



Department of
Agriculture and Food



Strawberries – not a host of Mediterranean fruit fly

Supporting your success

Introduction

Strawberry (*Fragaria x ananassa* Duchesne) is a hybrid derived from crossing Chilean strawberry (*Fragaria chiloensis*) with *Fragaria virginiana* from eastern North America. Australia is the 28th largest strawberry producer by volume worldwide and Western Australia (WA) produces 12.5% of the total Australian production (Strawberries Australia Inc. 2015). In WA, strawberries are cultivated within the geographic distribution of the Mediterranean fruit fly (medfly, *Ceratitidis capitata* Wiedemann), which is considered to be one of the most serious economic pests of fruit and vegetables (Syneisewska and Tatem 2014).

Medfly is a pest of quarantine concern and strawberry is regulated by some markets as a host of medfly, requiring postharvest treatment to access those markets. Within Australia for domestic trade, the regulation of a fruit or vegetable as a fruit fly host is usually based on published host lists. The inclusion of strawberry as a medfly host is questionable. No Australian publications list strawberry as a medfly host (Hancock et al. 2002; DEPI 2015). MEDHOST, an international database that lists all known hosts of medfly (Liquido et al. 2014) implicates strawberry as a host in Europe (Austria, Germany) and the United States of America (Florida, Hawaii; Table 1). However the original European records could not be validated and no contemporary records have been published. In Hawaii, Kobayashi and Fujimoto (1975) reared medfly from Chilean strawberry (*Fragaria chiloensis*) collected from a backyard. In Florida, Thomas et al. (2010) list strawberry as 'occasionally infested by medfly', but do not provide further details such as crop location (e.g. commercial or garden), fruit condition, or other factors that affect host suitability. Interestingly, strawberry is not included in the International Crop Protection Compendium datasheet on medfly (CABI 2015). Additionally, the United States Department of Agriculture does not consider strawberry fruit to be a Medfly host according to the APHIS Manual for Agricultural Clearance (APHIS, 2005)

Table 1: Published records of strawberries as a medfly host

Country	Species	Sample	Reference
Europe (Austria, Germany)	?	?	Thiem (1932), Böhm (1958), Baas (1959) cited in Liquido et al. 2014
Florida, USA	<i>Fragaria x ananassa</i>	?	USDA (1930); Thomas et al. (2010)
Hawaii, USA	<i>F. chiloensis</i>	1,386 g, 26 adults, 4 pupae (dead)	Kobayashi and Fujimoto (1975)

To determine the status of cultivated strawberry as a host of medfly in Western Australia, we followed a protocol that has been developed for determining host status (FAO 2005).

Materials and Methods

The protocol proposed by the FAO (2005) to determine non-hosts status follows a series of steps (Fig. 1). Step 1 requires laboratory tests with punctured or unpunctured fruit. If no development occurs in punctured fruit, it is described as a conditional non-host. If fruit flies develop from unpunctured fruit, it is described as a conditional host. If punctured and unpunctured fruit become infested, it is described as a potential host. Step 2 requires field tests with no adult emergence, or fruit collection with no larvae, pupae or adults recovered to prove non-host status (Fig. 1).

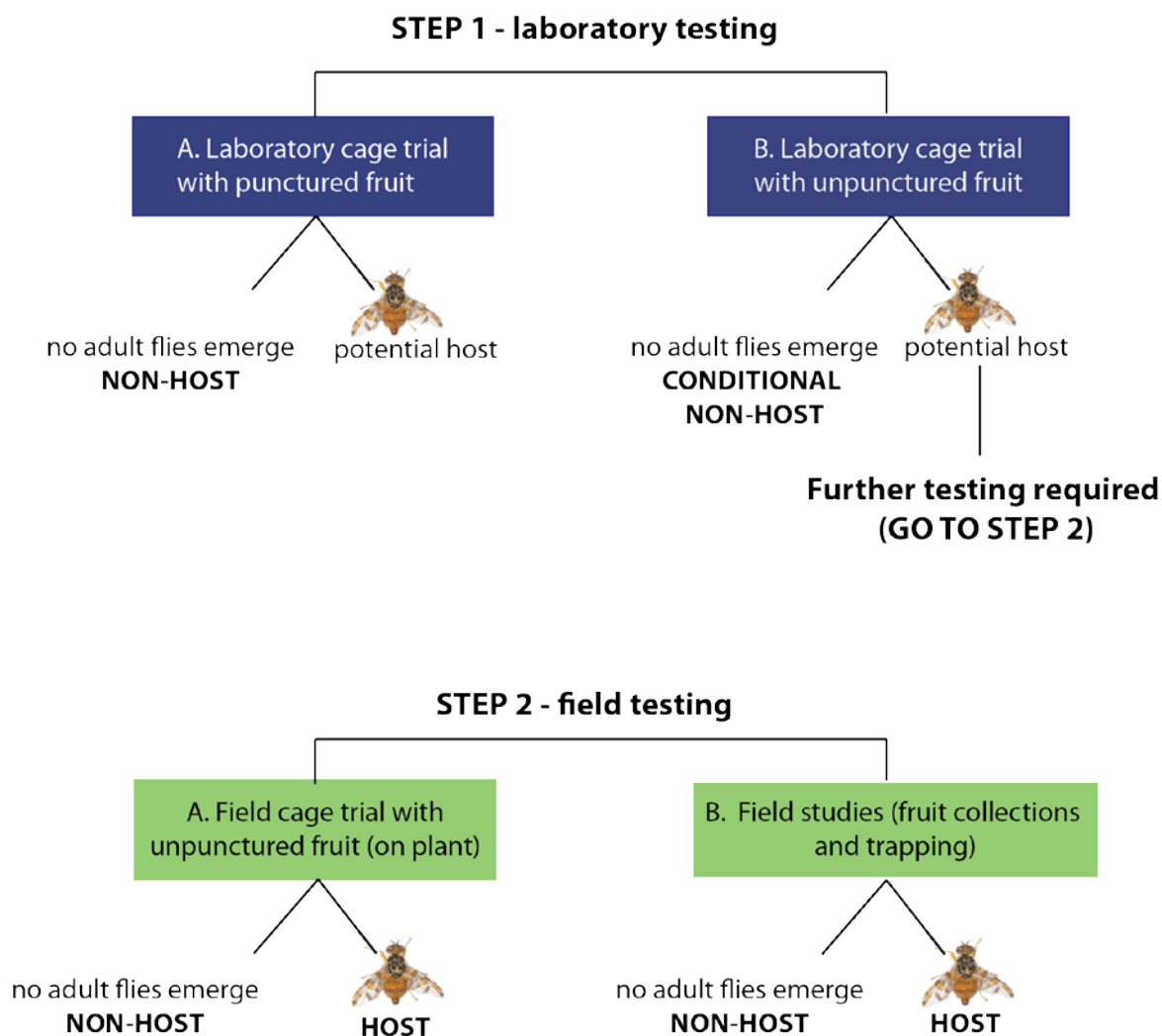


Figure 1. Pathway to provide evidence of non-host status (adapted from Cowley et al. 1993 and FAO 2005).

Step 1: Laboratory Cage Trials

Adult fruit flies were obtained from a laboratory colony maintained at South Perth. The colony is replenished each year from field collected fruit. Prior to experimentation, fecundity tests were conducted on gravid females to determine the potential oviposition load as per the protocol (FAO 2005). These tests indicated that 220 females were required to produce 1500 viable eggs.

Fruit of the varieties Camarosa, Albion, and Driscoll were collected at harvest maturity (hard, coloured) from a commercial field in Bullsbrook, Western Australia, on the 19 August 2014. No pesticides had been applied for fruit fly control. Cumquats (*Citrus japonica*) were included as the positive control, and were collected from trees planted at South Perth, DAFWA. A sub-sample of cumquats was held separately to determine if they had been infested prior to testing.

Strawberry and cumquats were divided into five groups of 500 g each, and then placed into individual cages 300 mm x 300 mm x 300 mm (BugDorm, Taiwan). The sides of the cage were covered with fine mesh. Females (8-10 days old) were released into cages and provided with sugar and water. After 24 hour exposure, fruits were removed from cages and held over vermiculite for 3 weeks in a constant temperature room (25°C). Each replicate was held separately in plastic tote boxes (195 mm deep x 295 mm wide x 390 mm long) with a wire mesh to hold fruit above the pupation medium. Pupae and adults were collected and counted; samples of 10 pupae were weighed from each cage.

Step 2: Field studies

Site selection

Eighty per cent of Western Australia's strawberry production is located in the Swan Coastal Plain north of Perth harvesting fruit from July to December. The main locations are Wanneroo, Carabooda and Bullsbrook. The remaining 20 per cent of production occurs in the Great Southern area around Mount Barker/Albany which harvests from October to May. Strawberry production takes place in areas where medfly is known to occur. There are approximately 45 commercial growers in WA, with a mean property size of 4 hectares.

Most strawberries are grown as an annual plant from bare-root transplants in the ground over black plastic mulch. Growers often use low clear plastic cloches to protect their crop during winter and spring, whilst a few are also using Haygrove tunnels. All strawberry production uses drip irrigation. The main varieties grown in WA are Camarosa and Festival, followed by Albion, Fortuna and San Andreas.

Farms were selected for monitoring and fruit sampling on the basis of medfly population pressure. Strawberries grown in the Perth area have a moderate to high endemic medfly population, whilst Mt Barker/Albany has a lower population. In Albany, Carabooda, Wanneroo, and Gnangara, farms were less than 1.3 km from likely sources of medfly (farm trees or urban area). Medfly monitoring traps were installed in August 2012 and August 2013 on six farms in the Perth Metropolitan area and on two farms in September 2012 and January 2014 in the Mount Barker/Albany region (Table 2, Figure 1).

Trapping protocol

The trapping protocol was developed by the South Australia Research and Development Institute (Appendix 1). Briefly, the protocol requires 10 traps installed on a property, that traps are located near the host (or a host tree), and spaced at least 25 m apart.

Traps were placed within the strawberry crop wherever possible, and approximately 1 m from the ground. Traps were suspended from tunnel structures, fences on the perimeter of the crop, or on stakes in the middle or edges of the crop (Fig. 2). In 2012 traps were checked weekly from 9/8/12 –14/12/12 in the Perth area, and from 20/12/12–15/5/12 in Albany/Mt Barker. In the 2013/14 season traps were checked weekly from 30/8/13–25/11/13 in the Perth area and 7/2–29/5/14 February in Albany/Mt Barker.

Table 2: Trap locations

Location	Area (Ha)	Distance to nearest medfly hosts	medfly pressure	Production type
Bullsbrook Farm 1	39.4	3 km	Moderate	Open field, Haygrove tunnel
Bullsbrook Farm 2	34.8, 50.2*	6 km	Moderate	Open field, low cloche
Carabooda	8.12	0.4 km	High	Open field
Gnangara Farm 1	4.09	1.08 km	High	Open field, low cloche
Gnangara Farm 2	12.84	1.1 km	High	Open field
Wanneroo	4.37	0.6 km	High	Open field
Wanneroo 2	8.2	1.2 km	High	Low cloche, field
Albany	36.25	1.3 km	Low	Open field, low cloche, Haygrove tunnel
Mount Barker	3.9	29 km	Low	Open field

*The first number indicates the size of strawberry planting in 2012, the second = 2013 planting. High >0.2 flies/trap/day, moderate 0.05-0.2 flies/trap/day, low 0-0.1 flies/trap/day

Trap and lure type

Male-targeted traps were used for monitoring medfly abundance. The trap used was a modified version of a Lynfield trap, which is the standard trap used for monitoring medfly in Australia (Wijesuriya and De Lima 1995; Figure 2). The trap body consists of a one litre clear plastic jar (100 mm diameter, 124 mm high) with an opaque white screw-on lid. Four 25 mm diameter entry holes are placed equidistant around the trap 50 mm below the top of the jar (Figure 3). The male lure consisted of two cotton dental rolls in a large paper clip loaded with 3 mL of capilure. A 10 mm² strip of dichlorvos was placed in the bottom of the trap as the killing agent. Lures and dichlorvos were replaced every 8 weeks.

The total number of flies per month, and the number of flies per trap per day were calculated for each property.



Figure 2. Lynfield trap hung in a low cloche.

Fruit collections

Cultivated strawberry was collected from the Perth area only as it has the highest medfly pressure. Fruits were collected from 23/9/12–30/11/12 in 2012 and 6/9/13–11/11/13 in 2013. Cowley et al. (1992) recommends that at least 1,000 fully ripe fruit are assessed per growing region: this gives 95% confidence that infestation levels greater than 0.3% would be detected. Berries were randomly collected in the field and consisted of 600 individual fruits (approx. 9kg) which were ripe, unwashed, unsorted, and ungraded. Berries were collected into new plastic zip-loc bags, then transported to the laboratory in cooled containers.

In 2012 berries were transferred to a constant temperature room for fruit fly rearing. They were placed on a metal grid in a plastic tote box (432mm x 324mm x 127mm) labelled with farm and collection date, then placed over another tote box of the same size containing vermiculite. This allowed any juices to drip from the top to the bottom box where it was absorbed by the vermiculite, preventing any larvae from drowning in the berries. The top of the tote box was secured with muslin to prevent ferment flies (*Drosophila* spp.) from infesting fruit. Vermiculite was sieved after 15 days for pupae. In 2013 fruits were dissected under a binocular dissecting microscope for eggs and larvae.

Statistical analysis

Lab trial data were analysed with one-way ANOVA with blocking effect (replicate) following log (x +1) transformation of the data or square root transformation (percentage) if appropriate. If significant, treatment means were separated using the Bonferonni-post hoc test ($P=0.05$). All analyses were performed with GenStat Windows® 16th edition software.

Confidence level

The probit 9 (99.9968%) standard is used to express the efficacy of a disinfestation treatment to kill 99.9968% of eggs and larvae in fruit. This standard ensures that adequate quarantine security is being provided. Follett and Hennessey (2007) have provided an equation to determine the confidence level associated with different sample sizes for host status determination:

$$C = 1 - (1 - p_u)^n$$

Where C is the confidence level, p_u is the acceptable level of survivorship, and n is the number of test insects (or fruit).

Results

Step 1: Laboratory Cage Trials

Larvae developed through to adulthood from unpunctured strawberry fruit, but did not perform as well as cumquat. Pupal deaths were significantly higher in strawberry than the control (cumquat), with mortality ranging from 39-85% compared to 4-9% for cumquat ($F= 16.10$, $df=3,12$, $P<0.001$). Two to four times more pupae were produced from cumquat than strawberry ($F=89.03$, $df=3,12$, $P<0.001$), and 5-8 times more adults emerged per 500 g of fruit ($F= 26.54$, $df=3,12$, $P<0.001$). Adult emergence from pupae ranged from 42.7-57.5% in strawberry, compared to cumquat where over 90% of pupae emerged ($F=22.34$, $df=3,12$, $P<0.001$). Pupae that emerged from cumquats were also twice the weight of pupae from strawberry ($F=7.24$, $df=3,12$, $P=0.005$; Table 3).

Table 3. Mean number \pm standard error of the mean of pupae and adults produced per 500g of fruit. Within a column, means followed by the different letters indicate significant differences at $P=0.05$

Cultivar	# pupae per 500 g fruit	Weight of 10 pupae (g)	# adults per 500 g fruit	Adult emergence from pupae
Albion	95.2 \pm 13.41a	0.164 \pm 0.017a	51.6 \pm 9.19a	53.2 \pm 2.6%a
Camarosa	69.4 \pm 5.05a	0.154 \pm 0.019a	30.6 \pm 6.49a	42.7 \pm 8.3%a
Driscoll	84.0 \pm 9.49a	0.144 \pm 0.016a	48.0 \pm 4.91a	57.5 \pm 0.9%a
Cumquat	274.0 \pm 12.53b	0.300 \pm 0.045b	260.4 \pm 13.74b	94.9 \pm 0.9%b

Step 2: Natural infestation

Trapping

Medfly was trapped in all strawberry growing areas except Mt Barker (Table 4). In the northern growing area, 123 males were caught in 2012 and 32 in 2013, mainly in farms located in Carabooda and Wanneroo (Table 4). Most flies were caught in November 2012 and October 2013. The number of flies per trap per day ranged from 0-0.25 in 2012 and 0-0.16 in 2013. In Albany 59 males were trapped in 2012-13 and 103 in 2014, with highest numbers in March 2013 and April 2014. The number of flies caught per trap per day ranged from 0-0.14 in 2013 and 0-0.18 in 2014.

Fruit collections and confidence level

Fruit collections spanned the period during which medfly was trapped on farms. No medfly were reared from the 25,200 individual fruits collected in 2012. Similarly, no medfly eggs or larvae were found in 27,600 individual fruits dissected in 2013. Sampling of 52,800 strawberry gave a confidence level of 99.99% at 95% that there was no successful fruit fly maturation. At probit 9 (99.9968%) the confidence level was 81.2%.

Table 4: Total number of male *C. capitata* caught in traps on strawberry farms in trial areas. N/A indicates that the farm was not sampled.

Month (number of trapping occasions)	Carabooda	Wanneroo 1	Wanneroo 2	Gnangara 1	Gnangara 2	Bullsbrook	Bullsbrook 2	Albany	Mt Barker
Aug 2012 (4)	15	3	0	0	n/a	0	0		
Sept (4)	16	0	0	4	n/a	0	0		
Oct (4)	12	9	0	3	n/a	0	0		
Nov (4)	46	-	0	0	n/a	0	1		
Dec (2)	2				n/a	0	2	0	0
Jan 2013 (4)								0	0
Feb (3)								9	0
Mar (4)								20	0
Apr (4)								5	0
May (3)								0	0
Aug 2013 (1)	5	1	1	n/a	0	0	0		
Sept (4)	2	0	0	n/a	0	0	0		
Oct 4)	15	1	0	n/a	0	0	0		
Nov (4)	8	-	-	n/a	-	0	0		
Feb 2014 (4)								15	0
Mar (4)								24	0
Apr (5)								55	0
May (4)								9	0

Table 5: Total number of strawberries collected from strawberry farms in trial areas.

Month	No. of harvesting dates	No. fruits sampled	No. farms sampled	<i>C. capitata</i> eggs or larvae detected in samples
August 2012	2	4800	6	0
September	4	6600	4	0
October	3	6600	3	0
November	3	7200	3	0
September 2013	4	15000	6	0
October	4	12000	6	0
November	1	600	1	0
	21	52800		

Discussion

We have demonstrated that strawberry is a potential medfly host under laboratory conditions, albeit a poor one. In cumquats 260 adults developed per 500 g of fruit compared to 43.4 adults per 500g of strawberry, and pupae reared from strawberry were 50% lighter than pupae reared from cumquat. However infestation under artificial conditions does not accurately represent what happens in the field, as laboratory trials can overestimate the risk of potential infestation. In the laboratory females are given no choice of host, and are exposed to hosts at a higher population density than what occurs in the field. For this reason the absence of natural infestation in the field in the presence of medfly is the strongest indication of non-host status.

Medfly was caught on farms in the Perth and Albany strawberry production areas. Strawberries grown in Albany, Carabooda, Wanneroo, and Gnangara are located within 1.3 km of farm orchards or urban areas which are the most likely sources of medfly. In the northern growing area, medfly populations were highest in mid-late spring (October-November) and in autumn (March-April) in the southern growing area. From 52,800 strawberries collected from August through to November, encompassing eight farms and 21 sampling dates, no eggs or larvae were found. The corresponding confidence level calculated from our field collections is 95% at 99.99% probability that survival of medfly was less than 0.0001: at probit 9 (99.9968%) the confidence level is 81.2%.

Our conclusions are also supported by anecdotal information. Between 2003/04 and 2008/09, 8711 tonnes of strawberry fruit was exported from WA with no medfly detections reported (DAFWA 2014). The United States also permits the entry of strawberries without specific mitigation measures for medfly from countries where it occurs including Israel, Morocco, Turkey, France, Spain (USDA 2010a). Strawberry is listed as a non-host of medfly by the USDA, and does not require it to be sealed in vessel's stores when transported from Central America, South America or West Mediterranean countries (USDA 2010b).

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APPENDICES

Appendix 1. Trapping Protocol

10 traps installed on a property in each district.

1. Traps located on the nearest fruit fly host tree with ripening fruit.
2. Distance between traps to be at least 25 metres and not on the same tree.
3. If no host trees within a distance of 25 metres of the site, the nearest leafy tree or fence post to be used.
4. Wherever possible, traps to be in semi-shade and well clear of the foliage to allow fruit flies easy access to the trap.
5. Each trap to be labelled on the lid.
6. All trap site to GPS recorded
7. Traps to be monitored for the period that fruit is exported to South Australia.
8. Inspection frequency to be once per week – summer (1 November to 30 April) and once per fortnight – winter (1 May to 31 October).

Appendix 2. Fruits collected from commercial farms in the Perth area 2012

Property	Date collected	No fruits collects	Number of larvae/pupae
Bullsbrook 1	23/08/2012	600	0
Bullsbrook 2	23/08/2012	600	0
Carabooda 2	23/08/2012	600	0
Wanneroo	23/08/2012	600	0
Bullsbrook 1	30/08/2012	600	0
Bullsbrook 2	30/08/2012	600	0
Wanneroo	30/08/2012	600	0
Gnangara	30/08/2012	600	0
Bullsbrook 1	6/09/2012	600	0
Bullsbrook 2	6/09/2012	600	0
Carabooda 2	6/09/2012	600	0
Wanneroo	6/09/2012	600	0
Bullsbrook 1	13/09/2012	600	0
Bullsbrook 2	13/09/2012	600	0
Carabooda 2	13/09/2012	600	0
Bullsbrook 1	21/09/2012	600	0
Bullsbrook 2	21/09/2012	600	0
Carabooda 2	21/09/2012	600	0
Wanneroo	21/09/2012	600	0
Bullsbrook 2	5/10/2012	600	0
Carabooda 2	5/10/2012	600	0
Wanneroo	5/10/2012	600	0
Bullsbrook 2	12/10/2012	600	0
Carabooda 2	12/10/2012	600	0
Wanneroo	12/10/2012	600	0
Bullsbrook 2	19/10/2012	600	0
Carabooda 2	19/10/2012	600	0
Bullsbrook 1	25/10/2012	600	0
Bullsbrook 2	25/10/2012	600	0
Carabooda 2	25/10/2012	600	0
Bullsbrook 1	2/11/2012	600	0
Bullsbrook 2	2/11/2012	600	0
Carabooda 2	2/11/2012	600	0
Bullsbrook 1	9/11/2012	600	0
Bullsbrook 2	9/11/2012	600	0
Carabooda 2	9/11/2012	600	0
Bullsbrook 1	23/11/2012	600	0
Bullsbrook 2	23/11/2012	600	0
Carabooda 2	23/11/2012	600	0
Bullsbrook 1	30/11/2012	600	0
Bullsbrook 2	30/11/2012	600	0
Carabooda 2	30/11/2012	600	0

