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Effect of heat shock and quarantine cold treatment with a warm temperature spike on survival of Mediterranean fruit fly eggs and fruit quality in Hawaii-grown ‘Sharwil’ avocado

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Abstract

The effects of a transient (warming) temperature spike on efficacy of an APHIS approved quarantine cold treatment, T107 (a), against Mediterranean fruit fly, *Ceratitis capitata*, was tested on Hawaii grown ‘Sharwil’ avocados. Avocados infested with late stage eggs were subjected to a warming temperature spike (ca. 4.2°C for 1 h) at 6–9 days into the treatment and subsequently allowed to resume the treatment until conclusion (12 days at < 1.1°C, 14 days at < 1.67°C or 16 days at < 2.2°C). Insertion of a ca. 4.2°C temperature spike into the treatment at 6–9 days had no effect on the efficacy of the quarantine cold treatment when fruit were allowed to resume the treatment to completion. Infested fruit which did not receive a ‘heat shock’ treatment (recommended to improve fruit quality) and subjected to cold treatment for 6–16 days at either < 1.1, < 1.67 or < 2.2°C (fruit center temperature) had no survivors in the fruit by the 9th day of cold treatment. Infested avocados subjected to a ‘heat shock’ treatment for 10–12 h at 38°C prior to cold treatment (as above) had no survivors in the fruit by the 8th day of cold treatment. Results of this study indicate that a transient (warm) temperature spike of ca. 4.2°C of the type experienced during an in-transit cold treatment of Hawaii grown ‘Sharwil’ avocados will not compromise the efficacy of the treatment. This study also reconfirmed that the T107 (a) cold treatment (as stated in the APHIS treatment manual) is efficacious against Mediterranean fruit fly eggs in ‘Sharwil’ avocados, and that use of a ‘heat shock’ to prevent chilling injury during the cold treatment did not extend survivorship of fruit fly eggs. Studies on the effects of prolonged (18–28 day) cold storage on fruit quality indicated that avocados can be stored at quarantine cold temperature (pulp, 1.1–2.2°C) for up to 24 days without significant loss of external and internal quality compared to fruit quality at 12–16 days storage. Also, shelf life, Gray Flesh discoloration (of internal tissue), and disease were not affected by the prolonged storage duration. © Published by Elsevier Science B.V.

Keywords: *Persea americana*; Avocado; Heat shock; Quarantine cold treatment; Mediterranean fruit fly; *Ceratitis capitata*; Fruit quality

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1. Introduction

The United States Department of Agriculture, Animal Plant Health Inspection Service (USDA-APHIS) requires that avocados (*Persea americana*) from Hawaii which are hosts of fruit flies be free of such pests prior to their entry into the contiguous US. Cold treatments are used for disinfestation of fruit flies in a number of commodities including citrus, carambola, lychee and kiwifruit (Gould, 1994). The treatment requires that fruit be exposed to a minimum fruit center temperature (FCT) of 12 days at $< 1.1^{\circ}\text{C}$, 14 days at $< 1.67^{\circ}\text{C}$ or 16 days at $< 2.2^{\circ}\text{C}$. In March 1996, use of the cold treatment, T107 (a), was approved for Hawaii grown 'Sharwil' avocados by USDA-APHIS-PPQ (APHIS, 1998). In January 1998, a commercial shipment of 'Sharwil' avocado was treated 'in transit' to its final destination of Seattle, WA. During the shipment, the maximum fruit center temperature (2.2°C) for the longest treatment time (16 days) was temporarily breached resulting in a few fruit having a measured fruit center temperature (FCT) of up to 3°C before returning to below 2.2°C for the duration of the treatment. According to the APHIS regulations, at that point in time, the fruit did not pass certification and thus was rejected at destination. The action resulted in a significant financial loss for the shipper.

The rejection criteria for failure to meet the cold treatment requirements were based on historical efficacy data at specific treatment times and temperatures. There have been no reports on the effects of limited temperature breaches on insect survival. The purpose of this study was to assess if transient (warming) temperature spikes result in appreciable risk to quarantine security when allowed to subsequently complete the treatment at approved temperatures and times. In addition, we retested the efficacy of the cold treatment without temperature spikes and assessed if the 'heat shock' (used to improve quality of cold-treated avocado) had an appreciable effect on insect survival.

In the event a second cold treatment was required to rectify an interrupted initial cold treatment, the effects of a prolonged treatment at quarantine temperatures on fruit quality were un-

known. Thus, fruit quality studies were also included to determine if back-to-back cold storage treatments would be detrimental to fruit quality.

2. Methods and materials

2.1. Insects

Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (medfly) pupae from the USDA-ARS, Pacific Basin Agricultural Research Center (PBARC) mass-rearing facility in Honolulu, HI were shipped by air to Hilo, HI where they were placed in $1 \times 1 \times 1$ m aluminum screened emergence cages containing sugar, water and hydrolyzed protein. Flies were held at $24\text{--}26^{\circ}\text{C}$, RH of 60–70% and a 12-h light:12-h dark photoperiod. Eggs were collected from adult stock colonies that were 10–12 days old using the previously published techniques (Jang, 1996). Only late stage ($> 80\%$ developed) eggs were used in this study. Late stage medfly eggs were reported to be most resistant to cold temperatures (Armstrong et al., 1995) of the three species (Mediterranean fruit fly, oriental fruit fly, (*Bactrocera dorsalis* Hendel) and melon fly, (*B. cucurbitae* Coquillett)) that infest avocado in Hawaii. A control consisting of three groups of 100 eggs were held on moist blotter paper in covered petri dishes to determine the hatch rate of the eggs from mass-reared flies.

2.2. Avocados

Avocado (*Persea americana*) var. 'Sharwil' obtained from Kona Gold Farm, Captain Cook, HI, were washed, waxed, and sorted under commercial conditions. For each test, 12 boxes of medium sized fruit (315–355 g per fruit) were transported to the ARS laboratory in Hilo one day prior to initiation of the test and held overnight in the lab ($22\text{--}23^{\circ}\text{C}$).

2.3. Infestation of fruit with *C. capitata*

One hundred and thirty avocados were infested with late Medfly eggs (approximately 48 h old at the time of the cold treatment) according to the

previously published methods (Jang, 1996). Each avocado was 'cored' through the fruit just above the seed using a 7-mm cork borer. The resulting transectional 'plug' was removed and a 1-cm section was removed from the center. One endocarp end of the halved plug was placed back into the avocado. Approximately 900 eggs in 10–20 μ l of water were inserted (1–2 cm) into the center of the fruit through the open end. The second portion of the plug was then replaced into the fruit and both ends of the fruit plug were secured with masking tape. Ten infested avocados were held at room temperature (22–23°C) for the duration of the longest treatment time as a control sample for the cold-treated avocados. In addition, a second non-treated control consisting of either an artificially infested peach fruit or *C. capitata* eggs placed on a standard artificial diet (wheat, torula yeast, sugar and preservatives) was included as a 'positive control' due to the poor host suitability of avocado fruit (Jang, 1996).

At the conclusion of the treatments, insects were removed from the infested avocado by removing the plug of the fruit and gently flushing out insects with water onto the prepared artificial diet. The tissue immediately surrounding the core of the avocado fruit was also cut from the fruit and along with the 'plug' placed on diet to insure that all the treated insects would be retrieved. Diets containing eggs, avocado plugs (cores) and surrounding tissue were placed over a bed of clean sand in screened plastic containers (Jang, 1996) and held in a fly-free room at 24–26°C. After 7–10 days the sand was screened for the presence of pupae and the diet media checked for the presence of live insects. Insect survival for each treatment was determined by the presence of live larvae and pupae in the diet/tissue. Estimated treated populations (ETP) and total treated population (TTP) were calculated according to the method of Jang (1996).

2.4. Experiments and treatments

Four tests (replicates) were conducted from February to April, 1998 and consisted of four experiments.

2.4.1. Experiment A

This consisted of six treatments (five fruit each), and determined if the heat shocked (conditioned) 'Sharwil' avocados prior to cold treatment affected the survival or mortality of Mediterranean fruit fly eggs. Treatments included heat shock (HS) only, HS + 6 days cold treatment (d CT), HS + 7 days CT, HS + 8 days CT, HS + 9 days CT, and HS + 12–16 days CT (exposure period varied from 12–16 days, depending on quarantine treatment temperature).

2.4.2. Experiment B

It consisted of four treatments (ten fruit each), and determined if the interruption of quarantine cold treatment with a single temperature 'spike' affected survival or mortality of Mediterranean fruit fly eggs. Treatments included HS + spike on day 6, HS + spike on day 7, HS + spike on day 8, HS + spike on day 9. All the treatments were returned to cold treatment for the completion of the exposure period (12–16 days) required for the quarantine treatment temperature (1.1–2.2°C).

2.4.3. Experiment C

This consisted of six treatments (ten fruit each) and determined the exposure period (length of time in days) required to kill *C. capitata* eggs at quarantine treatment temperatures (1.1–2.2°C, FCT). Treatments included 6, 7, 8, 9 and 12–16 days at quarantine treatment temperature.

2.4.4. Experiment D

Consisting of seven treatments (about 25 fruit each), it determined the effect of duration (days of storage) of quarantine cold treatment (pulp temperature, 1.1–2.2°C), after heat shock, on shelf life (days to ripen at room temperature, 22–23°C) and fruit quality. Treatments included HS + 12–16 days, HS + 18 days, HS + 20 days, HS + 22 days, HS + 24 days, HS + 26 days and HS + 28 days cold temperature, each stored in separate fiberboard cartons. After cold storage treatment, fruit were allowed to ripen at room temperature (22–23°C).

2.5. Heat shock (conditioning) and quarantine cold treatments

Avocado fruit were 'heat shocked' to condition the fruit against chilling injury which would normally occur as a result of a cold treatment (Nishijima et al., 1995). The 'heat shock' is not considered a part of the quarantine treatment for fruit fly disinfestation but results in fruit of improved quality compared to cold-treated only fruit. Heat shock treatment was conducted in a heat chamber designed and constructed by the University of Hawaii-Manoa, College of Tropical Agriculture and Human Resources (CTAHR) at the Waiakea Research Station in Hilo, Hawaii. The heat chamber was described in previous studies (Hara et al., 1997).

Avocados were packed into vented cartons (three cartons for infestation studies and seven cartons for quality studies), heat shocked at 38°C (air) for 10–12 h in the heat chamber, transferred and cooled in a cooling room at ca. 21°C until pulp temperatures were <28°C. Avocados not subjected to heat shock were held at room temperature (22–23°C).

After the heat shock treatment, infested fruit for Experiments A and C were kept in the same carton, but infested fruit in Experiment B were marked and then mixed with non-infested 'filler' fruit and placed in separate boxes by spike treatment. The seven boxes of infested fruit and seven boxes of quality control fruit were cold treated in a walk-in refrigerated room (Kysor//Kalt, Portland, OR) at $0.68 \pm 0.07^\circ\text{C}$.

The 14 boxes were stacked in a staggered design, two-layers high, and supported off the concrete floor on wooded 'slats' to allow airflow. A circulating fan was used to enhance air circulation through the room.

Quarantine cold treatment commenced when pulp temperatures near the seed reached <2.2, <1.67, or <1.1°C, usually about 1–2 days after exposure to cold storage temperatures. Pulp temperatures of the 'warmest' fruit determined the exposure period (days) of the quarantine treatment. According to the APHIS schedule, pulp temperatures of <1.1, <1.67 or <2.2°C require exposure of 12, 14 or 16 days, respectively.

2.6. Spike temperature treatment (Experiment B)

A single box of infested avocados (mixed with non-infested 'fillers') was removed from the cold treatment on the 6th, 7th, 8th or 9th day and placed in a walk-in refrigerator set at $7.6 \pm 0.10^\circ\text{C}$. After the pulp temperature reached a minimum 4.2°C, the fruit were returned to the cold treatment walk-in and the 12–16 days exposure time was continued for quarantine treatment. Actual mean pulp temperatures recorded were $4.48 \pm 0.28^\circ\text{C}$, for 1 h or $>2.2^\circ\text{C}$ for 3 h.

2.7. Monitoring temperatures

Omega OM-481 (Omega, Stamford, CT) and Omnidata polycorder (Omnidata International Inc., Logan, UT) temperature recording devices with thermistor or thermocouple probes were used to record air and pulp temperatures. Fruit were probed through the flesh to seed depth using a metal spike or screwdriver and the hole or cavity was sealed with orange oil and masking tape.

During heat shock in the heat chamber, pulp temperatures of single fruit were monitored for each box of infested fruit and at least two boxes of quality fruit. Air temperatures were monitored near air intake vents and near the stack of boxes.

During cold treatment in the walk-in refrigerated room, pulp temperatures were similarly monitored for each box of infested fruit and at least one box of quality fruit. Air temperatures were monitored near the middle of the room (on the wall) and on top of the stack of boxes. A sample temperature profile showing a transient temperature spike to ca. 4.2–5.0°C and return to the treatment temperature is shown in Fig. 1.

2.8. Fruit quality evaluation

External and internal quality of avocados was determined after fruit were ripened at room temperature (22–23°C) following 12–16, 18, 20, 22, 24, 26 and 28 days cold storage (previously treated by heat shock). External appearance was evaluated on a rating scale of 5–1 (5, excellent; 4, good; 3, acceptable, marketable; 2, marginally

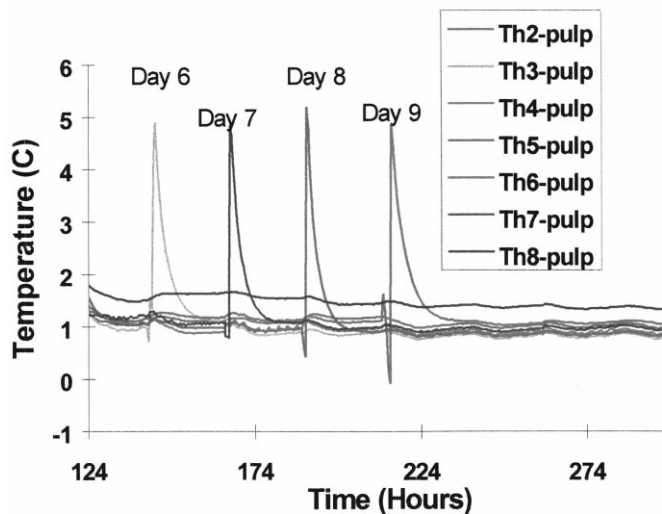


Fig. 1. Air and pulp fruit center temperatures for 'Sharwil' avocados subject to quarantine cold treatments exhibiting a temperature spike of 4.2°C on days 6–9 of the 12–16 day treatment protocol.

acceptable and 1, unacceptable) and internal quality was based on acceptable or not acceptable flesh appearance and texture (Sanxter et al., 1994; Nishijima et al., 1995). Gray Flesh (a specific discoloration of internal tissue associated with chilling injury) and incidence of disease (stem-end and/or body rot) were also recorded.

2.9. Analysis of data

Survival of cold treated insects was based on percent estimated treated population (ETP) which was the total number of individuals that survived in untreated control fruit. Although the APHIS treatment protocol allows for different exposure times based on the fruit center (pulp) temperature, this temperature cannot exceed 2.2°C. Analysis of variance (ANOVA) found no difference ($P > 0.05$) between tests (replications) within any of the experiments (A–C) carried out using cold treatment (Table 1). Thus, data from all of the tests (replicates) within an experiment were combined for the purpose of the analysis. Data were analyzed for significant differences among treatments using the general linear models procedure (Proc GLM) and comparison of means were based on Fisher's unprotectd LSD test at $P = 0.05$ level of significance (SAS Institute Inc., 1989–1996).

Fruit quality data were analyzed using the general linear models procedure (Proc GLM) and regression analysis procedure (Proc Reg), and comparison of means, where appropriate, was based on Fisher's unprotectd LSD test at $P =$

Table 1

Analysis of variance of Experiment A — heat shock (10–12 h at 38°C, air) treatments (heat shock only, heat shock+6 days cold treatment (pulp at <2.2°C), heat shock+7 days cold treatment, heat shock+8 days cold treatment and heat shock+12–16 days cold treatment), and block on survival^a of Mediterranean fruit fly eggs in 'Sharwil' avocados

Source	df	F value
Survival of Mediterranean fruit fly eggs		
<i>Experiment A</i>		
Treatment	5	12.93****
Test	3	0.83
<i>Experiment C^b</i>		
Treatment	5	2721.10****
Test	3	1.49

^a Survival based on percent estimated treated population (ETP).

^b ANOVA of Experiment C — cold treatment only (0, 6, 7, 8, 9 or 12–16 day at pulp temperature <2.2°C), and block on survival of Mediterranean fruit fly eggs in 'Sharwil' avocados.

^c F value for main (treatment) or block (test) effect, by experiment, significant at $P = 0.001$ (***)

0.05 level of significance. (SAS Institute Inc., 1989–1996).

3. Results

3.1. Survival of *C. capitata* eggs in ‘Sharwil’ avocado subject to a heat-shock conditioning prior to cold treatment (Experiment A)

Heat shock of infested avocado fruit without cold treatment resulted in a mean of 86% of the fruit fly eggs surviving compared to non-heat shocked controls (Table 2, day 0). Cold treatment of heat shocked fruit for 6–16 days at $< 2.2^{\circ}\text{C}$ showed a large increase in mortality at 6 and 7 days (4 and 0.5%, respectively) with no survivors recovered after 8 days of treatment (Table 2). Statistically there were no significant differences among the heat shock fruit that had been cold treated. The total estimated treated population (ETP) for these tests were 8386 for the four tests, while the total treated population (TTP) for the four tests was 75 810. No survivors were found in infested avocado held for 8 days and beyond in these tests.

3.2. Effects of transient temperature spike (4.2°C) on survivorship of *C. capitata* in heat shocked and cold treated ‘Sharwil’ avocado (Experiment B)

For each of the four tests (12 days at $< 1.1^{\circ}\text{C}$ (test 1), 16 days at $< 2.2^{\circ}\text{C}$ (test 2), or 14 days at $< 1.67^{\circ}\text{C}$ (tests 3 and 4)), exposure of infested fruit to a temperature spike (pulp at $4.48 \pm 0.28^{\circ}\text{C}$, for 1 h) during the treatment had no effect on survivorship of *C. capitata* eggs when the fruit were subsequently allowed to complete the required treatment protocols. No eggs or larvae survived regardless of whether the temperature spike occurred on day 6–9, and regardless of which protocol was used, as long as the treatment was allowed to resume treatment at the required temperatures and times until completion. No tests of temperature spikes on days earlier than day 6 were conducted, since it was deemed reasonable that if a spike occurred early in the treatment

Table 2

Effect of heat shock (10–12 h at 38°C , air) and cold treatment or cold treatment only on survival^a of Mediterranean fruit fly eggs in ‘Sharwil’ avocado — Experiment A and C

Treatment	Number of Tests ^b	Survival of Medfly eggs exposed to cold treatment (pulp $< 2.2^{\circ}\text{C}$) Mean% ETP
<i>Experiment A</i>		
Heat shock (HS) only	4	86.35 a ^c
HS+6 days cold treatment	4	3.99 b
HS+7 days cold treatment	4	0.46 b
HS+8 days cold treatment	4	0.00 b
HS+9 days cold treatment	4	0.00 b
HS+12–16 days cold treatment	4	0.00 b
<i>Experiment C</i>		
0 days cold treatment	4	100.00 a
6 days cold treatment	4	5.70 b
7 days cold treatment	4	0.68 b
8 days cold treatment	4	0.09 c
9 days cold treatment	4	0.00 c
12–16 days cold treatment	4	0.00 c

^a Survival based on percent estimated treated population (ETP).

^b Each test consisted of five to ten fruit which were artificially infested with approximately 900 eggs in each fruit.

^c Values in column, by experiment, followed by the same letter are not significantly different according to Fisher’s un-protected LSD test at $P = 0.05$.

protocol, the treatment would start over since the fruit quality would not be adversely affected (Sanxter et al., 1994). A total ETP of 12 924 eggs survived from non-treated controls in infested av-

ocado. However, based on the cumulative TTP of 102 080 treated insects without survivors over the course of the four tests, probit 9 efficacy at the 95% confidence limits (CL) for the cold treatments was exceeded.

3.3. Survival of *C. capitata* eggs in 'Sharwil' avocado at various cold temperatures (Experiment C)

For non-heat shocked avocados infested with eggs of Mediterranean fruit fly, mortality at 6 and 7 days were significantly different from 8, 9 and 12–16 days (Table 2). Comparing results of tests in which infested fruit were heat shocked and non-heat shocked prior to cold treatment we found that application of heat shock contributed to overall mortality of eggs. When averaged over all the four tests, the results showed no survivors of *C. capitata* eggs after 9 days of cold treatment at any of the three temperature protocols. The mean survivorship was 5.7% at 6 days of treatment and less than 1% for days 7 or 8. The estimated treated population (ETP) based on survivorship of *C. capitata* eggs in avocado was 13 541 treated insects for the cumulative total of the four tests. However based on the total treated population (TTP) (which was based on the estimated number of eggs placed into treated fruit and corrected for hatch rate) the cumulative total number of eggs treated was 126 350.

3.4. Shelf-life (days to ripen) and fruit quality of 'Sharwil' avocados that were heat shocked then stored at quarantine cold temperature for various lengths of time (Experiment D)

Overall, approximately 5 days at room temperature (22°C) was the ripening period for all the heat shocked, cold storage treatments, Tables 3 and 4, regardless of duration regime (12–28 days) (Table 4). External quality rating (5, excellent to 1, not acceptable) were of average and slightly above average rating (slightly above 3) for up to 24 days. Storage for 26 and 28 days resulted in slightly lower external quality. Regression analysis showed a negative linear relationship ($R^2 = 0.3626$) between external quality rating (y variable) and duration of cold treatment (x variable), $y = -0.0403x + 3.9815$, with significant parameter estimates, $P = 0.0001$ for intercept and $P = 0.0007$ for the treatment coefficient. Internal acceptance (percent of fruit with acceptable quality internal tissue) was at least 84% for fruit stored for up to 24 days, but was slightly less acceptable (77–80%) for 26 and 28 days storage. Regression analysis showed a negative linear relationship ($R^2 = 0.2491$) between percent internal acceptance (y variable) and duration of cold treatment (x variable), $y = -0.8517x + 104.03$, with significant parameter estimates, $P = 0.0001$ for intercept and $P = 0.0069$ for treatment coefficient. Incidence of Gray Flesh (discoloration of internal

Table 3

Analysis of variance of cold storage treatment (days at <2.2°C, pulp) following heat shock (10–12 h at 38°C, air), and block on shelf life (days to ripe at 22–23°C) and fruit quality of 'Sharwil' avocado — Experiment D

Source	Df	F value				
		Days to ripen	External quality rating ^a	Internal acceptance ^b (%)	Gray flesh ^c (%)	Disease (%)
Treatment	5	1.69*	3.80* ^d	7.27***	2.52	2.20
Test	3	4.23*	1.84	14.05***	6.04**	3.51*

^a External quality rating based on 5, excellent; 4, good; 3, acceptable/marketable; 2, marginally acceptable, and 1, unacceptable.

^b Internal tissue was acceptable if absent of defects or disease.

^c Gray flesh was an unacceptable gray discoloration of internal tissue.

^d F value for main (treatment) or block (test) effect significant at $P = 0.05$ (*), $P = 0.01$ (**), or $P = 0.001$ (***).

Table 4

Effect of cold storage treatment (days at <2.2°C, pulp) following heat shock treatment (10–12 h at 38°C, air) on shelf life (days to ripen at 22–23°C) and fruit quality of ‘Sharwil’ avocado — Experiment D

Cold storage (days)	Means ^a				
	Days to ripen	External quality rating ^b	Internal acceptance ^c (%)	Gray flesh ^d (%)	Disease (%)
12–16	5.00 ab ^e	3.42 a	91.6 a	5.9	11.2
18	5.50 a	3.19 ab	87.6 ab	19.5	13.5
20	4.92 ab	3.36 a	92.6 a	7.6	16.4
22	4.91 ab	3.10 abc	84.2 bc	19.1	14.3
24	4.66 b	3.19 a	88.3 ab	17.9	10.9
26	5.15 ab	2.81 bc	76.8 d	15.2	24.6
28	4.60 b	2.76 c	79.5 cd	14.6	17.8

^a Data represent means of four tests, with about 25 fruit in each test.

^b External quality rating based on 5, excellent; 4, good; 3, acceptable/marketable; 2, marginally acceptable, and 1, unacceptable.

^c Internal tissue was acceptable if absent of defects or disease.

^d Gray flesh was an unacceptable gray discoloration of internal tissue.

^e Values in columns followed by the same letter are not significantly different according to Fisher's unprotected LSD test at $P = 0.05$ level of significance.

tissue) which ranged from 6–19%, and incidence of disease, which ranged from 11–25% were not significantly different among the cold storage duration regimes.

4. Discussion

Results of this research successfully established three important parameters that support the use of the APHIS approved cold treatment for disinfestation of tephritid fruit flies in ‘Sharwil’ avocados. First, the cold treatment as listed in the APHIS treatment manual will meet or exceed probit 9 efficacy for late stage *C. capitata* eggs. Since *C. capitata* eggs are more cold tolerant than other developmental stages of *C. capitata*, *B. dorsalis*, or *B. cucurbitae* in cold treated carambola (Armstrong et al., 1995), the treatment protocol should be effective against these species in avocado. Our results showed no survivorship of *C. capitata* eggs after 9 days of cold treatment. Second, we determined that a heat-shock treatment used to prevent skin discoloration and improve fruit quality of ‘Sharwil’ avocados followed by exposure of up to 16 days of cold treatment had no conditioning effect on *C. capitata* eggs. Our

findings suggest that *C. capitata* eggs showed a lower survival in every case when exposed to a heat-shock prior to the cold treatment as compared with non-heat-shocked infested fruit. In our experiments, heat-shock of infested fruit resulted in no survivors in the test fruit after 8 days of cold for any of the four tests conducted. Third, a transient (ca. 2–3 h) breach in the temperature during the course of a simulated in-transit cold treatment did not affect the overall efficacy of the treatment as long as the treatment was allowed to resume, and the full treatment schedule allowed to proceed to completion. We based these results on a temperature spike of ca. 4.2–4.5°C which approximated an actual commercial situation in which a temperature spike of 3°C occurred during in-transit cold treatment of ‘Sharwil’ avocado shipped from Hawaii to Seattle in 1998.

Current APHIS protocols allow that if a given cold treatment temperature is breached after having achieved a FCT of, e.g. <1.1°C that the treatment may continue based on the next (higher) temperature (e.g. <1.67°C), as long as FCT of the fruit did not exceed the next higher temperature and the time is extended to meet the requirements of the treatment at the new temperature (e.g. to 14 days at <1.67°C). However, if the

FCT of 2.2°C is exceeded at any time during the treatment, the treatment must then start over again or the shipment is deemed to have not met the 'minimum' treatment protocol. Our results show that transient temperature spikes (in excess of 2.2°C) of the type tested in this study will not compromise the efficacy of the treatment and that no additional days of treatment are needed if the temperature is subsequently returned to the (lower) temperature and the treatment allowed to continue for the remaining required days of treatment. We do not have information on the length of time that the temperature breach may last during a treatment or the highest temperature that could be achieved without requiring additional days of treatment. These questions are outside the scope of this study but are clearly warranted and necessary if adjustments to cold treatments for a variety of conditions are desired. However, we do recommend that in-transit cold treatments of 'Sharwil' avocado which experience temperature breaches of no more than 4.2°C (for no longer than 3 h) at day 6–9 (after having previously attained a designated FCT) be certified as 'successfully treated' as long as temperature and duration regimes are resumed and completed.

Temperature spikes on days 1–5 were not tested in this study, however, our fruit quality studies indicated that even after 28 days, there was little reduction in the quality. Thus, if a temperature spike occurs during days 1–5, the fruit quality would not be adversely affected if the treatment were to start again from day 0. Our studies also indicate that at day 9 and beyond (day 8 and beyond with preconditioned fruit) no eggs survived the cold treatment at any of the three treatment temperatures tested in this study. Thus temperature spikes that may occur beyond day 9 should not pose any additional risk to quarantine security. We recommend, however, that if temperature spikes occur beyond day 9, that the same protocols (as the 6–9 day protocol) for monitoring sequential (hourly) temperature readings are followed and that the consignment must still complete the required treatment regime (days and temperature) to be considered fruit fly disinfested.

Shelf life and fruit quality results in these studies confirmed results in previous studies (Sanxter et al.,

1994). Fruit ripened in approximately 5 days after being held at pulp temperature < 2.2°C for up to 28 days in our studies, which was identical to previous studies for 17–21 days duration. External quality rating of 3.0 in the previous studies was comparable to our results for external quality rating of about 3. However, 60% internal quality acceptance of previous studies was less than our results, which was 85–93% internal acceptance for 12–24 days duration. In summary, this study demonstrated that fruit quality was not diminished when stored up to 24 days at quarantine cold temperatures.

We believe that allowing for a transient temperature spike of the type reported in this study will not compromise quarantine security or fruit quality provided the treatment regime be allowed to continue. In addition, allowing for such deviations from the current protocol will facilitate the export of 'Sharwil' avocados to the US mainland while continuing to insure that quarantine pests (such as fruit flies) are kept out. To our knowledge, this study is the first to examine the effects of temporary breaches in quarantine cold treatment protocols on the resulting efficacy of the treatment. This study, while limited to a narrow temperature spike, documents the scientific basis for additional flexibility in ensuring farm-to-store availability of fresh, high quality produce such as avocados. As a result of this study, USDA-APHIS, PPQ subsequently revised their T107 (a) protocols to allow for a transient temperature spike similar to that reported in this study.

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