fruit were examined and no fruit fly eggs or larvae were found.

Unfortunately, one of the requirements of the SA protocol was for samples to be submitted two weeks before the official season started. None of the Victorian farms had mature fruit available at this time, so they were unable to use the protocol. While there was interest in extending the timeline for the trial protocol, this was not going to be available until January – by which time the Victorian packhouses interested in sending to SA had already finished for the season.

Monitoring and evaluation

The project has achieved its aims, in that we have successfully developed a standardised protocol for BSF of cherries and then tested, adapted and implemented this protocol at farms and packhouses.

As stated in the Background section, there were two key objectives for encouraging adoption of BSF to test cherries for the presence of fruit fly:

- Determine actual levels of fruit fly infestation in cherries, before and/or after packing, as well as in fruit rejected at the packing shed.
- Enhance market access through adoption of BSF within a systems approach

While there has been limited adoption of the method by the growers themselves, there has been strong adoption at the state biosecurity level. As a result, a good dataset has already been collated demonstrating that Qfly rarely infests cherries. This is extremely encouraging; such data is essential to understanding the true level of risk involved in exporting cherries from Qfly (or Medfly) endemic areas to fruit fly sensitive markets.

Quarantine treatments are usually developed with the assumption that there are extremely high levels of infestation in the exported fruit. Hence, Probit 9 security may be required, equivalent to observed mortality of 99.9968%, or no survivors from 93,750 treated insects[5]. If, through BSF, we can demonstrate that the rate of infestation of cherries by Qfly is negligible, it should logically follow that a lower level of security (than probit 9) should be required.

Collaboration between this project team, NSW Biosecurity and the CSIRO “Systems Approach for Market Access” project has enabled the development and testing of BSF as part of an ICA (Interstate Certification Assurance) for domestic marketing. While small scale and still on trial, the protocol was used by at least three NSW businesses this season.

Project progress and results have been communicated to the cherry industry market access working group (through Andrea Magiafoglou, Charlotte Brunt and Hugh Molloy). Milestones have been submitted as required

[5] Corcoran RJ. 2000. Use of Probits and “Probit 9” in quarantine entomology. Queensland Horticulture Institute discussion paper.

Recommendations

Implementation of BSF has already yielded some excellent data and demonstrated that this technique can be used as part of a systems approach for market access. If repeated over the next few seasons, this information could be used to implement a more widely accepted systems approach for market access. It could even support conditional non-host status for cherries exported from fruit fly endemic areas and/or a reduction in quarantine treatment severity based on more accurate assessment of risk.

However, the protocol still needs some refinement. The major issue identified was the requirement to submit both sets of BSF results two weeks prior to when fruit shipments were expected to commence. Most farms did not have fruit available for testing at this early stage. This issue was made even more problematic by hot weather in December, which meant the season was extremely short. Moreover, tests conducted two weeks or more before harvest do not provide proper verification that cherries are indeed free of fruit fly.

1. It is recommended that Hort Innovation, CGA and the State Biosecurity agencies continue the testing conducted this season and work towards wider acceptance of the proposed ICA protocol. This will ensure that data on infestation rates continues to be collected into the future by both private businesses and state authorities. The protocol also needs to be refined to so that BSF testing occurs within the normal harvest window, in parallel with fruit exports.

A number of other pests were found during the BSF procedure. These were identified as beetle eggs, thrips and other small insects. It could be useful to also gather this data as another indicator of what (non-quarantine) pests are active in the orchard. To make this information easy to collate, an accompanying guide to identifying eggs and larvae of beetles etc could be provided to growers.

2. Investigate whether BSF can be used to provide baseline data of insect pests generally, and produce a reference page / poster of eggs and larvae that may be found using this method.

It was noted during the course of this project that modern colour vision sorting machines include NIR systems. These are used to detect fruit softening and, with proper calibration, can be extremely accurate. Cherries infested by fruit fly almost immediately start to soften due to introduction of bacteria as well as larval feeding. If these fruit could be detected and rejected during sorting, this could provide another element to be used in a systems approach.

3. It is recommended that Hort Innovation and the cherry industry consider funding a small research activity examining whether modern NIR sorting machines can detect fruit fly larvae in cherries and, if so, from what life stage.

Monitoring of fruit fly populations in traps is used to determine whether an area is fruit fly free, as well as trigger declaration of outbreaks. However, pheromone traps only attract male flies. Without data that links trap catches to larvae in fruit, assumptions regarding the risk of fruit infestation are purely speculative. This linking data is rarely collected because rearing fruit fly out of large volumes of fruit is expensive, time consuming and requires large amounts of storage space and equipment. However, BSF now offers an alternative way to detect fruit fly eggs and larvae in fruit.

Linking data on trap catches with actual infestation rates would be valuable not only for the cherry industry, but to all industries affected by fruit fly.

4. It is recommended that Hort Innovation consider developing a multi-industry project using BSF to examine the relationship between prevalence of flies in traps and presence of eggs and larvae in fruit. Industries that could benefit from investment in such a project include citrus, stonefruit and berry crops.

Refereed scientific publications

DRAFTS ONLY, prepared for the Journal of Economic Entomology.

Balagawi S, Broughton S, Liang W, Archer J, Cruickshank D, Cruickshank C, Barchia I. 2018. Evaluation of brown sugar flotation procedure for detection of Queensland and Mediterranean fruit fly (Diptera: Tephritidae) infestation in Australian cherry fruits.

(Note: This paper is based on data from the previous project conducted by NSW DPI, but has been reviewed as part of the current project)

Balagawi S, Cruickshank D, Cruickshank C, Ekman J. 2018. Effect of parameters of the brown sugar flotation procedure on its ability in detecting Queensland fruit fly eggs in a cherry fruit.

Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

Acknowledgements

This project was greatly improved by the involvement of Cherry Growers of Australia and members of the Cherry Market Access Working Group. It would also not have been possible without the support of the cherry packhouses who participated in the workshops and training and gave valuable feedback relating to the standard protocol.

The project team would particularly like to thank:

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Hugh Molloy – Antico International

Joe Falco – Nordiko

Matt Smyth – Department of Agriculture and Water Resources

Lloyd Kingham – NSW DPI Biosecurity

Kathryn Fielder – CSIRO Systems Approach for Market Access

Appendices

Confidential

Appendix 1 – Results of trials conducted by NSW DPI

Appendix 2 – Procedure for BSF for cherries

Appendix 3 – Balagawi et al journal article abstract only; refinement of the BSF procedure for cherries