Fermentation for disinfesting fruit waste from *Drosophila* species (Diptera: Drosophilidae)

R Noble, A Dobrovin-Pennington, B Shaw, DS Buss, JV Cross, MT Fountain

Abstract

Economic losses in a range of fruit crops due to the spotted wing drosophila, *Drosophila suzukii* (Matsumura), have become severe. Removal and treatment of fruit waste, which may harbour *D. suzukii*, is key in preventing re-infestation of fruit production. Natural fermentation for disinfesting fruit wastes from *D. suzukii* was examined at ambient air temperatures of 12–20 ºC. Soft and stone fruit wastes infested with eggs, larvae and pupae of *Drosophila melanogaster* (Meigen) or *D. suzukii* were placed in sealed vessels containing fruit wastes and samples were retrieved at intervals and tested for the emergence of adults. Mean temperatures of the fruit waste in the sealed vessels during fermentation were 15–23 ºC. Fermentation for three days was effective in disinfesting waste from different life stages of *D. suzukii*. Treatment for four days also ensured that the waste was free of viable life stages of *D. melanogaster*, which could be used as an indicator species for disinfestation of waste from *D. suzukii* due its greater tolerance of fermentation. The O_2 concentration of the headspace air in the vessels became undetectable after 13-16 h, with a corresponding increase in CO_2 concentration which exceeded 80 % vol/vol. The resulting hypoxia and/or hypercapnia may explain the efficacy of the fermentation treatment in disinfesting the waste. Fermented fruit remained attractive to *D. suzukii* and retained its capacity to rear a life cycle. Covering or mixing fermented fruit with a sufficient depth (0.1 m) or volume (×9) of soil or coir prevented the re-infestation of treated waste.

The spotted wing drosophila (*Drosophila suzukii*), a pest endemic to South East Asia, has recently invaded western countries. Unlike the common fruit or vinegar fly (*Drosophila melanogaster*), its serrated ovipositor enables it to lay eggs in undamaged, ripening fruit, thus significantly threatening fruit production. *D. suzukii* has spread rapidly and economic losses for a wide range of fruit crops are severe (Cini et al. 2012). Removal and treatment of fruit waste, which may harbour *D. suzukii*, is a key step in preventing re-infestation of fruit production on a local scale (Walsh et al. 2011). Composting, which has been used to disinfest crop wastes (Noble et al. 2009), is difficult for fruit due to the very high moisture content, and may worsen the problem by hastening the development of surviving larvae in the warm conditions of decomposition (Cini et al. 2012). Covering infested waste with a 0.3 m deep layer of sand or soil can remove the risk of re-infestation (Isaacs et al. 2013) but must done as soon as the waste is produced and does not utilise land efficiently. Covering or mixing treated fruit waste with spent (waste) growing medium such as coconut fibre (coir), followed by composting, could process two significant disposal problems from soft fruit production into usable organic compost (Drakes et al. 2001).

In a survey of fruit growers in the US (Anon. 2012), 1-20% of the cherry (*Prunus avium* L.) crop and 10-30% of the plum (*Prunus domestica* L.) crop was pre-harvest waste, which is susceptible to *D. suzukii* (Cini et al. 2012); crop losses during packing were 2-20% and 2-30% respectively. Research in the UK showed that pre-harvest waste for strawberry (*Fragaria* × *ananassa* Duchesne) and raspberry (*Rubus idaeus* L.) crops was 2-3%, with grading and packing losses of 3-4% (Terry et al. 2011). Given typical yields per hectare for the above fruit crops in a growing season in the US and UK (Anon. 2014, 2015), fruit waste production per hectare can be over one tonne for raspberries, two tonnes for cherries, five tonnes for strawberries and seven tonnes for plums. If infested with *D. suzukii*, these

quantities of fruit waste are a significant risk to the ripening crop (Walsh et al. 2011; Cini et al. 2012; Isaacs et al. 2013).

Fermentation is the conversion of organic compounds such as sugars into ethanol, acetic acid and carbon dioxide (CO_2) in the absence of oxygen (O_2) by yeasts and bacteria (Chakir et al. 1993). In fermenting fallen fruits, the ethanol and acetic acid concentrations can result in significant environmental stress for fruit-breeding *Drosophila* species (Chakir et al. 1993). However, *Drosophila* species including *D. melanogaster* that breed in fermenting fruits and man-made fermentations are more tolerant to these fermentation products (David and Van Herrewege 1983; Chakir et al. 1993). *D. suzukii* is less tolerant to ethanol than *D. melanogaster*, probably due to lower alcohol dehydrogenase activity (Chakir et al. 1993; Sampson et al. 2016).

Absence of O_2 (anoxia), depletion of an adequate O_2 supply (hypoxia) and/or exposure to an elevated, toxic CO_2 atmosphere (hypercapnia), which occur during fermentation, have been used to control insect storage pests. Moderately severe hypoxic and hypercapnic treatment, i.e. 2% O_2 + 18% CO_2, 80% N_2 led to cessation of development of eggs, larvae and pupae of cowpea bruchids (*Callosobruchus maculatus* F.). Larvae and pupae could survive longer durations of this treatment than eggs or adults (Cheng et al. 2012).

D. melanogaster larvae can survive in a completely O_2 depleted atmosphere for up to six hours (Krishnan et al. 1997). Zhou et al. (2008) found that although 70% of 27 wild-type lines of *D. melanogaster* embryos were able to complete their life cycle at 6% O_2, an atmosphere containing 4% O_2 was lethal. Exposure to a CO_2 atmosphere for 10.7 h resulted in the mortality of 56-76% of melon fly (*Bactrocera cucurbitae* Coquillett), 64-77% of oriental fruit fly (*Bactocera dorsalis* Hendel) and 99-100% of Mediterranean fruit fly (*Ceratitis capitate* Weidmann) adults (Keiser et al. 1982). Bouletreau et al. (1984)

demonstrated that *D. melanogaster* was more resistant to CO_2 concentrations of 2-20% than the closely related *Drosophila simulans* (Sturtevant) with regard to egg-to-adult mortality.

The aim of this work was to determine if hypoxic/hypercapnic treatment resulting from natural fermentation under ambient conditions could be used to disinfest fruit waste from *D. suzukii*. Comparisons of the tolerance of *D. suzukii* and *D. melanogaster* to fermentation were made to determine whether *D. melanogaster* can be used as an indicator species for disinfestation of fruit wastes from *D. suzukii* following specific fermentation treatments. A further aim was to determine whether the fermented fruit waste was attractive for *D. suzukii* egg laying and thereafter able to support a further life cycle, and if so, whether mixing or covering the treated waste with soil or used growing medium could prevent it from being a source of infestation.

Materials and Methods

Fruit fermentation vessels

Fruit waste consisted of soft fruit (strawberries or raspberries) or stone fruit (plums or cherries), kept at ambient temperatures (12–19 °C) for up to two days or at 4 °C for up to 10 days from harvesting. The majority of the fruit was undamaged but classed as unmarketable due to over- or under-size, uneven shape or ripening, or blemishes; less than 5% of the fruit in any of the batches was mechanically damaged or rotting. Fruit wastes were fermented either in 615 litre plastic pallet boxes (1.12 x 0.93 x 0.59 ht m) (All Pallets Ltd, Eastbourne, East Sussex, UK) or in 220 litre plastic barrels (1.00 m ht x 0.56 m diam, with rounded base and shoulders) (Metaloplastiki, Agrinioy, Greece). Fruit waste was filled into the pallet boxes (460 kg soft fruit or 410 kg stone fruit) or barrels (180 kg soft fruit or 160 kg stone fruit) to within 100 mm of the top. The pallet boxes were then sealed with a single layer of polyethylene stretch shrink wrap (500 mm wide x 25 micron thick, Agricare UK Ltd,

Canterbury, Kent, UK) followed by a plastic lid. The lids were then sealed on to the pallet boxes around the rim with a further layer of shrink wrap. The barrels were sealed with screw-on lids fitted with an aquarium air pump check valve (type Betta AV040, J&K Aquatics Ltd, Huntworth, Somerset, UK) to prevent excessive pressure developing inside the sealed vessels. Closable 6 mm diameter plastic tubing fitted through a hole in the pallet box lids and shrink wrap layer or barrel lids enabled gas measurements of the vessel headspace air to be made with a gas detector (Dräger Safety AG, Lübeck, Germany). Concentrations of O_2 and CO_2 were measured with Dräger gas detector tubes 6728081 and CH20301 respectively at 1–12 h intervals for up to two days and at daily intervals thereafter for up to six days, or until the end of each test. Concentrations of carbon disulphide (CS_2), hydrogen sulphide (H_2S), methane (CH_4), acetic acid, alcohols, dimethyl sulphide, and mercaptans were measured with Dräger gas detector tubes 8101991, 8101461, CH 20001, 6722101, 8101631, 6728451 and 6728981 respectively, after three days in 10 of the tests. The accuracy of the above gas detector tubes is stated as a standard deviation of ±10 to 15% by Dräger Safety AG. Temperatures of the waste and ambient air were monitored with temperature probes connected to a data logger (Grant Instruments Ltd, Cambridge). At the start and end of tests, samples of each type of fruit waste were analysed for pH and electrical conductivity (EC) using 1:5 dilutions with distilled water, and for dry matter content, (Anon., 1986).

Drosophila eradication tests

Until the UK quarantine pest status of *D. suzukii* was lifted in 2014 (Anon. 2016) outdoor experimental work starting in 2013 could only be conducted with *D. melanogaster*. To examine the effect of ambient air temperature on the eradication of *Drosophila*, tests were conducted outside in May, July and October in 2013, 2014, 2015 and 2016. Batches of soft fruit (raspberry or strawberry) waste were tested in each of the above months and years; an

additional batch was tested in July 2015, producing a total of 13 soft fruit waste batches. Similarly, 10 batches of stone fruit (cherry or plum) waste were tested at the above times, except in May 2013 and 2014, due to unavailability of sufficient stone fruit waste early in these seasons. All of the tests were conducted in pallet boxes, except the *D. melanogaster* tests on one batch each of soft and stone fruit wastes in July 2014, the May 2013 batch of soft fruit waste and the October 2016 batch of stone fruit waste which were conducted in plastic barrels.

To increase the natural infestation of fruit waste with *D. melanogaster* eggs, larvae and pupae, 50 kg samples of the same type of fruit waste used in each batch were placed in open pallet boxes to allow the ingress of egg laying females. After seven days, when high populations of *D. melanogaster* adults (>500) were observed on the waste, 2 kg was removed from top layer and spread on the surface of each batch of fruit waste in the vessels before sealing. All *Drosophila* adults were removed from the samples at the start of each test to avoid subsequent egg laying and false positives in the emergence numbers. The natural infestation of the samples of waste used in the tests with eggs, larvae and pupae of *D. melanogaster* was confirmed by observation of two replicate 50 g samples under a stereo microscope. From these observations, the calculated number of larvae and pupae ranged from 60 to 150 per kg fruit waste.

Tests on *D. suzukii* eradication were conducted in each of four of the batches of strawberry and cherry wastes used for tests on *D. melanogaster* in May and July in 2015 and 2016 with *D. suzukii* eggs, larvae and pupae artificially reared in 500 g of the same type of fruit. The samples were placed in open containers on the surface of the fruit waste in the vessels, but kept separate from the *D. melanogaster* in the waste. The *D. suzukii* were reared by placing fruit not infested with *D. melanogaster* in net cages with populations of *D. suzukii* adults for 10 days in a room at 20 °C with a 16 h:8 h light:dark cycle, and the presence of

eggs, larvae and pupae in the samples then confirmed as for *D. melanogaster*. From these observations, the calculated number of larvae and pupae ranged from 70 to 170 per kg fruit waste. Adult *D. suzukii* were removed from the samples before the start of each test.

Each replicate test was conducted with separate batches of soft fruit waste and stone fruit waste and consisted of samples of infested fruit that were kept for two different durations in separate sealed vessels of waste, or left untreated and used as positive controls. To examine the effect of the duration that samples were kept in the sealed vessels on *D. melanogaster* eradication, samples were allotted in pairs to different duration treatments: (a) 0.25 to 1 d (b) 1.5 to 2 d (c) 3 to 6 d. Each duration treatment was examined in the same month in two or three different years, and was paired with the other two duration treatments, each on four occasions, at least once in every month and year. This produced a 2 fruit waste type × 3 duration × 3 month factorial treatment design, with two or three replicates of each factorial combination in different years. As previously described, there was an additional batch of soft fruit waste, and two missing batches of stone fruit waste but no missing fruit waste type × duration × month factorial treatment combination. The duration treatments and fruit waste batches used for *D. suzukii* samples were (a) 1 d and (b) 2 d in July 2015 and May 2016, (b) 2 d and (c) 3 d in May 2015, and (a) 1 d and (c) 3 d in July 2016. Samples of *D. suzukii* were prepared in both types of fruit on each occasion except for the 1 d (July 2015) and 2 d (May 2015) treatments where no stone fruit was used. Concentrations of O_2 and CO_2 in the headspace air of sealed vessels were measured in all except two of the 23 batches of fruit waste used in the *D. melanogaster* tests, and in all of the *D. suzukii* tests, as previously described. The number of gas measurements varied between tests depending on their duration which ranged from 6 h to 6 d. The concentrations of the other headspace gases mentioned previously were measured on five batches each of soft and stone fruit wastes, in pallet boxes in May 2015, July 2016 and October 2013 and 2015, and in barrels in May 2013. At the end

of each measured duration in the sealed vessels, 500 g samples of fruit waste and containers with *D. suzukii* infested fruit were removed from the surface of the vessels and placed in open five litre plastic containers and covered with a fine mesh to allow ventilation but exclude any *Drosophila*. The containers were kept in a room with a 16 h:8 h light:dark cycle at 20 °C for three weeks and checked at 2-3 day intervals for any *D. melanogaster* or *D. suzukii* adults that may have emerged from the waste. Similar 500 g samples of *D. melanogaster* infested fruit waste or fruit with reared *D. suzukii* that were not placed in fermentation vessels were used as positive controls in each test.

The effect of the following treatments on the numbers of emerged *D. melanogaster* and *D. suzukii* adults from samples retrieved from sealed vessels and positive controls was analysed using ANOVA: (a) fruit waste type (b) duration in sealed vessel (c) month of test and (d) year of test.

Regression analyses of the number of emerged adults from each sample against the duration that infested samples were kept in the sealed vessels, the fruit waste type, and the mean temperature of the waste were conducted. A $\log_e$ transformation of the adult emergence numbers (n) was used due to the non-orthogonality of the data. To avoid problems with $\log_e$ transformed values of zero, (n/2) of the smallest n value other than zero (0.5) was added to all the adult emergence numbers. Explanatory models of adult emergence, including just main factor effects (duration in sealed vessel, fruit type, temperature) were considered, as well as models involving two- and three- factor interactions. Improvements in fit due to adding extra terms (duration, fruit type, temperature) were included in the hierarchical structure, and the best fitting yet parsimonious model was identified for each variable. A similar regression analysis of the headspace air O_2 and CO_2 concentrations against the duration that the vessels were sealed, fruit type and temperature was conducted with untransformed values.

D. suzukii attractiveness and disposal of treated fruit wastes

The attractiveness for *D. suzukii* egg laying and ability of the fermented strawberry waste to rear a complete life cycle, before and after mixing with soil or spent growing medium coir, was determined to establish what disposal methods could be used for the treated and disinfested waste. Strawberry waste fermented for two days in the above sealed vessels was tested by placing 10 g samples in Petri dishes in clear plastic cages with four female and two male *D. suzukii* two- to four-day old adults. The cages (200 × 120 ×100 [deep] mm) with a mesh lid to allow ventilation but exclude *Drosophila*, were kept in the same lit incubation room previously described. The effect of adding proportions (33, 67, 80 or 90% vol/vol) of soil or waste coir to the fermented waste on its attractiveness for *D. suzukii* egg laying and ability to rear a complete life cycle was tested in the same way. The same weights of fresh strawberries tested in the same way were used as positive controls. The same weights of fermented strawberry waste and fresh strawberries without the introduction of *D. suzukii* adults were used as negative controls. Four replicate cages of each treatment were prepared. The adults were removed after six days and the cages tested for the presence of further adults after three weeks.

Results

Gas measurements and fruit wastes

The range in mean temperature of the fruit waste in the sealed vessels during fermentation was 15–23 ºC and was on average 3 ºC higher than the mean ambient air temperature (Table 1). Differences in temperature between types of fruit waste were not significant.

The O_2 concentration of the headspace air in the vessels containing soft or stone fruit waste declined after the vessels were sealed and generally became undetectable after 13–16 h (Fig. 1a). There was a corresponding increase in CO_2 concentration which exceeded the

detection limit of the gas detection tubes (80 % vol/vol) after a similar period (Fig. 1b). The changes in headspace O_2 and CO_2 concentrations occurred in both the pallet boxes and plastic barrels at similar rates; both types of vessel allowed the release of excess air pressure within the headspace. The correlation coefficients for all the regression equations shown in Fig. 1 were significant at $P< 0.001$. The decrease in O_2 concentration and increase in CO_2 concentration in vessels containing soft and stone fruit wastes were similar, except in four batches of soft fruit waste where the changes in these gas concentrations were more rapid than in other fruit waste batches. Here, the O_2 concentration was depleted after 3–5 h, with a corresponding increase in the CO_2 concentration to above 60% vol/vol (Fig. 1 a and b). Two of these four batches were in pallet boxes and two were in plastic barrels. The effect of fruit waste temperature in the range 15-23 ºC on the change in headspace O_2 and CO_2 concentrations was not significant.

Mean concentrations (n = 10) of acetic acid (2.4 ±1.9 ppm), alcohols (912 ±723 ppm), dimethyl sulphide (9.6 ±8.7 ppm) and CH_4 (0.29 ±0.25 % v/v) were recorded after three days in the headspace air of the sealed vessels. CS_2, H_2S and mercaptans could not be detected.

The initial and final moisture contents of the soft fruit wastes were higher than those of the stone fruit wastes (Table 1). The strawberry, raspberry and plum wastes in the vessels separated into solid and liquid fractions, with about one third liquid after four days (Table 1) and about equal proportions after six days. Cherry waste remained largely intact after six days in the vessels, with only a small amount of liquid fraction after six days. The infested fruit waste samples placed in containers on the surface of the bulk of the fruit waste in the vessels remained intact after a six-day period.

The pH of the fruit wastes remained at 4 (± 0.4) during treatment for four days in the sealed vessels. The electrical conductivity (EC) of the waste also did not change significantly

during the fermentation process, although strawberry waste had a slightly higher EC than the other types of fruit waste (Table 1).

Drosophila eradication tests

In four of the 13 batches of naturally infested soft fruit waste and in two of the 10 batches of naturally infested stone fruit waste, less than three *D. melanogaster* adults emerged from the untreated positive control samples. Even though all of the corresponding fermentation treatments resulted in no adult emergence from samples, the effect of fermentation treatment on emergence in these six batches could not be determined reliably. Due to absence or low adult emergence numbers in corresponding untreated samples, their inclusion could overestimate the control efficacy of the fermentation treatment and they were therefore removed from the *D. melanogaster* analysis. Of the six batches, one (July 2014 soft fruit) was in plastic barrels. All of the other 17 infested untreated fruit waste samples used in the tests resulted in the emergence of 7–59 *D. melanogaster* adults. Positive controls from six of the eight batches of fruit waste containing fruit samples artificially infested with *D. suzukii* resulted in the emergence of 14–75 *D. suzukii* adults. Of these six batches, five were within the 17 batches that were retained for *D. melanogaster* analysis, and they contained ten sealed vessel duration samples, each with corresponding time zero positive controls. No *D. suzukii* adult emergence was observed from either the positive controls or fermentation treatment samples from the remaining two batches of fruit waste which were therefore removed from the analysis, as explained above. The number of emerged *D. melanogaster* and *D. suzukii* adults from positive controls is shown in time zero data points in Figs. 2a and 2b.

There were no significant effects of month or year of test on the numbers of emerged *D. melanogaster* adults in samples retrieved from sealed vessels or in positive controls. There was no significant effect of fruit waste type on the numbers of emerged *D. melanogaster* or

D. suzukii adults in samples retrieved from sealed vessels or in positive controls. The effect of duration in sealed vessels on the numbers of emerged adults was highly significant (P<0.001) for both *D. melanogaster* and *D. suzukii*. For *D. melanogaster*, there was a significant (P<0.01) difference in adult emergence between all three fermentation duration treatments: (a) 0.25 to 1 d (b) 1.5 to 2 d (c) 3 to 6 d, and with the positive controls. For *D. suzukii*, there was a significant difference (P<0.01) in the number of emerged adults between the positive controls, 3 d and 1 or 2 d treatments, but not between the 1 d and 2 d treatments. There was no significant difference in infest rate in the positive controls corresponding with *D. melanogaster* samples that subsequently resulted in emergence or no emergence following the same duration treatment in sealed vessels.

The numbers of emerged *D. melanogaster* and *D. suzukii* adults from retrieved samples of infested fruit waste declined logarithmically with increasing duration in the sealed vessels (Figs. 2a and 2b). The rate of decline in adult emergence was not significantly different between soft and stone fruit wastes or between wastes at 15–18 ºC or 19–23 ºC so that combined linear regression equations were fitted for all (a) *D. melanogaster* and (b) *D. suzukii* data as follows:

(a) $\log_e n = -0.6591t + 2.3240$ $\quad R^2 = 0.5607$ ($P< 0.001$)

(b) $\log_e n = -1.476t + 3.0024$ $\quad R^2 = 0.7544$ ($P<0.001$)

where n was the number of emerged adults from fruit samples retrieved after duration t days that the samples were kept in sealed vessels of fruit waste.

No emergence of *D. melanogaster* adults was observed from fruit waste samples that were in sealed vessels for four or more days. In five of the batches of soft fruit waste, no adult *Drosophila* emergence was observed after 1–2 d. Four of these five batches corresponded with the batches described above in which depletion in O_2 concentration and

increase in CO_2 concentration occurred more rapidly than in the other batches of soft fruit waste (gas concentrations were not monitored in the fifth of these batches).

No emergence of *D. suzukii* adults was observed from fruit waste samples that were in sealed vessels for three days (Fig. 2b). The more negative regression coefficient in Fig. 2b than in Fig. 2a shows that the effect of duration in the sealed vessels of fruit waste was more acute for *D. suzukii* than for *D. melanogaster*.

Drosophila suzukii attractiveness and disposal of treated fruit wastes

Following the temporary exposure of fruit samples to egg laying *D. suzukii* females, the mean numbers of adult *D. suzukii* recorded in cages with fresh strawberries (7.8 ± 3.8) or fermented strawberry waste (7.6 ± 3.7) were not significantly different. Adults were also recorded in cages with 33:67, 67:33 and 80:20 vol/vol mixtures of soil or spent coir and fermented strawberry waste but not in cages with 90:10 vol/vol mixtures (Fig. 3). No adults emerged from the negative control samples of fresh strawberries or fermented waste that were not exposed to *D. suzukii* adults at the start of the test.

Discussion

This work has shown that *D. suzukii* is more sensitive to fermentation than *D. melanogaster*. This may be due to a greater sensitivity to hypoxia and/or hypercapnia of *D. suzukii* than *D. melanogaster* since differences in tolerance are known within and between *Drosophila* species (Bouletreau et al. 1984; Zhou et al. 2008). Lower tolerance to ethanol and acetic acid in fruit-breeding *Drosophila* species than in *Drosophila* species associated with fermentation (Chakir et al.1993; Sampson et al. 2016) may also be partly responsible. However, the culture medium concentrations of ethanol and acetic acid they found to be lethal for *D. melanogaster* (6–17%) and for fruit- breeding *Drosophila* species (1–5%) after two days exposure are much

higher than those we detected in the vessel headspace air after three days. The concentrations are also higher than those that are encountered and survived by *D. melanogaster* in fermenting fruits, which typically contain 4-5% ethanol (Gibson et al. 1981; Dudley 2004). *D. melanogaster* has also be found to survive in wineries in fermentations containing 7% ethanol (McKenzie and McKechnie 1979). We found that *D. suzukii* was capable of breeding in similar numbers in fermented strawberry waste and fresh strawberries. Toxicity of ethanol or acetic acid in the fermented waste was therefore not solely responsible for eradicating *Drosophila* from the waste in the fermentation vessels. However, the potential toxic effects on *Drosophila* of depleted O_2 and/or elevated CO_2 atmospheres we measured in the fermentation vessels are similar to those described in previously cited work on life stages of various insect species in the absence of ethanol (Keiser et al. 1982; Bouletreau et al. 1984; Krishnan et al. 1997). AliNiazee (1972) found that adults of two flour beetle species (*Tribolium confusum* Jacquelin duVal and *Tribolium castaneum* Herbst) were most susceptible to anoxia, followed by larval, egg and pupal stages; mortality of pupae occurred within five days of exposure. The LT_{95} of codling moth (*Cydia pomonella*) eggs in anoxic/hypoxic atmospheres (0–2% O_2 in N_2) was 1.2–1.7 days and in hypercapnic atmospheres (40 – 100% CO_2 in air) was 1.3–1.4 days (Soderstrom et al. 1991). Soderstrom et al. (1990) found that elevated CO_2 (60% in air) killed life stages of *C. pomonella* more quickly than low O_2 (0.5%, 10% CO_2 , balance N_2) except for pupae. By contrast, Margam (2009) discovered that decreasing O_2 levels while keeping CO_2 concentration constant triggered a gradual decrease in feeding activity of *C. maculatus* whereas insect feeding was not impacted by increasing CO_2 at constant O_2.

The greater tolerance to fermentation of *D. melanogaster* than *D. suzukii* means that it can be used by growers as an indicator species for disinfestation of fruit waste for *D. suzukii* following fermentation treatment. The independent and interacting effects of hypoxia,

hypercapnia and ethanol toxicity on *Drosophila* species mortality could be determined by rearing *Drosophila* in controlled atmospheres using an artificial medium that does not readily ferment. We found the changes in vessel headspace O_2 and CO_2 concentrations to be stoichiometric, as would be expected from other fermentations (Tsai and Lee 1989). Artificially changing the ratio of O_2 and CO_2 concentrations to achieve a more rapid *Drosophila* kill, for example by introducing dry ice in the vessels, would add to cost.

Fruit waste temperature within the range 15–23 ºC had no effect on the duration required in sealed vessels for disinfestation of *Drosophila*. Schilman et al. (2011) found that the effect of temperature in the range 20-30 ºC during anoxia on recovery time of *D. melanogaster* adults was small compared with the effects of the duration of the anoxia, up to 60 min. Recovery time was shorter and percentage survival higher when the anoxic period was at 25 ºC compared with 20 or 30 ºC. Conversely, AliNazee (1972) recorded a greater susceptibility of different life stages *Triboloium* species to anoxia at 26.7 ºC than at 15.6 ºC. It is possible that temperature outside of the range used here may have a significant effect on the duration required for disinfestation, for example in fruit that has been cold-stored or harvested in warm climates. Similarly, no difference in the required duration for disinfestation between soft and stone fruit wastes was detected although other types of wastes, such as unripe fruit or fruit with a lower respiratory quotient (Fonseca et al. 2002), may require longer durations.

From figures presented earlier, strawberry fruit waste production can be up to five tonnes per hectare. If a five-day processing period is used, and fruit is harvested over a one month period, two pallet boxes, each containing 420 kg waste, would be needed per hectare. However, if fruit is harvested over a 10 day period, then nine pallet boxes would be needed per hectare. The numbers of pallet boxes needed for raspberries and cherries are likely to be lower than those needed for strawberries, whereas for plums, the number may be greater. If

the shorter processing period needed for some of the fruit waste batches in this work could be explained, this would reduce the number of vessels needed by a grower to treat fruit waste.

Observations made at a field site within a week where fermented strawberry waste from sealed pallet boxes had been incorporated at 100t/ha into the soil to a depth of 0.15 m did not show any *Drosophila* adults being attracted to the site. Field observations also showed that heaps of treated fruit waste covered with a layer of manure or spent growing medium coir at least 0.1 m deep did not attract *Drosophila* adults. Soil or green waste compost could also be used for this purpose. This depth is significantly less than the 0.3 m recommended by Isaacs et al. (2013).

This work will be applicable to the safe disposal of fruit and other crop wastes that may be infested with various pests, for example olive fruit fly (*Bactrocera oleae* Rossi) (Crohn et al. 2008), following comparative experiments. The lethal effects of hypoxia and/or hypercapnia on *D. suzukii* larvae may also reduce the spoilage of overwrapped packaged fruit that has a low egg infestation but is substantially sound (Fonseca et al. 2002).

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Table 1. Analysis of fruit wastes before and after fermentation for four days in sealed vessels, and maximum and minimum temperatures of all waste batches during fermentation. Each analytical value is the mean of four replicate batches of waste and two determinations per batch.

Fruit waste type	Moisture % wt/wt			pH		EC mS/cm		Temperature ºC	
	Start	End solids	End liquid	Start	End	Start	End	Min.	Max.
Strawberry	92.9	92.0	97.2	3.63	3.96	3.88	3.77	18.6	23.3
Raspberry	87.6	82.7	93.9	4.46	3.94	3.03	3.15	16.6	22.6
Plum	81.1	78.5	91.1	3.84	3.63	2.84	2.80	15.3	21.4
Cherry	80.5	79.8	90.4	4.41	4.12	3.15	2.98	15.3	19.5
LSD (P = 0.05)	2.1	2.4	2.3	0.36	0.47	0.63	0.71	3.7	3.9

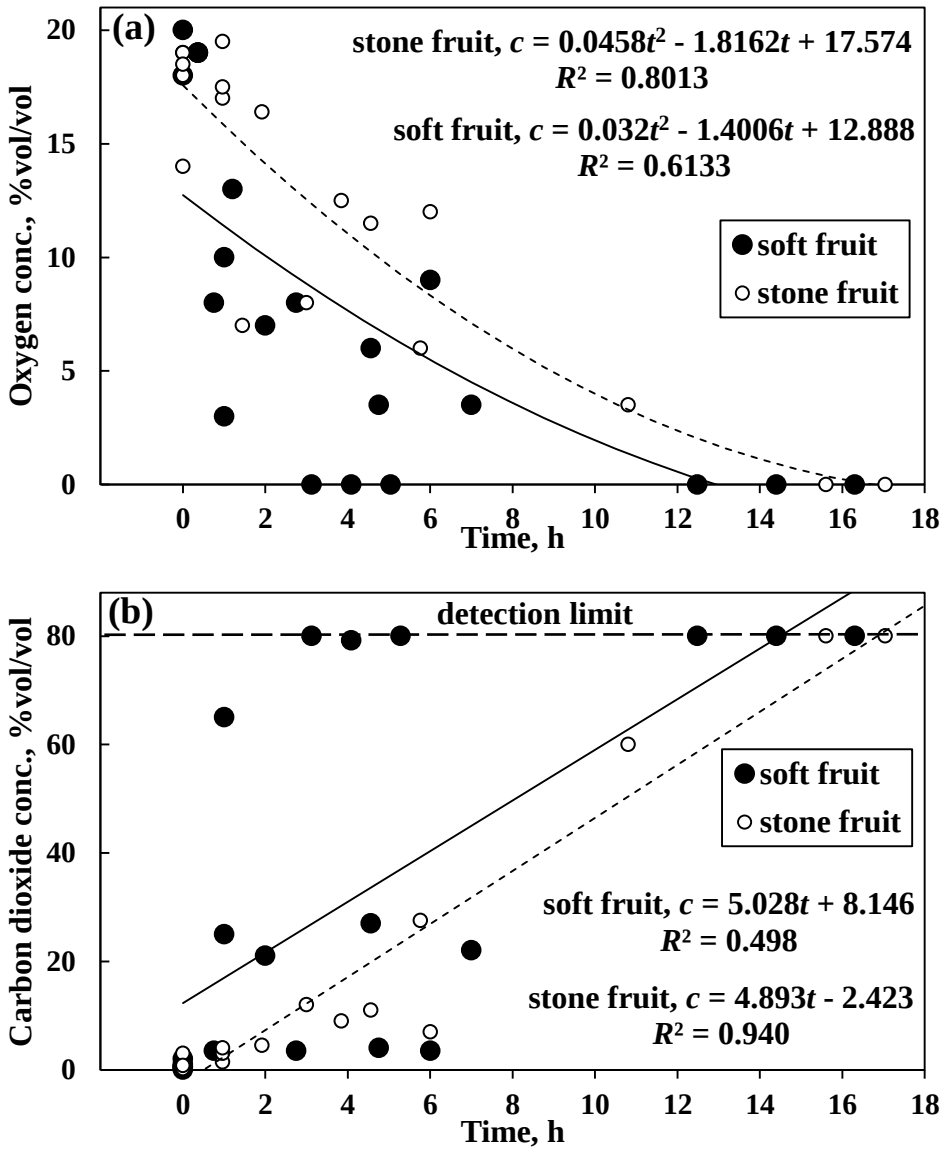

Fig. 1. Concentrations, *c* of (a) oxygen and (b) carbon dioxide in the headspace air of vessels containing soft fruit (solid symbols and lines) or stone fruit (open symbols and dashed lines) wastes; the vessels were filled and sealed at time $t = 0$.

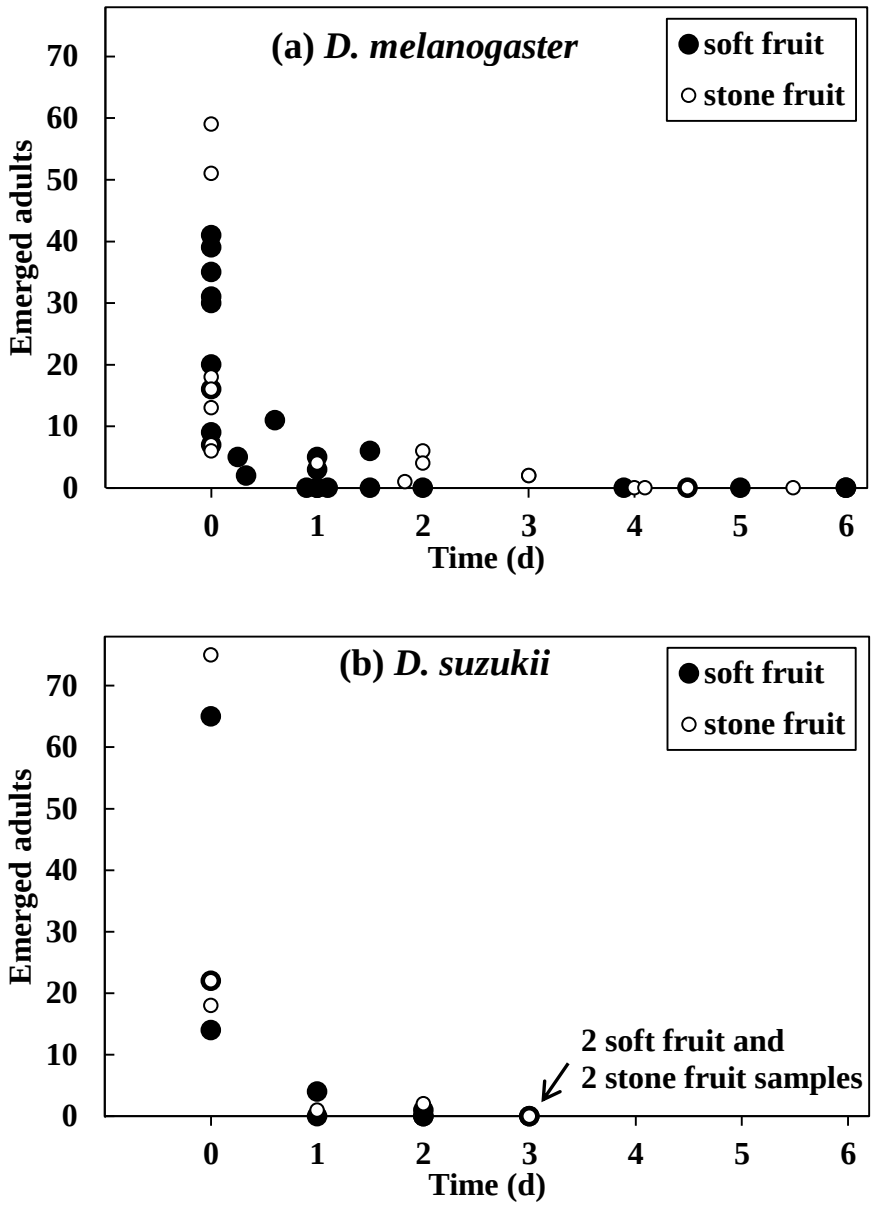

Fig. 2. Emergence of (a) *Drosophila melanogaster* and (b) *Drosophila suzukii* adults from samples of infested soft fruit (solid symbols) or stone fruit (open symbols) after different durations in sealed vessels containing wastes of the same types of fruit.

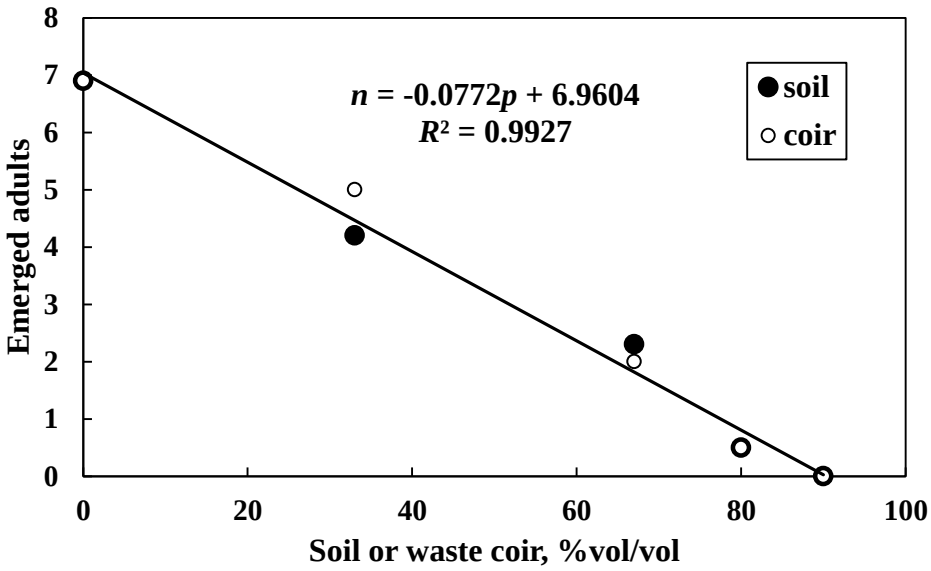

Fig. 3. Emergence of *Drosophila suzukii* adults, *n* from samples of fermented strawberry waste mixed with different proportions of soil or spent growing medium coir, *p*. Samples were exposed to egg laying females after fermentation. No adults emerged from non-exposed fermented waste. Each value is the mean of four replicate samples. The fitted linear regression is for combined data of soil and coir.