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Final Report

Evaluating the sugar flotation method for testing cherries for Queensland fruit fly

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Summary

The sugar flotation procedures have been used for many years to separate insects from other substrates. In this study we investigated whether the brown sugar flotation (BSF) procedure could be used for detecting the eggs and larvae of *Bactrocera tryoni* (Froggatt) (Qfly) and *Ceratitis capitata* (Wiedemann) (Medfly) in the cherry fruits grown in Australia. The study was undertaken in the laboratory under optimal conditions (26^{o}C, 60% RH) for fruit fly development. The first set of experiments compared the egg and larval detection ability of the BSF procedure between hand and machine fruit crushing techniques. The second set of experiments focused on determining the sensitivity of the BSF procedure in detecting fruit fly infestation, while the final experiments investigated the cherry fruit sampling regime for use in the BSF test procedure. In the first two set of experiments, either one or three infested fruits were placed among 35 fruits and crushed either by hand or machine. The crushed fruits were subjected to the BSF, water sieving and pupation procedures and the proportion of insects recovered by the BSF procedure was determined to validate the effect of hand and machine fruit crushing techniques. This same data was further used to determine the sensitivity of the BSF procedure for detecting fly infestation in cherry fruits. In the third experiment, various quantities of uninfested cherry fruits were combined with one infested fruit and crushed with the machine and subjected to the BSF procedure to determine the appropriate fruit sampling regime. The results from this study showed that the BSF procedure had 100% capability of detecting at least one insect in an infested sample irrespective of the fruit crushing technique. However when the BSF procedure was used to detect the total quantity of insect infested into a fruit sample, it was more efficacious in detecting a higher proportion of the total insect infested from fruits crushed with the machine than that crushed by hand. The sensitivity analyses of the BSF procedure suggests that it is highly sensitive and can detect at least one larva from all the samples tested (100% probability of detection) even when cherry fruit has a total of five individual larvae. However, the probability of detecting all the insects infested into a cherry fruit is less than 100%. The study also showed that a fruit sampling regime of 42 fruits provided a higher probability of detecting an insect by the BSF procedure. From all the results, it was evident that the BSF procedure is an effective tool in detecting at least one Qfly and Medfly insect in cherry fruit and should be incorporated, together with the existing sampling practice, as part of the system approach protocol for detecting fruit fly infestation in Australian cherry fruits.

Keywords

Brown sugar flotation, Queensland fruit fly, *Bactrocera tryoni* (Froggatt), Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), cherry

Introduction

Fruit flies (Diptera: Tephritidae) are important economic pests throughout the world as their infestation on fresh horticultural commodities causes considerable damage on host commodities and restricts market access of these hosts (White & Elson-Harris 1992). The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Qfly) is the main quarantine pest fruit fly species in the South Eastern region of Australia while the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Medfly) is the main pest species in the Western region of Australia (Drew 1989, Smith 1988). Both species infest a wide range of horticultural host commodities, hence restricting both the domestic and international markets of these hosts. As a consequence, various pest management strategies and market access protocols have been established and implemented inorder for specific host commodities to gain access to these market (e.g. Cherry Growers Australian Inc 2014). These market access protocols may range from pre-harvest techniques such as area freedom and field chemical sprays through to post-harvest treatments such as cold storage and vapour heat (Sharp 1993, Jessup and Baheer 1990). However, the recent banning/ restriction on the use of the key chemicals, dimethoate and fenthion, which have been successfully used to manage these pests over many decades, and the increasing demand for pest freedom from the importing countries/ states, has meant that improved systems approach protocols to gain market access are needed. This improvement may require some quick and robust tests for validating the risk of Qfly and Medfly infestation along the supply chain of the host commodity before it reaches its target market. One such group of tests include those that exploits specific density differences between insects and aqueous solutions of various salts and sugars which results in dislodging and floating the insect from its substrates (Pask and Costa 1971).

Various flotation methods have been used to separate invertebrates from aquatic and soil system (Edwards and Fletcher 1971; Fast 1970). Such flotation techniques have also been used for separating fruit flies from their host fruit/ substrate. For example, Yee (2012, 2014) suggested that the sugar flotation method or the hot water flotation method could be included in a systems approach protocol for ensuring cherries are free from western cherry fruit fly (*Rhagoletis indifferens* Curran). The brown sugar flotation (BSF) procedure has been recommended for use on farm or for interstate and international trade in the USA, Canada and Mexico (Anonymous 2014, CFIA 2015a, CFIA 2015b, Hueppelsheuser 2011, Steeves 2013).

In this study, we assessed the efficacy of BSF procedure in detecting infestations of Qfly and Medfly on Australian cherry fruits. Cherry is an important fresh horticultural commodity grown in Australia with high market value in both domestic and overseas market. We therefore aim to investigate the ability of the BSF procedure in detecting infestation of the various life stages of Qfly and Medfly on cherry fruits. The study further delved into investigating the sensitivity of the BSF procedure in detecting infestation in cherry fruit, and determination of the appropriate cherry fruit sampling regime to be used for the BSF procedure in Australia.

Methodology

Part 1. Hand fruit crushing technique

Fruit infestation & test conditions:
Marketable cherry fruits were placed on the top of meshed cages and gravid female fly oviposited into the fruit by protruding their ovipositor through the cage mesh and piercing into the fruit. For experiments involving Qfly, infested fruits were allocated to one of four groups and stored at temperatures between 25°C to 27°C for optimal fruit fly development. These four groups represented fruits that were held for one day, three days, five days or seven days. These periods corresponded to the developmental period of egg, first instar larvae, second instar larvae and third instar larvae. A total of ten replicates were undertaken. For the experiment using Medfly, the BSF procedure for the egg stage was carried out using 12 replicates of infested fruit which had been kept at 26°C for one day. The other 33 replicates of fruits were kept at 26°C for between 3 and 7 days and this allowed the fruits to be tested when they were hosting a mixture of the three larval instars. A second set of experiment with a different source of cherries and 1 infested:34 uninfested fruit ratio was carried out to further test the BSF procedure

Test fruit sampling, crushing & brown sugar flotation:
After each of the fruit holding periods, replicates for each life-stage were formed by adding 3 randomly chosen infested fruits to 32 uninfested fruits. This simulated the Canadian protocol where 35 fruit from each 5kg carton are treated (CFIA 2015a). Several brown sugar solutions with 4kg of sugar in 20L water were made up and 4L were used for each replicate. This sugar:water mixing ratio provided a brix reading of 15-18%, and our preliminary observation showed that the eggs and larvae of both species will float at this range of percentage brix in the brown sugar solution. The infested and uninfested mixed fruit samples for each replicate were placed on a ceramic bowl and crushed to the fruit pip with a hand held ceramic club. These crushed fruits were then placed in a bucket and 4L of the brown sugar solution was poured over the crushed fruits.

Egg & larvae recovery procedures:
Three procedures were applied in a sequential order to each of the crushed fruit sample (replicate) to detect eggs and larvae of the two fly species. The crushed fruits were first subjected to the brown sugar flotation (BSF) procedure, followed by the water serving procedure and then the pupation procedure.
i) BSF procedure:
Each of the 35 crushed fruits for each replicate was assessed for egg and larval recovery using the BSF procedure. Fruits crushed were suspended in the brown sugar solution to float eggs and larvae. Any eggs and larvae that floated on the surface of the sugar solution after the prescribed minimum 10 minute settling time were removed with a pipette and counted.
ii) Water sieving recovery:
After completion of the BSF procedure, all fruit slurry and liquid resulting from the BSF procedure were sieved through a series of different sizes of mesh so that eggs and larvae that remained in the fruit were recovered. The fruits were further assessed for egg/ larval presence by a water sieving process. Each fruit was held in one hand over a beaker and under a running stream of water which dislodged any egg/ larvae in the fruit pulp. The water was strained with fine sieves and all solid materials were transferred onto black cloth in a Petri dish for examination under a dissecting microscope. Any fruit fly eggs and larvae recovered were counted and recorded.
iii) Pupation procedure:
After the fruit was assessed by the BSF and the water sieving procedures, they underwent the final assessment of pupation. The remaining fruit pulp for each replicate were placed on mesh gauze suspended over slightly damp vermiculite in plastic trays covered with fine mesh and kept for two weeks at room conditions until pupation. The vermiculite from each infested fruit sample was then sieved and the number of pupae recovered were counted and recorded.

For each replicate, numbers of insects recovered via the three techniques described above were added together to get the total initial eggs that would have been deposited into the infested fruits in the test samples. The proportion of the total eggs or larvae recovered using the BSF procedure was then calculated to evaluate the detection ability of the BSF procedure.

Part 2. Machine fruit crushing technique

A. Machine detection ability

All experiments in Part 2 were done with the use of a specially designed machine to crush cherry fruits (see photo below) and therefore repeated the protocol described for the hand fruit crushing technique above. Both the 3:32 and 1:34 (infested:uninfested) fruits ratios were tested using the machine fruit crushing technique and 30 replicates were done for each life stage (i.e., egg & 2nd instar larvae) for each of the two infested:uninfested fruit ratios. All test fruits for each replicate for the two fruits infestation ratios were placed in the machine and crushed. Approximately 4-5L of the brown sugar solution was poured through the top of the machine and into the container and all the three egg/ larvae sampling procedures (i.e., BSF, water sieving, pupation) were undertaken for 15 replicate for each fruit infestation ratios and life stages while only the presence/ absence data of egg/ larvae of the BSF procedure was recorded for the other 15 replicates.

Machine fruit crushing

Egg/ larvae in BSF solution

Hand fruit crushing

B. Sensitivity of the BSF procedure

Sensitivity determination - fruit infestation and grouping:
Using the information gathered from a preliminary study on the oviposition behaviour of Qfly on cherry, we exposed the experimental fruits to a 3, 5 and 10 minutes period of Qfly oviposition by placing the tests fruits on top of a cage with gravid 2-3 weeks old Qflies. This provided varying quantities of initial eggs deposited into the experimental fruit that ranged from low through to high. For each test sample/ replicate, a single infested fruit was mixed with 34 uninfested fruit (1:34, infestated:uninfestated fruit ratio) and crushed using the machine. The crushed fruits of each sample were then subjected to the BSF, water sieving and pupation procedures. The total initial quantity of eggs in each infested fruit for each replicate was then determined by combining the quantity of eggs, larvae and pupae recovered using the three procedures. Since proportional data was used to determine sensitivity of the BSF procedure, the total eggs/ larvae from each replicate were ranked into various infestation levels that ranged from low to high. The grouping range for the eggs included replicates that had total eggs between 1-5, 6-10, 11-20, 21-30, 31-50, 51-70, 71-100, 101-130, 131-160. The mean proportion of eggs/ larvae detected by the BSF procedure from replicates within each infestation level (range) was then calculated and used to determine the sensitivity of the BSF procedure.

C. Determination of the cherry sampling regime for the BSF procedure

To determine an appropriate sampling regime, an additional Qfly detectability tests was undertaken using a stratified sampling regime based on the Canadian protocol. The Canadian protocol states a sampling regime of 35 fruits from a 5kg box for the BSF procedure (CFIA 2015a). If the individual Australian cherry weight range is from 8g to 12g and if we use 10g as an average weight per fruit, it would imply a sampling regime of 350g of fruit from a 5kg box. That is 7% of each 5kg box to be subjected to the BSF test procedure. Therefore from the current 600 fruits inspected per box, only 42 fruits (7%) would be subjected to the BSF procedure. Hence, the BSF procedure using 42 fruits per test sample was tested (i.e., one infested fruit with 41 uninfested fruit). To construct a

prevalence curve, we investigate four sample densities of infested:uninfested fruit ratios, and these included 1:34, 1:42, 1:81, 1:99. To avoid overcrowding of crushed fruits on the surface and making it difficult to detect eggs/ larvae, the surface area of the container used for each sampling regime was proportionately increased with increasing sample size where by the surface area of the container was double the total surface area occupied by the uncrushed cherry fruits.

The fruit crushing and brown sugar flotation procedure for each of the test samples/ replicate followed that described for the machine fruit crushing technique above. However, only one Qfly infested fruit was used for each test sample for the respective uninfested fruit densities. A total of 30 replicates were used for egg and 2nd instar larvae for each of the above infested:uninfested fruit ratios and that the data was only collected for the presences or absences of Qfly egg/ larvae on the surface of the BSF solution

Statistical analysis

All analyses were done using IBM SPSS Statistic v24. Data were generally subjected to either parametric or non-parametric tests. The data from the experiments investigating whether an egg/ larvae was present or absent on the BSF solution was subject to a non-parametric bionomial test (chi-square). The data for the experiments involving the actual count of the number of egg/ larvae were subjected to a one-way analysis of variance (ANOVA) when comparing the mean proportion of egg/ larvae detected by the BSF procedure across three or more groups. When the mean of only two groups were compared a two-tailed t-test was performed. Prior to any parametric tests (ANOVA/ t-test), all data were checked for normality, and homogeneity of variance was tested using Levene's test. Where the assumptions of the ANOVA were violated, in spite of standard transformations, the non-parametric equivalent of ANOVA, Kruskal–Wallis test, was used instead. When a significant difference was detected by the ANOVA, Tukey's HSD test or Games-Howell test (when variance was heterogenous) was used for post hoc, pair-wise comparisons of means. All proportional data were subjected to an arcsine square root transformation prior to analysis and back-transformed for graphical presentation. Alpha was set at 0.05 or 95% confident level for all analyses.

Outputs

1. Power point presentation:
   Balagawi, S., 2016 (May). Evaluation of the brown sugar flotation procedure for detecting infestation of the Queensland fruit fly and the Mediterranean fruit fly (Diptera: Tephritidae) in Australian cherries. Fruit Fly and Systems Approach Workshop, Melbourne, Australia (see attachment)

2. Manuscript:
   Balagawi, S., Broughton, S., Liang, W., Archer, J., Cruickshank, D., Cruickshank, C., 2016. Evaluation of the brown sugar flotation procedure for detecting infestation of the Queensland fruit fly and the Mediterranean fruit fly (Diptera: Tephritidae) in Australian cherries. Will be submitted to *Journal Applied Entomology* (currently under NSW DPI internal review), see attachment.

Outcomes

Ability of BSF in detecting at least one Qfly or Medfly egg/ larvae in cherry fruits

Irrespective of the fly species, type of fruit crushing techniques (machine or hand), fruit infestation ratios (3:32 or 1:34, [infested:uninfested]) or the fly life stages, the BSF procedure was able to detect at least one egg/ larvae in all the samples tested, hence showing a 100% rate of detection with a highly significant difference ($p < 0.001$) between the number of detected and undetected samples at 95% confidence level (Table 1).

Table1.

Fruit fly species	fruit crushing technique	infested: uninfested test fruit ratio	life stage	percent (%) detected	percent (%) undetected	Statistic (α=0.05) *n*	*df*	*Chi-Square*	*p*
Qfly	machine	3:32	egg	100	0	30	1	56.06	< 0.001
		1:34	egg	100	0	30	1	56.06	< 0.001
	machine	3:32	2nd instar	100	0	30	1	56.06	< 0.001
		1:34	2nd instar	100	0	30	1	56.06	< 0.001
	hand	3:32	egg	100	0	10	1	16.20	< 0.001
		1:34	egg	100	0	10	1	16.20	< 0.001
	hand	3:32	1st instar	100	0	10	1	16.20	< 0.001
		3:32	2nd instar	100	0	10	1	16.20	< 0.001
		3:32	3rd instar	100	0	10	1	16.20	< 0.001
		1:34	1st instar	100	0	10	1	16.20	< 0.001
		1:34	2nd instar	100	0	10	1	16.20	< 0.001
		1:34	3rd instar	100	0	10	1	16.20	< 0.001
Medfly	hand	3:32	egg	100	0	12	1	20.17	< 0.001
		3:32	all instars	100	0	33	1	62.06	< 0.001

Ability of BSF in detecting total quantity of Qfly or Medfly egg/ larvae in cherry fruit

When investigating the ability of the BSF procedure in detecting all the eggs/ larvae in an infested cherry fruit sample, it failed to detect all the eggs or the larvae. The probability of detecting all Qfly eggs or larvae in a crushed cherry sample of 35 fruits ranged from 33% through to 80% for the hand and machine fruit crushing techniques respectively (see Table 2). When the fruits infested with Medfly at the infestation ratio of 3:32 were crushed by hand and subject to the BSF procedure, the proportion of eggs detected by the BSF procedure was significantly lower than the proportion detected for all the three larval instars combined (Table 2).

Table 2.

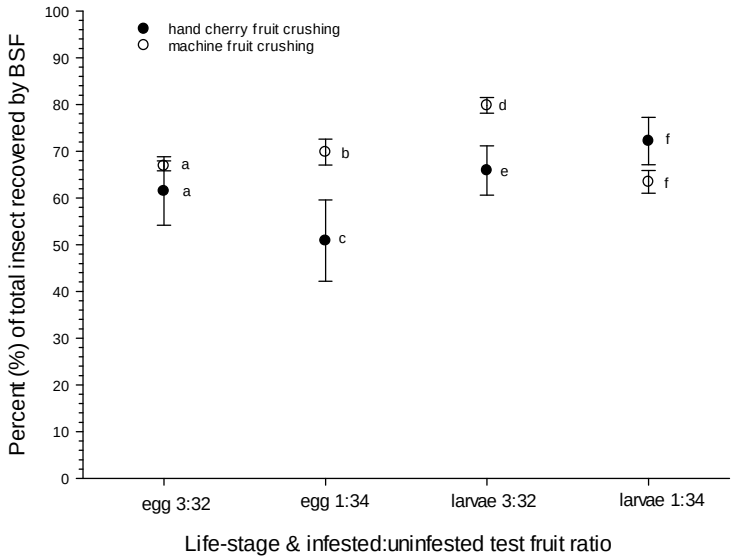
Fly species	fruit crushing technique	infested: uninfested test fruit ratio	mean (±SE) percent detection per life-stage egg	1st instar	2nd instar	3rd instar	Statistic (α=0.05) *test*	*p*
Qfly	machine	3:32	66.9 ± 1.1a		79.8 ± 1.7b		$t_{21.1} = 6.088$	< 0.001
		1:34	69.8 ± 2.8a		63.5 ± 2.4a		$t_{28} = 1.766$	0.088
	hand	3:32	61.5 ± 7.4a	57.6 ± 6.5a	65.9 ± 5.3a	33.4 ± 4.7b	$F_{3,36} = 5.647$	0.003
		1:34	50.9 ± 8.7a	65.6 ± 6.8ab	73.7 ± 4.7b	56.6 ± 4.5ab	$F_{3,36} = 1.882$	0.150
Medfly	hand	3:32	57.8 ± 2.1a		*67.3 ± 4.3b		$t_{39.2} = 2.486$	0.017

*, mean proportion for the combinations of all three larval instars

Comparison of hand vs machine fruit crushing techniques

While there were no significant differences in the BSF procedure in detecting at least one Qfly egg/ larvae from cherry fruit samples crushed using either the hand or the machine crushing technique (Table 1), there were significant differences between these two fruit crushing techniques when the BSF procedure was used to evaluate the proportion of detecting the total egg/ larvae initially infested into the cherry fruits (see Figure 1).

Figure 1



The comparison between the hand vs machine cherry fruit crushing techniques was done using the egg and 2nd instar larvae of Qfly for both the 3:32 and 1:34 (infested:uninfested) fruit infestation ratios (see Figure 1). The machine fruit crushing technique allowed the BSF procedure to detect a significantly higher proportion of egg (t_{23} = 2.422, p = 0.024) and 2nd instar larvae (t_{23} = 2.963, p = 0.007) for the 1:34 and 3:32 fruit infestation ratio respectively. However, there was no significant difference in the detection ability of the BSF procedure between the two fruit crushing techniques for the egg stage at the 3:32 fruit infestation test ratio (t_{23} = 0.882, p = 0.387) and for the 2nd instar larvae at the 1:34 (t_{23} = 1.725, p = 0.098) fruit infestation test ratio (see Figure 1).

Sensitivity of the BSF procedure

There was a 100% probability of the BSF procedure in detecting at least one Qfly 2nd instar larvae in a 35 crushed cherry fruit sample irrespective of the initial quantity of larvae hosted by the test fruit sample (Figure 2a). Although the probability of detecting at least one egg by the BSF procedure was low (36.3%) when an infested cherry fruit sample of 35 fruits had one to five eggs deposited into it, any test sample that had 6 or more eggs provided the BSF procedure with a 85-100% probability of detecting at least one egg (see Figure 2a). However, when the sensitivity of the BSF procedure in detecting the total Qfly egg/ larvae in an infested cherry fruit sample of 35 fruits was investigated, the probability of detecting all the eggs or the 2nd instar larvae in an infested cherry sample was less than 100%. The probability of detecting the total 2nd instar larvae in an infested cherry fruit sample was again higher than the egg stage at all the ranges of total egg or larvae initially present in the fruit sample (see Figure 2b).

Figure 2a

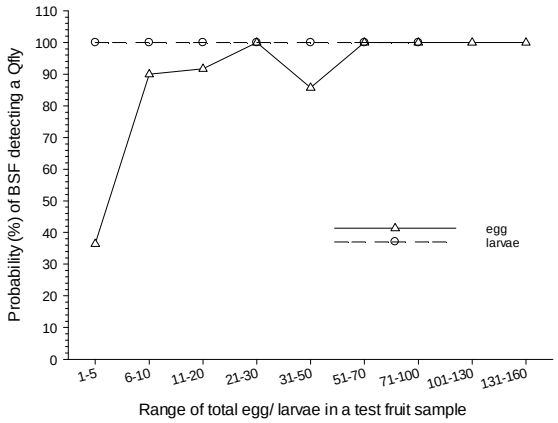
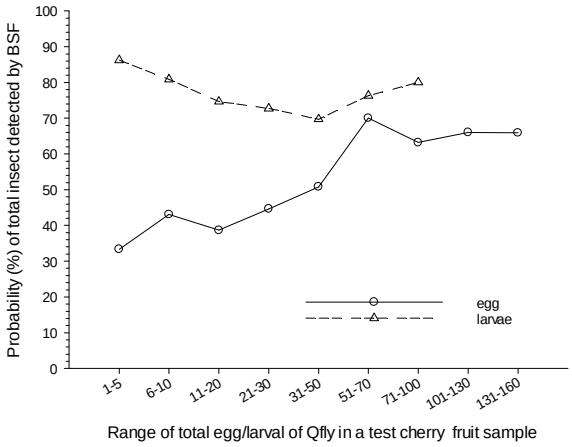


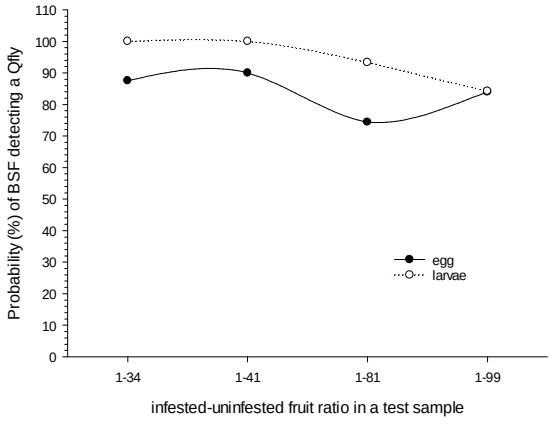
Figure 2b



Cherry fruit sampling regime for the BSF procedure

The probability of detecting at least one larvae was consistently higher than that for detecting at least one egg for the various infested:uninfested test fruit ratios (see Figure 3). There was a 100% probability of detecting at least one larva by the BSF procedure when an infested fruit was crushed with either the 34 or 41 uninfested fruits. However, this probability of detection gradually decreased when the uninfested fruit quantity was increased to 81 (93% detection) and 99 (84.2% detection), (see Figure 3). The probability of the BSF procedure in detecting at least one egg was high when the infested fruit was sampled with 34 (87.5% detection) and 41 (90% detection) uninfested fruits, decreased when sampled with 81 (74.4% detection) uninfested fruits but then increased when 99 (84% detection) uninfested fruits were used. The increase in egg detectability at the 1-99 sampling regime would most likely be a result of the use of softer fruits at this later stage of the experiment and this specific data should be used with caution (see Figure 3)

Figure 3



Evaluation and Discussion

Hand vs machine fruit crushing and BSF procedure

We investigated the efficacy of the BSF procedure in detecting eggs and larvae of two of the major pests in Australia (Queensland fruit fly and the Mediterranean fruit fly) using cherry fruits. Results from this study demonstrated that the BSF procedure effectively detect eggs and larvae of both species inside cherry fruits. The BSF procedure was able to detect at least one egg/ larvae inside all the infested cherry fruit samples tested irrespective of whether the fruits were crushed by hand or the cherry crushing machine, and irrespective of whether there was one or three infested fruit(s) among the 35 test fruits sample. The results showed a 100% probability of the BSF procedure in detecting at least one egg/ larvae within a 35 fruit sample. Given that the commercial market access protocol for cherry into non-endemic regions of Qfly or Medfly clearly highlights a zero tolerance of accepting any cherry consignment with either a single egg or larvae, the BSF procedure would be an effective tool to be used as part of the current fresh cherry systems approach protocol to validate the risk of Qfly and Medfly infestation in fruit destined for both the domestic and international markets.

Although the BSF ability in detecting the presences or absences of at least one egg or larvae between fruit samples crushed with hand and machine was similar, the machine fruit crushing technique was efficacious in exposing a higher proportion (64-80%) of the total egg or larvae in the crushed cherry test sample than the hand (51-74%) fruit crushing technique. The machine fruit crushing techniques consistently exposed higher proportion of eggs and the three larval instars. Given these comparative results as well as the lower labour input and the highly standardized nature of the cherry fruit crushing ability with the use of the machine, it is appropriate to suggest here that the machine fruit crushing technique should be used if the BSF procedure is to be used as part of the system approach protocol for detecting Qfly and Medfly in cherry fruits. Furthermore, the cherry fruit crushing machine used for this study was built (with similar dimensions) based on that used for detecting infestation of the western cherry fruit fly in cherries (Yee 2012, Brown 2009) and blueberries (Canadian Food Inspection Agency 2001) destined for export, hence providing an additional valid justification for its use for the Australian cherries.

Sensitivity of BSF procedure

The results from the Qfly oviposition behavioral studies showed that if uninterrupted, a gravid Qfly female is naturally capable of depositing six eggs during a single oviposition event of three minutes. The results from the sensitivity tests shows that the BSF procedure has a 100% probability of detecting a single larvae (esp. 2^{nd} instar) in a cherry fruit even at a very lower larval load of less than five larvae in a test sample. Although the probability of detecting a single egg was 36% if the test sample had five or less quantity of eggs, the probability of the BSF procedure in detecting an egg quickly jumped to 87-100% if the initial quantity of eggs in the test sample was greater than five. Given that the Qfly deposits a minimum of six eggs during its minimum oviposition time period, it could be concluded that the BSF procedure is highly sensitive in detecting a egg or a larvae even when a minimum quantity of eggs or larvae are deposited in a cherry fruit. Furthermore, the life history studies of Qfly in a cherry fruit shows that if five eggs were initially deposited into a cherry fruit, an average of one and half adult flies would emerge from these eggs. This implies that if the BSF procedure fails to detect a egg due to five or less eggs being deposited into a cherry fruit consignment, and that the consignment is exported to a Qfly non-endemic region, it is most likely that by the time the consignment reaches its destination, only a single fly (at most) would be surviving hence resulting in reproductive redundancy and no chances of pest population establishment. However, given the zero tolerance of allowing a consignment with at least one insect into the markets within a non-endemic region of the insect, we acknowledge that it may still be

difficult to get cherry consignment with five or less flies into this non-endemic market.

Cherry sampling regime for BSF procedure

As expected, the probability of the BSF procedure in detecting at least a single larva (predominantly 2nd instar) of Qfly gradually decreased with increasing sample size. A 100% probability of larval detection occurred when the test sample size was 35 and 42 fruits but decreased to 93% and 84% when the sample size increased to 82 and 100 fruits respectively. The probability of the BSF procedure in detecting at least one egg was lower than that of the larvae across all the fruit sampling size tested and ranged from 74.4 - 90% detection. The current cherry sampling regime for post-harvest quarantine test purposes undertaken is that 2% of the total consignment is selected and 600 individual fruits from each of the boxes that make up the 2% are sampled for pest and disease inspection. For example, if there are 1000 cherry boxes from one consignment, 20 boxes are selected and 600 fruits from each of the 20 boxes are inspected for pests and diseases. This implies that detecting presences or absences of Qfly or Medfly from a single infested fruit among the 600 fruits using the BSF procedure would require more time and resources.

The BSF procedure if adopted by the Australian Cherry Industry will be used as a tool for detecting fruit fly infestation within the already established systems approach protocol for cherry consignment. As such, it should be used as an additional tool for detecting infestation and should not replace any existing procedures such as visual inspection. Hence, being an additional tool would allow growers/ quarantine officials to subsample fruits from the current 600 fruits selected for inspection. If we than use the already established Canadian market access protocol for cherries with the use of the BSF procedure where 35 fruits are sampled from a 5kg box (~500 fruits) (CFIA 2015a), it does imply that a 7% subsample of the 600 fruits (i.e., 42 fruits) to be subjected to the BSF procedure. Surprisingly the results from this study also shows that the a sample size of 42 fruits subjected to the BSF procedure provided the highest probability of detecting the egg or the larvae compared to the other tested sample sizes. Thus from this observation, it would be appropriate to recommended that 42 fruits of the 600 fruits for post-harvest quarantine inspection should be subject to the BSF procedure. Alternatively, a sampling regime of 82 or 100 fruits is still feasible since the probability of detecting egg or larvae was mostly above 80%, but this may depend on the requirement of the target market.

In conclusion, the study shows that the BSF procedure is an effective tool for determination Qfly and Medfly infestation in Australian cherry fruits. If the post-harvest quarantine tolerance for fruit fly is such that a detection of an individual egg/ larvae from a cherry fruit consignment will restrict market access of this consignment, then the BSF procedure is 100% effective in detecting fly infestation and should be considered for incorporation into the current market access protocol for the Australian cherry fruits.

Recommendations

- The brown sugar flotation procedure be incorporated into the systems approach protocol for cherries and used as one of the many post-harvest tools to detect fly infestation in cherries.

- That the Machine fruit crushing technique be used for the BSF procedure rather than the hand fruit crushing technique.

- The BSF sampling regime for cherry 42 fruits for BSF test from the 600 fruits that are currently used for quarantine inspection.

- Staff using the BSF procedure has to be trained
    - To distinguish between fruit fly eggs/larvae in solution from white cherry fruit fibre
    - To distinguish between true fruit fly (Qfly & Medfly) egg/ larvae to those of the vinegar flies (*Drosophila spp*)

Scientific Refereed Publications

Journal article

Balagawi, S., Broughton, S., Liang, W., Archer, J., Cruickshank, D., Cruickshank, C., 2016. Evaluation of the brown sugar flotation procedure for detecting infestation of the Queensland fruit fly and the Mediterranean fruit fly (Diptera: Tephritidae) in Australian cherries. Will be submitted to *Journal Applied Entomology* (currently under NSW DPI internal review).

Intellectual Property/Commercialisation

No commercial IP generated

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Appendices

A. Power point presentation (see separate attachment to this report)

B. Final draft manuscript for peer-review publication (see separate attachment to this report)