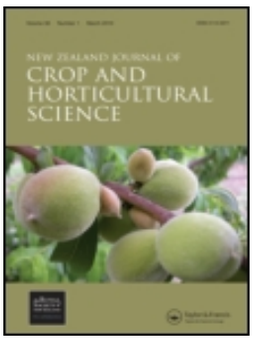

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Cold treatment of table grapes infested with Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and Queensland fruit fly *Bactrocera tryoni* (Froggatt) Diptera: Tephritidae

CPF De Lima[a]*, AJ Jessup[b], ER Mansfield[a] and D Daniels[b]

[a]*Department of Agriculture and Food, South Perth, WA, Australia;* [b]*New South Wales Department of Primary Industry, Gosford, NSW, Australia*

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A series of trials, using standard bioassay procedures followed by large-scale export tests, were conducted on five table grape cultivars at 1°C, 2°C and 3°C against the Mediterranean fruit fly (MFF) and the Queensland fruit fly (QFF). MFF was found to be more tolerant to cold treatment than QFF as shown in tests required to achieve complete mortality in >100,000 insects. MFF control was achieved in 16 days at 1°C, 18 days at 2°C and 20 days at 3°C. QFF control was obtained in 12 days at 1°C and 14 days at both 2°C and 3°C. These results provide flexibility for static and in-transit quarantine treatments for export and are an effective alternative to methyl bromide fumigation.

Keywords: Mediterranean fruit fly; *Ceratitis capitata*; Queensland fruit fly; *Bactrocera tryoni*; table grapes; quarantine treatments; cold storage

Introduction

Quarantine restrictions are placed on the export of table grapes due to the presence of the Queensland fruit fly (QFF), *Bactrocera tryoni* (Froggatt) and the Mediterranean fruit fly (MFF), *Ceratitis capitata* (Wiedemann) on the Australian mainland. Australian producers can supply fresh high-quality table grapes 'counter season' to meet the market demands in the Northern Hemisphere. QFF is endemic to the entire east coast of Australia extending inland as far as 600 km (May 1963), whereas MFF is present in pockets around major towns in Western Australia (White & Elson-Harris 1992). Quarantine disinfestation methods are fumigation using methyl bromide, cold treatment or irradiation (APHIS, 2006). Methyl bromide is phytotoxic to table grapes, reduces shelf life and is restricted in use because it is ozone depleting and subject to be phased out under the Montreal Protocol. Irradiation does not damage fruit but issues of live sterilized insects in produce have not been resolved. Cold storage of table grapes below 4°C is essential for maintaining good quality (Winkler et al. 1974; Crisosto et al. 1994; Cameron & Pasquale 2006). Since previous work (Hill et al. 1988; Jessup et al. 1993; Heather et al. 1996; De Lima et al. 2002; Lima et al. 2007) showed that cold treatment in the 1–3°C range was effective in disinfesting MFF and QFF in citrus, it was considered a good alternative to methyl bromide for treatment of table grapes.

QFF trials were conducted at the Horticultural Research Laboratories in Gosford, New South Wales. MFF trials were conducted at the Department of Agriculture and Food Research Laboratories in South Perth, Western Australia.

*Corresponding author. Email: francis.delima@agric.wa.gov.au

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Materials and methods

Test fruit

Harvested fruit was stored in waxed cartons containing sulphur dioxide generator pads at 1°C and 90% RH to preserve quality. All experimental work in both laboratories is conducted at 26 ± 1°C and 60–65% RH, the conditions under which the fruit fly colonies are maintained. To determine the course of development of the immature stages, test fruits were removed from cold storage and held for 48 h at 26 ± 1°C and 60–65% RH to equilibrate to a temperature suitable for egg and larval development.

MFF trials were conducted from 2003 to 2005 on Red Globe, Thompson Seedless and Crimson Seedless varieties from the Upper Swan Research Station and adjacent Swan Valley vineyards, 10 km north east of Perth in Western Australia. The fruit did not receive direct pesticide applications and were free from pesticide residues. They were of good quality—sugar: acid ratio and brix values are summarized as: Red Globe (23, 16.5), Thompson Seedless (25, 17.5) and Crimson Seedless (28, 18.5).

QFF trial fruit was sourced from the Fruit Fly Exclusion Zone a fruit fly-free production area in the Murray Valley in Victoria from estates in Cardross and Birdwoodton 1200 km south west of Gosford. 1°C trials were done in 1988–90 in Thompson Seedless, Flame Seedless and Ruby Seedless; while the 2°C and 3°C trials were conducted in 2006–07 in Thompson Seedless, Crimson Seedless and Red Globe. The fruit were free from pesticide residues and of good quality—sugar: acid ratio and brix values are summarized as: Flame Seedless (22:17.5), Ruby Seedless (22:17), Thompson Seedless (24, 18), Crimson Seedless (26, 18.5) and Red Globe (22, 16.5).

Development of immature stages of fruit flies in table grapes

MFF and QFF do not develop in table grapes to give sufficient number of pupae to prepare satisfactory disinfestation trials. The artificial infestation methods used in citrus disinfestation studies were unsuitable for table grapes. For MFF, the method found most suitable involved removing 0.5 ml of fruit pulp and inserting 0.2 ml of 6 h old eggs in paper medium using a forceps to give an average of 100–120 eggs per fruit. The eggs were obtained from a standard colony of about 1.2 million adult flies using previously published rearing methods (De Lima et al. 2007).

In MFF trials, the course of development of the immature stages was determined by infesting 500 fruit of each variety and placing in the incubating room at 26 ± 1°C and 60–65% RH. Thereafter at 24 hourly intervals, a sample of 20 fruit was taken for determination of the life stages present. This was done by dissecting the fruit over a series of sieves (Endecotts Ltd, London, UK) ranging in aperture size from 2.0 mm to 125 μm. The fruit pulp was washed with a gentle stream of deionized tap water to separate the eggs and larvae from the fruit medium. The numbers of live and dead individuals present in each stage were counted, and the proportion present in each stage recorded, each day after infestation. The larval stages were classified by examining the mouth hooks under a microscope (Anderson 1963; White & Elson-Harris 1992). Thus, the number of days after infestation at which each of the four target insect life stages (eggs, 1st, 2nd and 3rd instar) predominated in the infesting population was determined. These data were used to commence treatment of infested fruit at each life stage separately. The number of puparia obtained per fruit was determined by setting aside 200 fruit to incubate to pupation. This information was used to determine the number of fruit required to obtain a minimum of 200 insects per replicate.

QFF artificial infestation consisted of two methods. In 1°C tests, fruit were punctured in four spots and placed on top of gauze-covered cages containing about 20,000 adult QFF 3–5 weeks old. Females oviposited into the fruit through the gauze of the cage for 6 to

7 h. In 2°C and 3°C tests, eggs and 1st instar larvae were raised on damp filter paper at 26°C then placed, using a 5 mm wide stainless steel spatula, into the wound in the fruit caused by pulling the berry from its peduncle. The eggs were obtained from a standard colony of about 320,000 adult flies using previously published rearing methods (De Lima et al. 2007). 50 fruit of each cultivar were dissected daily by cutting open under a slow stream of tap water into a series of beakers to recover larvae. Larvae were fixed in 70% alcohol and life stages identified by examining their mouth hooks (Anderson 1963).

Most tolerant stage trials

MFF experiments consisted of 16 treatments at 1°C and 17 treatments at 2°C and 3°C including the untreated control. A total of 9600 fruits of each variety were used at 1°C, while 10,200 each were required at 2°C and 3°C. 28,800 fruit were treated at 1°C while 30,600 were required at 2°C and 3°C each, making 90,000 fruits for all three temperatures. Thus, 50 fruit were selected without conscious bias from the infested batch for each replicated treatment and placed in large labelled plastic 'tote' boxes. The boxes had a wire mesh bottom to permit emerging larvae to drop into the sand contained in a second box fitted below. The box containing the infested fruit was covered with Terylene voile to allow air exchange and sealed in place with a plastic lid having a large aperture in the centre. The fruit were held at 26 ± 1°C and 60–65% RH for the time required for development to each stage required for the experiment. Each exposure consisted of three replicates (150 fruit) for each stage. At each treatment, four stages were tested, requiring 600 infested fruits to be treated. Each replicated trial was set up in one of the three purpose-built (34 m^3) replicate cold rooms (thermostat accuracy of ± 0.1°C). Fruit pulp temperatures were recorded by placing thermistor probes into the core of uninfested fruit placed in similar plastic labelled 'tote' boxes in the cold rooms. The MFF treatments consisted of 15 periods of cold exposure at 1°C and 16 periods at 2°C and 3°C plus one untreated control. The 15 cold exposures were incremental doses of cold at 1°C beginning with 24 h as the lowest dose and increasing by 24 h up to 240 h (10 days); after this, the dose was increased by 48 h up to 480 h. The 16 cold exposures at 2°C and 3°C were incremental doses of cold beginning with 48 h at the lowest dose and increasing by 24 h up to 336 h (14 days); after this the dose was increased by 48 h up to 480 h. 100% mortality was achieved in 12 days at 1°C, 14 days at 2°C and 16 days at 3°C. For QFF experiments, there were 10 periods of cold exposure at 1°C and 17 periods at 2°C and 3°C plus one untreated control. The cold exposures were 24 h incremental doses from 1 to 10, or 1 to 17 days. 100% mortality was achieved in 7 days at 1°C, 13 days at 2°C and 3°C.

Timing of exposure period began when the last probe in the fruit reached 1.0 ± 0.5°C, 2.0 ± 0.5°C or 3.0 ± 0.5°C. This was achieved within approximately 6 h of starting each trial and these treatment conditions were held over the entire experimental period for each table grape variety. The additional doses for both MFF and QFF were used to confirm 100% mortality and to establish at least two successive dose levels at 100% to enable confidence in selecting the treatment for the large-scale trials. After exposure to the specified treatment, the box containing the fruit was removed to the controlled environment room containing the control fruit for collection of surviving stages as puparia. The number of puparia emerging at each dose was compared with the number from the untreated controls to obtain the percentage responding to the treatment. The criterion for survival was the formation of an apparently normal puparium (Baker 1939). Refrigeration for the three cold rooms is supplied by $3 \times$ Lovelock Luke F26-120A air-cooled belt-driven Condensing Units with R22 refrigerant $+ 6 \times$ Muller MNDE17 Induced Draught Evaporator with refrigeration capacity of 7500

Watts at 1°C with compressor speed 536 rpm. This capacity can be increased to 9500 Watts at 1°C by raising the compressor speed to 700 rpm. Defrost cycles are set at 12 h intervals but defrosting may occur on demand from a gas pressure monitoring switch. Six fans, two for each room (350 mm four-blade propeller type) circulate air across the evaporator at an airflow averaging 800 litres/second measured at various points in the room. The fans are switched off during the defrost cycle.

Temperatures were recorded on a 'Squirrel' (Grant Instruments, Cambridge, UK) data logger with an accuracy of ± 0.05°C. Each cold room was supplied with a logger to monitor a total of 16 thermistor probes, six to record air temperatures at various positions in the cold room. One probe each was placed to record the inlet and outlet air temperatures of the cooler. The remaining four probes were placed at various heights in other positions—top door-end, middle, and left and right sides to calibrate the cold room for uniformity of temperatures. The data from each probe site were recorded for the test report. The remaining 10 thermistors were used to record fruit pulp temperatures by placing the probes in the core of uninfested fruit at different positions in the experimental stacks containing the infested fruit. Temperature recordings were automatically logged at 60-min intervals throughout the trial. All thermistor probes were calibrated in melting ice with a certified mercury glass thermometer, before and after each trial to verify their accuracy.

Large-scale trials

For the MFF trials from 2004 to 2005, a total of 81,000 fruits were infested for Thompson Seedless, Crimson Seedless and Red Globe varieties at three temperatures, giving 27,000 each at 1°C, 2°C and 3°C. For QFF trials at 1°C from 1989–90, the number of infested fruits were 99,745 Thompson Seedless, 36,892 Ruby Seedless and 69,038 Flame Seedless. For QFF trials at 2 and 3°C in 2006–07, the number of fruit infested were: for 2°C trials 14,960 Thompson Seedless, 22,750 Crimson Seedless and 12,868 Red Globe; and for 3°C trials 16,746 Thompson Seedless, 21,750 Crimson Seedless and 15,750 Red Globe. In MFF the 2nd instar was shown to be the most tolerant stage to cold treatment, whereas in QFF it was the 1st instar.

Fruits for the large-scale trials were infested in the same way as described above for the most tolerant stage trials. The fruit infested for treatment and controls were held in wire cages over sand in a controlled environment room 26 ± 1°C and 60–65% RH and covered with custom-made Terylene voile covers. The infested fruits in each treatment replicate were incubated to the required development stage for testing. Selection of fruits for treatment and control was done without conscious bias. On the day of treatment, the specified number of fruits for each stage for treatment and control were separated. The control fruits were placed in wire cages for development to pupation. Extra fruit were taken from each replicate trial for dissection to estimate the number of live insects in the target stage treated and other life stages present.

The infested fruits for cold treatment were taken and placed in the centre of every export carton containing uninfested filler fruit and packed following standard export practice. Each replicate trial was set up in a separate cold room and consisted of 448 cartons each containing approximately 10 kg fruit stacked on pallets. There were six pallets in each replicate cold room. Each pallet carried 56 cartons arranged as eight cartons per layer and seven layers high to bring the treated volume to 39.53%. Thus there were 4480 kg fruit in each replicate cold trial in which 2000 fruits infested with 2nd instar MFF were treated and 1000 fruits were held as control. This was replicated three times for each variety and temperature. A similar procedure was adopted for 1st instar QFF. The infested fruits were placed in the centre of every carton throughout the stacks in the cold room so that a representative treatment could be obtained in all cartons.

The cool-down period was accelerated by use of a forced fan cooler placed in the cold

room. When the temperature of the last probe was within the specified treatment temperature (1.0 ± 0.5°C, 2.0 ± 0.5°C or 3.0 ± 0.5°C), the forced cooling process was stopped and normal cold room operations were allowed to continue. In MFF trials, treatments started when the last fruit probe recorded treatment temperature and cool-down times ranged between 47–57 h. In QFF trials, treatments started when half the fruit probes recorded treatment temperature and cool-down times ranged between 8–13 h. For MFF, table grapes were exposed to 1°C for 16 days, 2°C for 18 days and 3°C for 20 days. QFF treatments were for 12 days at 1°C and 14 days at 2°C and 3°C. After exposure to the cold treatment, the infested fruits were removed from the cartons and taken to the controlled environment room at 26 ± 1°C and 60–65% RH and placed in containers over sand to collect puparia.

Data analysis

MFF and QFF data obtained from a series of exposure periods (days) to 1°C, 2°C and 3°C of the four stages—eggs, 1st, 2nd and 3rd instar—were subjected to probit analysis (Finney 1971). The total number from all replicates were combined to obtain the number treated (puparia in control) and the number responding (from puparia obtained for each exposure period) and analysed using the GenStat package (GenStat Release 8.1 2005). For the analysis of MFF data, the probit model uses a generalized linear procedure, assuming a binomial distribution for the number of responses and a probit link function between the number of responses and the logarithm ($\log_{10}$) of the dose. Tests on data using the logit link function and the complementary log-log function did not significantly reduce the residual deviance and the probit link function was retained in analysis. For the analysis of QFF data, independent regression calculation was used for each stage in each fruit since most of the regression lines were not parallel. This method gave a better fit to the data. Testing the data using Wadley's analysis (GenStat Release 8.1 2005) did not give better insight into the data. The relative potency of cold to the fruit flies was calculated at the LD_{50} and LD_{99} estimates and given as a ratio of MFF:QFF.

Results

Development of immature stages of MFF and QFF in table grapes

The details of the life history of MFF and QFF were established before every series of trials. For MFF, approximately 50% of each stage was present on the following days after infestation—eggs, 1–2; 1st instar, 3–5; 2nd instar, 6–8; 3rd instar, 9–12. For QFF, >50% of each stage was present on the following days after infestation—eggs, 0–1; 1st instar, 2–3; 2nd instar, 4–5; 3rd instar, 6–9. The results were used to determine the stage of treatment as well as the date of treatment for each stage following infestation.

Most tolerant stage trials

The MFF data (Table 1) show that the 2nd instar is the most tolerant life stage followed by the 1st instar at the LD_{50} estimate. However, the LD_{99} 95% fiducial limits overlap at 2°C and 3°C for 1st and 2nd instars in Crimson Seedless indicating that these lines are not parallel compared with the other stages. Red Globe is the most susceptible variety to MFF.

The QFF data (Table 1) show that the 1st instar is the most tolerant life stage at the LD_{50} estimate for all varieties at 2°C and 3°C; but eggs are more tolerant at 1°C in Thompson Seedless. Crimson Seedless is the most susceptible variety to QFF followed by Red Globe. QFF exhibits very little change in response to cold treatment at 2°C and 3°C where the 95% fiducial limits frequently overlap at both the LD_{50} and LD_{99} points.

A comparison of data across all varieties (except Ruby Seedless and Flame Seedless for which data are only available at 1°C) for the two fruit fly species is given by the relative

Table 1 Comparison of the number of days exposure at 1°C, 2°C and 3°C required to kill 50% (LD_{50}) and 99% (LD_{99}) with 95% fiducial limits (FL) of the four immature life stages of Mediterranean fruit fly and Queensland fruit fly in five table grape cultivars. The analysis is based on three replicate trials for each life stage.

	Mediterranean fruit fly and grape cultivar infested		Queensland fruit fly and grape cultivar infested		
Treatment temperature and life stage treated	LD_{50} (95% FL) days	LD_{99} (95% FL) days	LD_{50} (95% FL) days	LD_{99} (95% FL) days	Relative potency ratio MFF: QFF LD_{50} – LD_{99}
1.0 ± 0.5°C	**Red Globe**		**Ruby Seedless**		
Eggs	2.633 (2.580, 2.689)	7.781 (7.615, 7.959)	2.432 (2.149, 2.706)	6.075 (5.391, 7.022)	–
1st instar larvae	2.916 (2.858, 2.969)	8.598 (8.421, 8.784)	2.895 (2.598, 3.171)	7.234 (6.525, 8.217)	–
2nd instar larvae	3.473 (3.416, 3.531)	10.261 (10.059, 10.477)	1.962 (1.725, 2.188)	4.901 (4.380, 5.606)	–
3rd instar larvae	2.854 (2.795, 2.909)	8.429 (8.251, 8.605)	1.804 (1.581, 2.016)	4.507 (4.031, 5.145)	–
	Crimson Seedless		**Flame Seedless**		
Eggs	2.532 (2.475, 2.594)	7.298 (7.128, 7.460)	1.584 (1.440, 1.722)	4.596 (4.257, 5.007)	–
1st instar larvae	2.890 (2.833, 2.968)	8.337 (8.155, 8.538)	2.543 (2.331, 2.745)	7.380 (6.876, 7.994)	–
2nd instar larvae	3.159 (3.095, 3.223)	9.093 (8.897, 9.290)	1.702 (1.543, 1.855)	4.940 (4.567, 5.388)	–
3rd instar larvae	2.670 (2.614, 2.736)	7.681 (7.519, 7.862)	1.444 (1.291, 1.594)	4.191 (3.837, 4.613)	–
	Thompson Seedless		**Thompson Seedless**		
Eggs	2.465 (2.405, 2.538)	7.831 (7.640, 8.047)	1.991 (1.625, 2.337)	6.241 (5.472, 7.268)	1.24 – 1.26
1st instar larvae	2.865 (2.795, 2.931)	9.097 (8.885, 9.323)	1.684 (1.399, 1.937)	5.280 (4.814, 5.895)	1.70 – 1.72
2nd instar larvae	3.190 (3.120, 3.278)	10.167 (9.927, 10.412)	1.425 (1.185, 1.642)	4.468 (4.016, 5.073)	2.24 – 2.28
3rd instar larvae	2.732 (2.665, 2.799)	8.672 (8.460, 8.899)	1.248 (1.03, 1.451)	3.913 (3.457, 4.529)	2.19 – 2.22
2.0 ± 0.5°C	**Red Globe**		**Red Globe**		
Eggs	4.389 (4.335, 4.431)	9.760 (9.633, 9.918)	2.339 (2.248, 2.429)	7.407 (7.136, 7.703)	1.88 – 1.32
1st instar larvae	5.515 (5.455, 5.579)	12.280 (12.123, 12.465)	3.267 (3.132, 3.401)	10.347 (9.955, 10.773)	1.69 – 1.19
2nd instar larvae	5.831 (5.770, 5.908)	13.015 (12.830, 13.199)	2.490 (2.391, 2.587)	7.885 (7.606, 8.188)	2.34 – 1.65
3rd instar larvae	4.171 (4.124, 4.228)	9.302 (9.174, 9.447)	2.833 (2.723, 2.942)	8.972 (8.659, 9.312)	1.47 – 1.04
	Crimson Seedless		**Crimson Seedless**		
Eggs	4.047 (3.989, 4.095)	9.240 (9.102, 9.387)	2.062 (1.917, 2.206)	7.117 (6.679, 7.622)	1.96 – 1.30
1st instar larvae	4.924 (4.861, 4.988)	11.278 (11.116, 11.430)	3.134 (2.928, 3.335)	10.814 (10.174, 11.557)	1.57 – 1.04
2nd instar larvae	5.068 (5.007, 5.121)	11.588 (11.412, 11.747)	2.207 (2.049, 2.362)	7.616 (7.155, 8.146)	2.30 – 1.52
3rd instar larvae	3.981 (3.937, 4.034)	9.112 (8.970, 9.245)	2.495 (2.315, 2.673)	8.610 (8.070, 9.233)	1.60 – 1.06

Table 1 (*Continued*)

Treatment temperature and life stage treated	Mediterranean fruit fly and grape cultivar infested		Queensland fruit fly and grape cultivar infested		Relative potency ratio MFF: QFF
	LD_{50} (95% FL) days	LD_{99} (95% FL) days	LD_{50} (95% FL) days	LD_{99} (95% FL) days	LD_{50} – LD_{99}
	Thompson Seedless		**Thompson Seedless**		
Eggs	4.605 (4.541, 4.669)	9.370 (9.236, 9.511)	1.653 (1.510, 1.790)	5.506 (5.177, 5.890)	2.79 – 1.70
1st instar larvae	5.527 (5.455, 5.588)	11.249 (11.096, 11.402)	2.270 (2.063, 2.470)	7.560 (7.093, 8.100)	2.43 – 1.49
2nd instar larvae	5.913 (5.846, 5.987)	12.057 (11.881, 12.225)	2.028 (1.828, 2.226)	6.755 (6.243, 7.351)	2.92 – 1.78
3rd instar larvae	4.525 (4.461, 4.580)	9.201 (9.071, 9.358)	2.126 (1.918, 2.332)	7.082 (6.567, 7.679)	2.13 – 1.30
3.0 ± 0.5°C	**Red Globe**		**Red Globe**		
Eggs	4.392 (4.359, 4.440)	9.726 (9.591, 9.869)	2.533 (2.440, 2.626)	7.614 (7.336, 7.916)	1.73 – 1.28
1st instar larvae	5.915 (5.857, 5.971)	13.073 (12.918, 13.252)	3.570 (3.445, 3.694)	10.731 (10.376, 11.115)	1.66 – 1.22
2nd instar larvae	6.188 (6.120, 6.247)	13.672 (13.500, 13.856)	2.701 (2.602, 2.799)	8.117 (7.843, 8.414)	2.29 – 1.68
3rd instar larvae	4.386 (4.334, 4.439)	9.702 (9.577, 9.831)	2.780 (2.675, 2.884)	8.354 (8.058, 8.676)	1.58 – 1.61
	Crimson Seedless		**Crimson Seedless**		
Eggs	4.211 (4.175, 4.262)	9.743 (9.610, 9.877)	2.128 (2.008, 2.249)	6.913 (6.533, 7.342)	1.98 – 1.41
1st instar larvae	5.360 (5.315, 5.429)	12.409 (12.240, 12.568)	3.333 (3.169, 3.494)	10.826 (10.303, 11.417)	1.61 – 1.15
2nd instar larvae	5.453 (5.399, 5.501)	12.599 (12.434, 12.757)	2.529 (2.394, 2.664)	8.216 (7.797, 8.687)	2.15 – 1.53
3rd instar larvae	4.346 (4.290, 4.398)	10.049 (9.902, 10.173)	2.530 (2.392, 2.668)	8.219 (7.785, 8.707)	1.72 – 1.22
	Thompson Seedless		**Thompson Seedless**		
Eggs	4.392 (4.349, 4.434)	9.932 (9.800, 10.069)	1.685 (1.550, 1.817)	5.673 (5.335, 6.061)	2.61 – 1.75
1st instar larvae	5.548 (5.496, 5.605)	12.556 (12.403, 12.715)	2.449 (2.253, 2.640)	8.246 (7.768, 8.792)	2.27 – 1.52
2nd instar larvae	5.798 (5.740, 5.852)	13.112 (12.955, 13.288)	2.246 (2.064, 2.425)	7.563 (7.116, 8.073)	2.58 – 1.73
3rd instar larvae	4.645 (4.590, 4.699)	10.510 (10.373, 10.659)	2.133 (1.949, 2.316)	7.182 (6.706, 7.725)	2.18 – 1.46

potency of cold treatment to MFF and QFF at the LD_{50} and LD_{99} points (Table 1). This demonstrates that MFF is considerably more tolerant to cold treatment than QFF at all three temperatures. In general, MFF tolerance ratio ranges from 1.238 to 2.916 times QFF at the LD_{50} estimate and 1.037 to 2.278 times at the LD_{99} estimate.

Large-scale trials

The selection of treatments for the large-scale trials was based on obtaining consecutive 100% mortalities in the most tolerant stage trials. For MFF, this was 16 days at 1°C, 18 days at 2°C and 20 days 3°C. In the control fruit (Table 2), the number of puparia recovered from all three varieties at each temperature exceeded 100,000 whereas no puparia were obtained from the cold treatment. In the QFF trials, total insect mortality was achieved in 12 days at 1°C and 14 days at both 2° and 3°C whereas the number of puparia found in the untreated control fruit for all three varieties at each temperature exceeded 100,000.

Discussion

Cold treatment was successful in disinfesting fruit flies in table grapes giving a temperature-time schedule as QFF 1°C 12 days, 2°C 14 days, 3°C 14 days; and MFF 1°C 16 days, 2°C 18 days, 3°C 20 days. In previous work (Hill et al. 1988; Jessup et al. 1993; Heather et al. 1996; De Lima et al. 2002; Lima et al. 2007) probit 9 level of security was determined in citrus as MFF (oranges and mandarins) 1°C 16 days, 2°C 18 days, 3°C 20 days; MFF (lemons) 1°C 14 days, 2°C 16 days, 3°C 18 days; and for QFF (oranges and mandarins) 1°C 14 days, 2°C 16 days, 3°C 16 days; (lemons) 1°C 12 days, 2°C 14 days, 3°C 14 days. Thus for QFF, the table grapes schedule is similar to that obtained for lemons whereas for MFF it is similar to that obtained for mandarins and oranges. QFF are more susceptible to cold treatment in grapes than in oranges and mandarins at the temperatures tested; whereas MFF are more susceptible to cold treatment in lemons than in grapes.

Powell (2003) analysed the MFF data from several workers dating from 1916 and found uncertainty was greater in the <1°C than in the 1.1–2.2°C temperature-time treatments and concluded that there was a high degree of confidence ($>95\%$) that a 2.2°C (36°F) 18-day APHIS treatment would achieve a probit 9 level of protection. He also found more variability in larval response to time-temperature-fruit combinations than to different hosts. In our data we find that larval response to hosts are more clearly defined in the 1–3°C temperature-time regimes than larval variation to temperature alone. The variation in response at 1°C is higher than at 2°C and 3°C especially for QFF. However, control in grapes and lemons is clearly achieved in shorter times than in oranges and mandarins for both QFF and MFF.

Heather and Hallman (2008) in reviewing cold disinfestation methods considered that experimental procedures where infested fruit is held at high temperature for insect development before they are cold treated leads to longer than normal cool-down times obtained in commercial practice. Australian export citrus is harvested in winter at <22°C and cooled down over a period of 72–96 h to optimum holding temperatures: oranges 7°C, mandarins 5°C, lemons 12°C. Table grapes are harvested in summer at >25°C and cool down to optimum holding temperatures 1–2°C is achieved within 18–36 h in commercial practice. While cool down in commercial practice may be shorter than in the disinfestation studies, the marginally longer exposure period (about 12 h) is viewed as a safeguard by quarantine authorities.

The APHIS schedule mandates temperature and duration of cold treatments that are pest species specific but not host specific. In contrast, MAFF (Ministry of Agriculture Forestry and Fisheries) Japan requires pest and host species specific treatments. In the APHIS generic approach, the commodities that are less supportive

Table 2 Large-scale trials showing the total number treated of 2nd instar MFF and 1st instar QFF. Comparison of the number of days required to kill >100,000 individuals in three replicate trials in five table grape varieties at 1°C, 2°C and 3°C.

	Mediterranean fruit fly			Queensland fruit fly		
Treatment temperature and variety	Days treated	Number of insects treated	Number of survivors	Days treated	Number of insects treated	Number of survivors
1.0±0.5°C						
Ruby Seedless	–	–	–	12	47,341	0
Flame Seedless	–	–	–	12	66,895	0
Red Globe	16	84,560	0	12	39,829	0
Crimson Seedless	16	81,006	0	–	–	–
Thompson Seedless	16	83,320	0	–	–	–
Total 3 varieties		248,886	0		154,065	0
2.0±0.5°C						
Red Globe	18	78,859	0	14	53,136	0
Crimson Seedless	18	84,684	0	14	48,196	0
Thompson Seedless	18	84,262	0	14	38,903	0
Total 3 varieties		247,805	0		140,235	0
3.0±0.5°C						
Red Globe	20	75,884	0	14	43,622	0
Crimson Seedless	20	73,236	0	14	70,831	0
Thompson Seedless	20	80,017	0	14	133,663	0
Total 3 varieties		229,137	0		248,116	0

of insects are accorded the same treatment as more productive hosts. For example, lemons are less hospitable to fruit fly than other citrus requiring 2 days less cold treatment (De Lima et al. 2007). When the LD_{50} data are examined (De Lima et al. 2007, and in this paper) the relative potency ratio MFF:QFF is highest in lemons (8.71 × at 2°C; 11.35 × at 3°C) and lowest in Thompson Seedless grapes (2.92 × at 2°C; 2.58 × at 3°C) indicating the wide variation in influence of hosts on comparative insect response. In this paper we show that in table grapes QFF can be disinfested under the same temperature-time treatment as lemons. This difference is acceptable to MAFF Japan as specific host treatments, but not to the United States Department of Agriculture (USDA) where the generic cold treatment is required. In terms of research requirements, the USDA approach can be simpler to handle since proof is based on cumulative tests of eggs and all larval stages totalling >100,000 insects with less than three survivors. The MAFF Japan approach can be more onerous since tests are required to prove the most tolerant stage after which this stage is selected for disinfestation of >30,000 insects with no survivors.

Data presented in this paper and published previously (Hill et al. 1988; Jessup et al. 1993; Heather et al. 1996; De Lima et al. 2007) have consistently shown QFF to be more susceptible to cold treatment than MFF. The greater susceptibility of other *Bactrocera* species to cold (as compared with MFF) has also been demonstrated by other researchers. For example, Burditt and Balock (1985) showed that all stages of the Oriental fruit fly *Bactrocera dorsalis* (Hendel) were killed in 12 days at 2.7°C and all

stages of Melon fruit fly *Bactrocera cucurbitae* (Coquillett) were killed in 10 days at 2.7°C. The *Bactrocera* species clearly are more susceptible to cold than the MFF and the case is made for a shorter cold treatment period for the QFF *Bactrocera tryoni*. In a review of the USDA APHIS Quarantine Treatment Manual of 1992, Gould (1994) referred to the finding of Meats (1976) that QFF acclimatises to cold temperatures. Current USDA-APHIS treatment schedules (APHIS 2006) for QFF are: <0°C, 13 days; 0.56°C, 14 days; 1.11°C, 18 days; 1.67°C, 20 days; 2.22°C, 22 days. However, the data presented here demonstrate that QFF are killed by continuous exposure for 14 days at all temperatures below 3°C and it is proposed that APHIS treatment schedules for the Queensland fruit fly in table grapes be modified to accept treatment for 12 days at 1°C and 14 days at 2°C and 3°C to benefit trade.

Advice from industry and shipping companies is that when issues arise in maintaining specified in-transit treatments, a temperature-time schedule provides exporters with more options in selecting upper limits for treatment temperatures. While table grapes store well for long periods at 0–2°C, the availability of temperature-time treatment schedules to 3°C gives industry more flexibility in exporting fresh harvest and disinfesting in-transit in refrigerated sea containers. In Australia, optimum table grape quality can be maintained for 5 months or more by storing fruit in the 0–2°C range and 85–95% RH (Cameron & Pasquale 2006). The research presented in this paper shows that these storage conditions are very suitable for disinfesting fruit flies.

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