Managing Fruit Fly Regional Grants Program – Round Two

Yarra Valley Regional Action Plan – Training and Research

Fruit Waste Management for QFF

Activity 1 – Literature review

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Abstract

Bactrocera tryoni (Queensland fruit fly) is present in much of Victoria and have recently been detected in the Yarra Valley Pest Free Place of Production threatening commercial and domestic fruit growing in the region. Limited information is available to small to medium size growers about on-farm waste fruit disposal methods that minimise the risk of fruit becoming infested. This study reviewed mechanical destruction, burial, augmentorium, heat and fermentation methods of fruit waste disposal to highlight studies that could help growers manage Queensland fruit fly risk.

Introduction

Bactrocera tryoni (Queensland fruit fly) is one of the most significant biosecurity pests in Australia's international and domestic horticultural markets (Reynolds, *et al.* 2017). *B. tryoni* are highly destructive as larvae and are polyphagous feeders that induce fruit decay and premature fruit-drop in a broad range of host fruit and vegetables (Clarke *at al.* 2011). *B. tryoni* are native to Queensland, although dispersal has led to the flies being widespread in Eastern Australia with occasional incursions in South Australia and Western Australia (Dominiak and Mapson 2017) (Raphael *et al.* 2014)

A variety of baits and insecticide cover sprays are traditional control techniques to manage pest insects (Raphael *et al.* 2014). Additionally, advanced techniques such as the Sterile Insect Technique (SIT) involving the release of large populations of sterile insects into small scale areas where pests are present has been implemented as a part of area wide management for fruit flies (Raphael *et al.* 2014). Little guidance is available for specific, short term, non-chemical approaches (Leach *at al.* 2018) that will hygienically manage waste fruit infested with *B. tryoni.*

Funding – Region grant explanation

The aim of this project was to investigate methodologies applicable for small to medium sized farms and commercial growers to hygienically disinfest and dispose of unwanted waste fruit in regions where fruit fly is present. As limited post-harvest fruit methodologies to specifically manage *B. tryoni* have been tested, the literature review explores a range of non-chemical techniques to manage species beyond *B. tryoni* across a variety of host fruit and vegetables. Previously recognised *B. tryoni* management strategies such as cold storage and chemical controls have been excluded from this paper as the purpose of this review was to determine alternative techniques that would be practical for a wide population of fruit and vegetable growers to implement.

Discussion

Mechanical control

Mechanically crushing infested fruit is a sanitation technique used to destroy the fruit and larvae within the fruit (Vargas *et al.* 2008). Different mechanical control techniques have been tested for their effectiveness at culling larvae to disrupt the fruit fly lifecycle and prevent the emergence of adult fruit flies. Klungness *et al* 2005 in Kula, Hawaii, used a metal soil compacter to smash zucchinis obtained from a field infested with the melon fly, *Bactrocera cucurbitae* (Coquillett). After applying the smashing technique to the infested fruit, the adult fly emergence rate was compared with fruit left whole. Results indicate that the smashing technique reduced the emergence rate of flies by 43.4%. There is limited information provided in the study regarding the density of fruit that was smashed and degree to which the smashing technique was undertaken.

In the Moncada area of Valencia, Spain, Chueca *et al.* (2013) trialled the difference between larval mortality rate when applying either smashing or grinding techniques to infested fruit. Oranges used were artificially infested with Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) larvae by puncturing a 10 mm diameter hole 20 mm deep into the fruit and removing 'a plug'. Larvae (15) were then placed into the fruit and the hole was filled with cotton.

To undertake the crushing treatment, infested oranges were introduced into a black plastic bag (54 x 60cm) and smashed by hand using a wooden roller (35 x 6cm). For the grinding treatment, infested oranges were cut into halves and ground for 8 seconds using an electrical household blender (455 rpm). Treated fruit was then placed into appropriate containers and stored in a climatically controlled chamber, optimal for the development of larvae into pupae. To determine larval mortality, the number of pupae that developed was compared to the number of larvae per fruit. Results from the treatments indicated that both treatments reduce the survival rate *C. capitata* larvae, although the grinding technique created a significantly higher larval morality rate (81%) than the crushing technique (29%).

A version of grinding was also tested under field conditions in an orchard using horizontal shaft-suspended wood shredders (Enguix, TRR-150 model) to treat fallen fruit. Several trials were undertaken using a variety of conditions to determine the settings that would produce the greatest percentage of ground fruit. The operational parameters included 2 tractor speeds (2.0 and 4.4 kph), 2 types of cutting tools (either 32 knives or 16 hammers), and 2 levels of shaft rotation speed (1,840 or 2,500 rpm). In each trial, either 300 mandarins or 200 oranges were placed into the middle of an orchard lane over a length of 100m. A shredder was applied to the field fruit at a set distance of 1cm between the ground and the base of the shredder throughout all trails.

Chueca *et al.* (2013) found all settings tested on the wood shredder produced relatively high percentages of completely ground fruit for both fruits trialled (89.2% to 97.6%). The greatest variation to completely grind the fallen fruit was observed between the type of cutting tools, where hammers produced a higher percentage (approximately 97%) of completely ground fruit in comparison to knives (approximately 89%). This highlighting that wood shredders fitted with hammer cutting tools are more effective to destroyed fallen fruit. No significant differences in the percentage of completely ground fruit was observed between the 2 tractor speeds (95 % at 2.0 kph and 95% at 4.4kph), and the 2 shaft rotation speeds (94% at 1,840 rpm and 96% at 2,500 rpm). Due to this finding, the authors suggest that growers use a combination of a shaft rotation speed that uses less power and a tractor speed that reduces operational time.

To determine whether a grinding treatment would affect the *C. capitata* population, Chueca *et al. (2013)* applied horizontal shaft-suspended wood shredders equipped with 15 hammers, a tractor speed of 4.4 kph and a shaft rotation speed of 1,840 rpm to dropped clementine fruit in fields. The number of adults captured in traps within the fields with ground fruit was compared to an adjacent field where no grinding treatments were applied. These conditions resulted in a 46% reduction of adult flies captured in the treated fields when comparing to control fields.

Based on the effectiveness of wood shredders completely grinding fruit and reducing field populations of adult flies, Chueca *et al.* (2013) suggests that this machinery is appropriate for growers to apply to larvae infested fruit in the field as a population management strategy.

Burial of infested fruit

To prevent the development of fruit flies into reproductive adults, infested fruit can be collected and deeply buried (Dhillon *et al.* 2005). As *B. tryoni* pupate within soil (Hulthen and Clarke 2006), the depth at which infested fruit is buried must exceed the depth of pupae survival. There is limited research currently available regarding soil depth for the survival of *B. tryoni*, and for this reason we looked into the effects of different burial depths on other species of Tephritidae that undergo similar developmental stages in soil.

Ali Ahmed *et al.* (2007) found that the deeper the burial of *C. capitata* pupae, the lower the number of emerged adult flies. Depths ranging from 2 cm to 20 cm were trialled to assess the number of pupae that develop into adult flies. Results demonstrate that depths of 2 and 10 cm had adult fly emergence rates of 69% and 58% respectively, whereas, depths of 15 and 20 cm had adult fly emergence rates of 25% and 23% respectively. The deepest burial tested produced over a 20% adult fly emergence rate, suggesting that pupae must be buried beyond 20 cm in fruit fly management.

Similar outcomes were observed in the emergence of *Bactrocera cucurbitae* (melon flies) in a field sanitation study conducted by Klungness *et al.* (2005). Burial depth tested included 0.15 m, 0.3 m and 0.46 m which recovered 103 ± na, 26 ± 8.7, and 0 numbers of adult flies respectively. As the trials used zucchinis collected from fields infested with melon fly larvae, the number of larvae within the fruit prior to the burial treatments is unknown. To capture adult flies, a window screen measuring 1.2 m^2 covered the treatment area, where 20 cm of the screen edges were covered with soil. Emerged adult flies trapped under the window screen were collected and counted.

The results from Klungness *et al.* (2005) suggest that burying infested fruit at a depth of 0.46 m below soil surface assisted in managing the disposal of fruit infested with *B. cucurbitae* and may provide insight into waste management of fruit infested with other species of fruit flies that undergo pupation in soil.

Deep burial of infested fruit produced a significant effect on pupal mortality when reaching adequate depths (Klungness *et al.* 2005). Alternatively, if sufficient burial depths aren't reached, pupation may proceed resulting in the emergence of adult flies. Additional measures such as fitting a window screen over the deep burial treatment areas may provide an extra form of protection against potentially emerging flies. Management of fruit fly via deep burial may also disrupt the lifecycle of other pest insects such as codling moth (*Cydia pomonella*) (Baughman *et al.* 2015).

Augmentorium

An augmentorium is a tent-like structure that has been developed for tephritid fly management while allowing the survival of natural enemies used for biological control. The augmentorium is designed to enclose fruit infested with tephritid fly larvae, capture the adult flies that develop and allowing secondary control by parasitoids (Jang *et al.* 2007).

Klungness *et al.* (2005) showed that an augmentorium effectively contained adult *B. cucurbitae* when trialling field sanitation treatments on infested fruit. The augmentorium was constructed using a translucent fine mesh (Lumite® 55 x 55) for a base that prevents larvae escaping from infested fruit into the soil. The top of the augmentorium was constructed from Phifertex® mesh (1 mm x 1.3 mm) that restricted the passage of melon flies but allowed parasitoids to pass though. Fruit were introduced into the augmentorium via a sock-like structure made from Lumite® mesh creating a simple 'airlock' entrance.

Augmentoria have also been demonstrated to be efficient in the field. In a study conducted by Jang *et al.* (2007), the implementation of augmentoria as a field sanitation tool as part of a Melon Fly Area Wide Project in Hawaii was assessed. To undertake field sanitation, farms involved were required to clear fields weekly of damaged fruit and place the fruit within an augmentorium.

The Hawaiian study team calculated that the 21,214 melon flies captured within bait traps inside augmentoria prevented a first-generation progeny of 3,367,722. The authors suggested that augmentoria were ideal for growers on small farms as little labour or costs were involved in comparison to other disposal methods.

Additional findings from other studies highlight the advantages of using field augmentoria including the resulting compost that can be used by the grower (Deguine *et al*. 2015).

Deguine *et al.* (2010) on Reunion Island, trialled a variety of mesh materials fitted on augmentoria to determine the most effective at capturing adult Tepheritid flies, while allowing the escape of parasitiods. All 3 sizes of mesh tested (1 x 1.5 mm, 1.2 x 1.3 mm and 1.9 x 1.9 mm), were 100% effective at capturing all 3 species of Tepheritid flies that emerged: *B. cucurbitae, C. capitata, Bactrocera zonata* (peach fruit fly). The largest mesh size (1.9 x 1.9 mm manufactured by Intermas Nets, South Australia) resulted in the highest percentage of parasitiods (*Fopius arisanus* and *Psyttalia fletcheri*) passing through. This suggests that a 1.9 x 1.9 mm mesh may be an appropriate size to use on an augmentoria if parasitiods are being used.

No studies were found to have evaluated using augmentoria for managing *B. tryoni,* although Ekman (2016) found a durable, lightweight, exclusion netting (Vegenet) with a mesh hole size of (1 x 3 mm) to successfully prevent the access of adult *B. tryoni* to vegetable crops.

Heat treatment

Heat is an alternative to cold treatment to disinfest waste fruit. As cold storage utilises a large amount of energy, costs associated with cold treatment can be prohibitively high and unpopular (Jessup *et al.* 1998). For heat to be an effective control strategy of *B. tryoni*, the temperature must exceed the heat-stress threshold of 36˚C (Yonow and Sutherst 1998). To ensure methods that utilise heat to disinfest fruit are effective, heat will often be combined with other factors.

Mild heat and packaging

Jessup *et al.* (1998) used mild heat (38°C) along with polyethylene bags (38µm, low-density) over a duration of 0 to 4 days as a simple method to disinfest various fruit. Fruit containing mature *B. tryoni* eggs or second instar larvae were placed into either unsealed or sealed polyethylene bags for heat treatment. In the sealed bags, the air between the fruit and the bag was pressed out manually then sealed using rubber bands, creating a modified environment. Fruit in the unsealed treatments were placed on trays, exposing the fruit to free air. Both treatments were stored at a constant temperature of 38°C, which was attained after 2 hours, then left for 0 to 4 days. After undergoing the required storage time, fruit were removed and stored in conditions that would allow for pupation.

Infested fruit placed inside sealed bags for more 3 days at a constant mild heat of 38°C resulted in the complete destruction of all mature eggs and second instar larvae within fruit as no insect survived to pupate. Infected fruits with less than 3 days of exposure to these conditions allowed for insect survival as pupation was observed after fruits were removed from treatment. This suggesting that the duration of exposure to mild heat has an influence on egg and larval mortality.

Other factors that influenced egg and larval survival was heat treatment and sealed packaging. The authors concluded that the combination of heat treatment and sealed packaging is more effective at killing insects than heat treatment alone. This suggested that mortality may be influenced by the modified atmosphere created inside the sealed bag caused by reduced oxygen and increased carbon dioxide concentrations. Additionally, relative humidity may also impact insect survival as humidity inside sealed bags (100%) was higher than the ambient relative humidity for unsealed bags (50-70%). Overall, Jessup *et al.* (1998) found combining mild heat and polyethylene bags as an effective, relatively inexpensive fruit disinfestation method.

A similar study conducted by Ndiaya *et al.* (2008) bagged fruit in 0.8 x 0.5m black plastic bags with exposure to solar heat to disinfest fruit from oriental fruit fly *Bactrocera invadens* (Drew) and mango fruit fly *Ceratitis cosyra* (Walker). The technique was found to be practical, however, bag resistance needs to be considered as tearing may occur. According to the authors, if bags are resistant against tearing, this technique may be an effective method to treat waste fruit before disposal.

Solar heat and plastic covering

The use of solar heat may be an appropriate heating method to provide sufficient internal temperatures within fruit to disinfest fruit. Jenkins *et al.* (2008) trialled the heat produced by solar energy as a sustainable heat treatment method to disinfest mangos containing West Indian fruit fly *Anastrepha obliqua* larvae. Abscised mangos collected from a field were placed into plastic bins containing vermiculite and stored in full sun position, covered and uncovered with a black garbage bag. As fruit were obtained from a field, the number of larvae per fruit prior to treatment was unknown. All treatments were undertaken over a duration of 3 days where temperature within the mangos was collected several times per day. After treatment, all fruit was removed and placed under laboratory conditions to monitor the survival of larvae and pupae.

Peak temperatures of fruit covered in the sun reached 59°C, whereas, uncovered fruit placed in the sun reached 52°C suggesting that the plastic bag covering assists in reaching higher temperatures more appropriate for larval control within fruit.

Whist exposure of larvae to these temperatures had a significant impact on larval mortality, not all larvae were killed. This may have been influenced by daily temperature fluctuations which ranging from 25°C and 59°C on clear days between 06:00 am and 14:00 pm. Moreover, cloud coverage may have reduced temperature to levels more conducive to larvae survival. The addition of black garbage bags covering fruit assisted in maintaining internal temperatures within fruit. Although the temperature through solar heating is not constant, covering waste fruits with plastic in sun-exposed positions can kill a large portion of larvae within fruit.

Heat through composting

An alternative heat treatment to disinfest fruit that incorporates the disposal of waste fruit is through composting. Composing is a biodegradation process of mixed organic substrates driven by microorganisms (Diaz and de Bertoldi 2007)**.** To ensure a sanitary product is produced through composting exposure time, internal temperature and several other factors are essential to eliminate high-risk organisms (Noble *et al.* 2009).

Kendra *et al.* (2007) composted fruit infested with *Anastrepha suspensa* larvae (Caribbean fruit fly) to determine the likelihood of adult flies emerging from residential compost piles. Compost heaps were constructed using wooden frames (1.2 x 1.2 m) filled with a 1:1 compost mixture of grass clippings and wood chips to replicate backyard composting. Prior to placing infested fruit 4-5 cm within each pile, the piles were turned on a weekly basis to promote decomposition. A pyramid screen structure was fitted above each compost pile 5 days after infested fruit were placed within the compost, creating a closed system to capture emerging adult fruit flies.

Control bins were constructed containing infested fruit stored at 25°C in a laboratory to compare adult emergence. In all testing conditions, the emergence of adult flies and internal temperature of the compost pile were recorded daily over the duration of the experiment. A total of 4 field trials were conducted from late summer to early spring for 30 days.

Composting resulted in an 11% average emergence of adult flies in all field trials relative to the controls indicating there is a small risk associated with residential composting on promoting development of fruit flies. Internal temperatures of the compost had a direct relationship with adult emergence, where the higher the temperature, the lower the emergence. Compost piles where temperature exceeded 48°C, which only occurred in late autumn, had the greatest impact on larval survival as the average emergence of adult flies was 0.2 ± 0.1. Spring conditions which had the lowest maximum compost temperature of 28.3°C produced highest emergence of 4.8 ± 1 adult flies.

The risk of adult emergence of fruit flies through composting may be reduced by promoting the internal temperatures of compost piles. This can be achieved by undertaking practices such as frequent pile turning and keeping the pile moist (Kendra *et al.* 2007)

Crohn *et al.* (2008) recommend that infested fruit should be placed deep within the pile as temperature increases and chance of survival reduced. No *Bactrocera oleae* (olive fruit fly) were able to survive more than 4 days at depths of over 30 and 100 cm within unturned chip yard waste piles. Cini *et al.* (2012) caution that if high internal temperatures aren't reached, the warm decomposing environment could in fact favour the development of fruit fly larvae within infested fruit.

Fermentation

Fermentation in an anaerobic process where yeast and bacteria convert organic compounds such as sugars into acetic acid, ethanol and carbon dioxide (Noble *et al*. 2017). The process of depleting oxygen (O_2) (hypoxia) and elevating carbon dioxide (CO_2) (hypercapnia) alone is a method used to control insect storage pests such as cowpea weevil as an alternative to chemical fumigation (Cheng *et al.* 2012). Similarly, exposure to high concentrations of acetic acid and ethanol can have toxic effects on insects (Charkir *et al.* 1993).

Noble *et al.* (2017) trialled a natural fermentation technique to disinfest fruit from spotted wing drosophila *Drosophila suzukii* and vinegar fly *Drosophila melanogaster*. Fermentation took place in either 615-liter plastic pallet boxes or in 220-liter plastic barrels using unmarketable soft and stone fruit. Additionally, fruit containing artificially reared *D. melanogaster* and *D. suzukii* larvae and pupae were placed within the fermentation vessels with the waste fruit. The pallets were sealed with plastic wrap, a plastic lid and additional plastic wrap and barrels were sealed with screw lids containing a pump check valve. Tubes were fitted through the lids to allow gas concentrations to be measured. Fermenting of infested fruit took place over different durations ranging from 0.25 to 6 days. After the required duration passed, 500g of waste fruit was removed to rear surviving *D. suzukii* and *D. melanogaster.*

Throughout the fermentation process the average temperate ranged from 15–23°C. The presence of oxygen in a majority of the vessels after sealing was undetectable after 13–16 h. As oxygen depleted, there was a corresponding increase of CO_2 which exceeded 80% vol/vol. The pH within the vessels maintained a relatively stable pH of 4 throughout all fermentations. After 3 days of fermentation, the average concentrations of acetic acid and alcohols in all vessels were 2.4 ± 1.9 ppm and 912 ± 723 ppm respectively.

Noble *et al.* (2017) found that fruit were disinfested from *D. suzukii* after 3 days of fermentation, whereas, *D. melanogaster* required 4 days of fermentation treatment. *D. suzukii* lower tolerance to fermentation have been explained as *D. suzukii* are non-saprophagous, therefore, they do not associate with fermented substrates containing ethanol, unlike *D. melanogaster* (Sampson *et al.* 2016). As *D. suzukii* prefers to oviposit within fresh fruit (Bolda, Goodhue and Zalom 2010), this may provide an insight to the sensitivity of fermentation on *B. tryoni.*

As the concentrations of ethanol and acetic acid within the fermentation vessels were much lower than lethal levels found in previous research by Chakir *et al*. (1993), hypoxia and hypercapnia may have influenced the efficacy of fermentation disinfesting the waste fruit.

Noble *et al.* (2017) also tested different field disposal methods for waste fruit that underwent 2 days of fermentation for its attractiveness to egg laying *D. suzukii* females. After temporary exposure to reproductive female flies, the 90:10 vol/vol mixtures containing soil or growing medium coir with the fermented waste fruit had no emergence of adult flies. The authors concluded that *D. suzukii* were unable to complete their lifecycle in such mixtures. When tested in the field, fermented strawberry waste incorporated into 100 t/ha of soil at a depth of 0.15 m showed no attraction to Drosophila adults. The authors concluded that after fermentation, ploughing treated fruit into the soil can be an appropriate method for disposal.

Conclusions

Several methods of waste fruit disposal have been shown to minimise the survival of several varieties of fruit fly larvae and in one case also boosting the population of parasitiods, further minimizing the risk of fruit fly.

- Mechanical controls reduced Mediterranean fruit fly larvae numbers, grinding methods being more effective than crushing (81% versus 29% mortality respectively).
- Burial methods where shown to be effective against Mediterranean and melon flies with depths of 46 cm completely stopping emergence of adult flies.
- Augmentoria were effective at preventing the escape of three varieties of fruit fly and allowed the entry and escape of parasitiods although no reports of use of augmentoria could be found for Queensland fruit fly.
- Heat has been shown to be effective against Queensland fruit fly. Bagging fruit and allowing internal temperatures to reach 38°C over 4 days destroyed larvae however some studies showed variable mortality rates possibly due to temperature fluctuations resulting from partial cloud coverage. Composting has also been shown to reduce fruit fly numbers although variation in temperatures within composting materials can give highly variable mortality rates.
- Fermentation of fruit infested with two species of *Drosophila* for 4 days effectively controlled fly populations which may indicate fermentation is an effective control measure for fruit fly.

Recommendations

To understand the practicality and effectiveness of the various non-chemical fruit disinfestation methods, each technique was critically reviewed with several fruit growers. Conclusions gathered from the deliberation indicate that:

- Mulching field fruits may not be applicable for all fruit growers as mulching machinery may not be existing or readily available for all fruit growers.

- Heat treatments and deep burial that rely on specific but variable parameters such as temperature and burial depths to disinfest fruit are difficult to provide assurance and confidence to growers 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 the netting becoming damaged, compromising the structures integrity.
- 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.

Further investigations as part of this project will evaluate the use of fermentation to manage rejected fruit on farms.

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