Fruit Waste Management for QFF – Scoping study

Milestone Activity Report

PROJECT NAME	Fruit Waste Management for QFF – Scoping study
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ACTIVITY 3	Small-scale feasibility study of one or more waste-management options

BOX HILL INSTITUTE

VICTORIA State Government

Fruit Waste Management for QFF – Scoping study

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Summary

Strawberries and raspberries were heavily inoculated with *Bactrocera tryoni* (Queensland fruit fly) and divided into control and fermentation treatment groups. Samples of controls and treatment groups were taken at regular intervals over a 2-week period, and placed in gauze-sealed beakers containing potting mix to allow larvae to pupate. Flies that emerged from sample beakers were counted up to 30 days from inoculation.

No flies emerged from treated raspberries or strawberries after 24 hr of treatment. Flies emerged from control samples up to 7 days from the beginning of treatment.

The mechanism of action for the killing of *B. tryoni* larvae was not established, however the study findings support the use of fermentation to manage rejected strawberries and raspberries that may be infested with *B. tryoni*.

Introduction

Disposal of rejected fruit from farm operations and fallen fruit in the field can increase the risk posed by *B. tryoni* in favourable conditions if good biosecurity practices are not followed. Appropriate disposal of all reject fruit is considered best practice for production and packhouse operations to ensure fruit fly risk is mitigated.

A literature review of fruit waste management options for QFF (Turner, 2019) found several methodologies that could be used to safely manage rejected fruit on small to medium sized enterprises (SME) without the use of chemicals, and where the threat of *B. tryoni* infestation exists.

To understand the practicality and effectiveness of the various reject-fruit management methods a panel of four fruit growers in Victoria's Yarra Valley region were engaged to critically review Turner's findings and give an opinion regarding their preferred option for managing rejected fruit.

Feedback from the panel concluded that:

- Mulching field fruits may not be applicable for all fruit growers as mulching machinery may not be owned or readily available for all fruit growers. Some fruit crops may not respond well to a pass with a typical 'mulching' implement at harvest time (for example, where crops are fragile and may be hit by machinery or where overgrown plants encroaching into the inter-row prevent easy entry by machine).
- Heat treatments and deep burial that rely on specific parameters such as temperature and burial depths to dispose of rejected fruit are difficult to provide assurance and confidence to growers in sensitive regions that fruit treatment has been successful.
- In-field augmentoria structures that rely on netting to prevent pupae and adult flies escaping into the environment may be impractical. Netting exposed to weather, machinery and equipment during harvest periods provides a risk of the netting becoming damaged, compromising the structure's integrity.

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- Cold storage of waste is not an option for some growers due to the absence of available isolated coolroom space, and the risks associated with the infested fruit's proximity to sound or first class product if placed in a shared chamber, e.g. disease and ethylene exposure.
- Fermentation to treat post-harvest fruits is practical but also produces a waste product after the fermentation process is complete. Determining the composition of the fermentation product would be necessary to understand whether the product can be utilised on-farm.

The grower panellists recommended that further investigation be made to understand the effectiveness of fermentation to manage reject fruit. As a result, two fruit trials were designed to evaluate the use of fermentation to manage rejected strawberries and raspberries, representing two fruits that are grown in the Yarra Valley which regularly have small volumes of reject fruit. Both industries are involved in the development market access protocol that requires appropriate disposal methods of waste fruit for property certification.

Methods

Fresh strawberries (14.4kg) and raspberries (14.9kg) were infested with *B. tryoni* at NSW Department of Primary Industries, Ourimbah, under supervision of Dr Solomon Balagawi (Figures 1, 2 and 3). Fruit was laid in a single layer on the top of mesh-covered cages containing Queensland fruit flies for approximately 45 minutes, and then checked visually to ensure adequate laying of eggs in the fruit (Figure 4).

Figure 1. Strawberries laid down for *B. tryoni* infestation.

Figure 2. Fruit flies infecting strawberries.

Figure 3. Raspberries laid down for *B. tryoni* infection

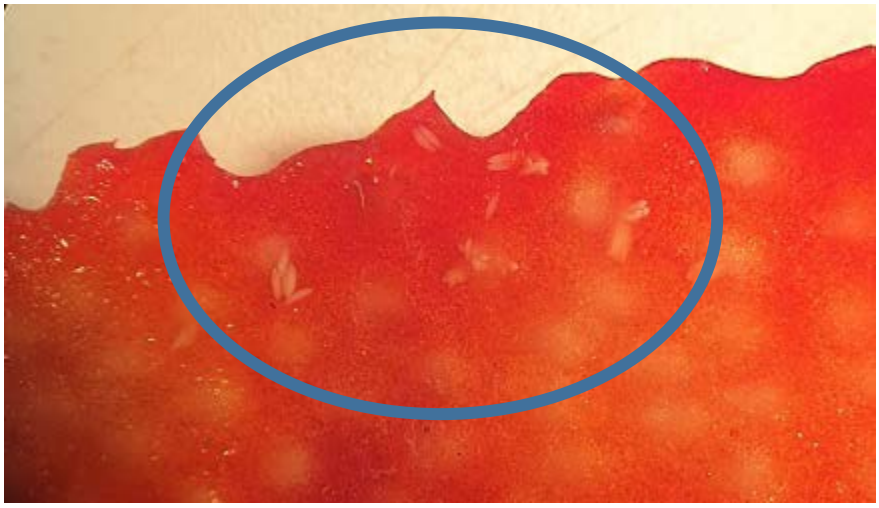

Figure 4. Eggs of *B. tryoni* in sample of strawberry.

Two 32 litre fermentation tanks were set up, each containing 13kg fruit that had been infested 3-4 days prior with *B. tryoni*, and then made up to approximately 25 litres total volume with distilled water. The fermentation tanks were sealed with a water-filled release valve in the lid to minimise contamination, however the lids of the vessels were taken off for each sample time point allowing for regular exchange of air within the headspace. Fruit in the fermentation tanks were gently agitated initially and prior to each sample collection.

A 1kg control sample of each of the infested fruits was kept separately in a 5 litre beaker covered with gauze. Control samples were not disturbed other than to take samples.

Liquid in the vessels was tested for pH, sugar, alcohol, and temperature. Ambient temperatures ranged between 22-27^{o}C.

Fruit samples (Approximately 21g representing 2 strawberries, or 14g representing 4 raspberries) were taken from the approximate centre of the floating fruit mass within the fermentation vessels. Similar samples sizes were taken from the top surface of the fruit in the control beakers without otherwise disturbing the fruit.

Duplicate fruit samples from controls and fermentation vessels were placed in beakers (600ml) containing 170g potting mix and wetted with 10ml of water (Figure 5). Beakers were covered with gauze to trap emerging flies and were checked daily for 30 days for emerging flies (except weekends and public holidays).

Initial samples were taken immediately after adding fruit and water and gentle agitating. Samples were taken at 0, 1, 2, 3, 4, 7, 10 and 14 days. At 14 days control samples showed extensive mould and pupae and sampling was ceased.

Figure 5. Fruit samples were added to 600 ml beakers containing 170g potting mix, covered with gauze, and then checked daily for emerging flies.

Results

Fruit flies began emerging after 17 days from infestation (Figure 6). Raspberry control samples were the first to have emerging flies. No flies were observed to emerge from raspberry fermentation samples. No further emergence from raspberry samples was observed after day 18.

Strawberry samples began to emerge at day 19 and emergence was observed in control samples until day 28. Flies emerged from samples taken from fermentation vessels over a three day period from day 20-22. No flies emerged from strawberry fermentation samples after day 22.

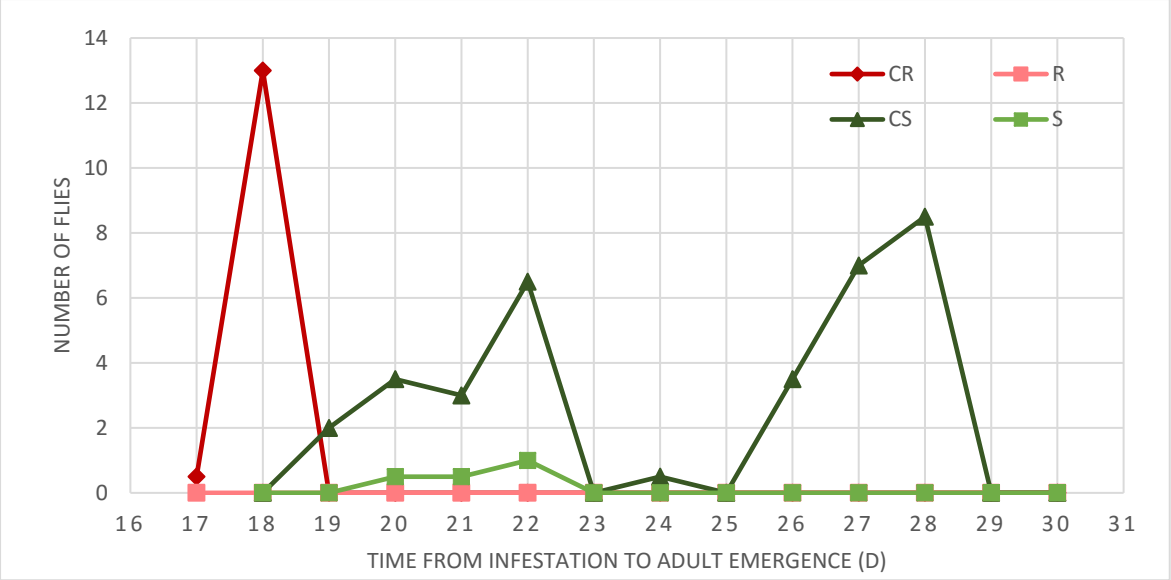

Figure 6. Emergence of *B.tyroni* flies from raspberry (R) and strawberry (S) samples and respective controls (CR, CS) over time from infestation.

Sugars in both the raspberry and strawberry fermentation vessels fell over the first day and alcohol increased to a final alcohol content of 6.0% consistent with expectations (Figure 7). A slight increase was observed in pH from 3.1 to 3.6 over the 14 days of treatment.

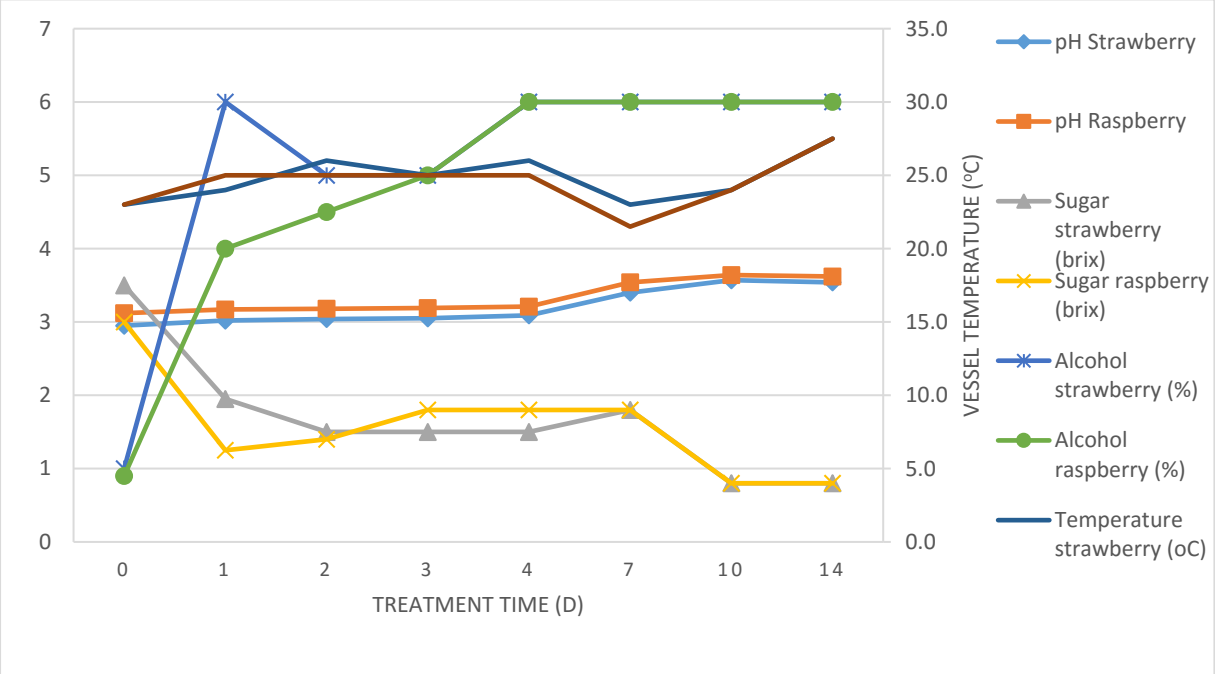

Figure 7. Changes in pH, sugar and alcohol (primary axis) and temperature (secondary axis) in the raspberry and strawberry fermentation vessels over the 14 day treatment time.

Raspberries

Flies emerged from raspberry control samples taken from the first four days of sampling (Figure 8). On average about 3 flies emerged from duplicate samples taken over this period. No flies were observed to emerge from control samples taken after 4 days.

No flies were observed to emerge from samples taken from fermentation vessels at any time.

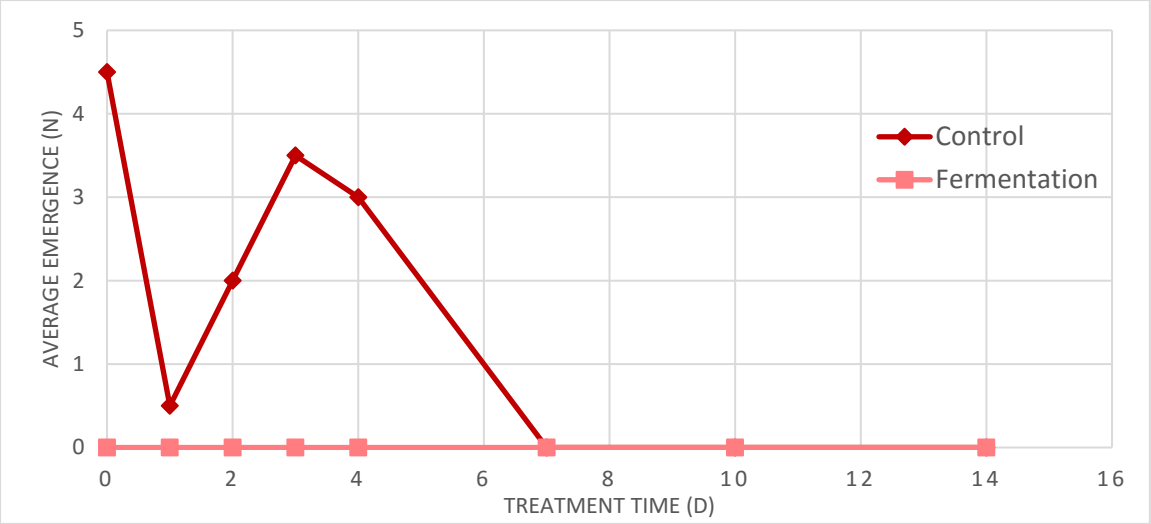

Figure 8. Effect of fermentation treatment on *B. tryoni* flies emerging from duplicate raspberry samples.

Strawberries

Flies emerged from strawberry control samples taken from the first 7 days of sampling (Figure 9). On average about 6 flies emerged from duplicate control samples taken over this period. No flies were observed to emerge from control samples taken after 7 days.

Flies emerged from strawberry samples taken from the fermentation vessels at time zero and at day 1. No flies were observed to emerge in fermentation samples taken after day 1.

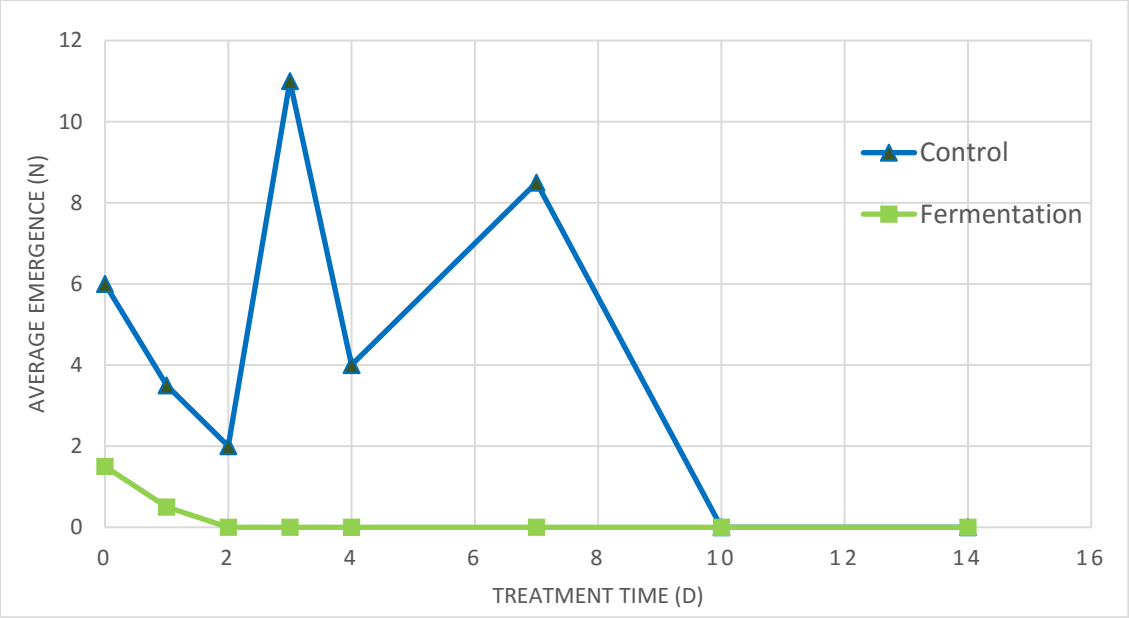

Figure 9. Effect of fermentation treatment on *B. tryoni* flies emerging from duplicate strawberry samples.

Discussion

Fruit was visually inspected during the infection process to check for 'sting' marks on fruit and *B. tryoni* eggs in the infested fruit. After approximately 45 minutes of infestation samples were deemed to be sufficiently infested. After infesting strawberries the fruit flies were given 24 hours to recover before being presented with raspberries to infest.

Samples of fruit from controls were visually checked at each sample time point to check for the presence of eggs or viable larvae. Unconfirmed *B. tryoni* larvae were observed in raspberry control samples up to 7 days, and in strawberries control samples up to 10 days. No visible *B. tryoni* larvae were observed in the 14 day control sample although many pupae were observed on the walls of the control vessel.

Some Drosophila contamination was observed in the control samples which were open to the laboratory environment though gauze covering. The first unconfirmed Drosophila larvae was noted at day 3 in the control sample and by 14 days there were many presumed Drosophila larvae noted.

Whilst observed larvae were not confirmed as either *B. tryoni* or Drosophila, presumed *B. tryoni* identifications were generally supported by the emergence of flies with the exception that numerous presumed *B. tryoni* larvae in 2nd or 3rd instar stage were observed in the raspberry control samples at day 7. No adult flies were observed to emerge from day 7 raspberry samples however no explanation could be given for this difference other than chance.

A lull in emergence of flies from day 23-25 was observed in the strawberry control samples (Figure 6) and may have been due to cooler nights experienced during this time. Samples in beakers were exposed to laboratory temperatures which were not controlled at night which may have contributed to the low emergence rates during these days.

The effect of the treatment on the viability of *B. tryoni* was observed from the first time point. No flies emerged from raspberries that had been submerged in the fermentation vessel, and no flies emerged from the strawberry treatment after one day of submersion. The immediate effect on viability may indicate that the cause of death is at least partly related to submersion and may be a combined effect of submersion and fermentation. However, the design of this project did not allow for these two variables to be distinguished.

The complete destruction of viable *B. tryoni,* in two days, in what would be considered in the field to be an extremely high level of infestation gives strong support for this methodology to be used for the management of reject raspberries or strawberries that are suspected to be infested with *B. tryoni*. Since the mechanism of destruction of *B. tryoni* cannot be determined from this study, no extrapolation of these findings should be extended to the treatment of other fruits.

Conclusions

This project set out to evaluate the use of fermentation as a biosecurity control to safely manage reject fruit that may contain *B. tryoni*. The study supported the use of fermentation to kill high levels of *B. tryoni* infestation in strawberries and raspberries but was unable to provide a mechanism for the destruction of the *B. tryoni*. Berries that were submerged, and then immediately sampled, strongly (but not completely) inhibited the emergence of flies indicating that alcohol is probably not the sole cause of larval death and that other factors such as anoxia are probably involved.

Without a good understanding of the mechanism of action, the findings of this study should only be applied to strawberries and raspberries under the same conditions as used in this trial.

Acknowledgments:

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References

Turner, L. (2019). *Fruit Waste Management for QFF.* Box Hill Institute.