Controlled Atmosphere/High Temperature Forced Air: A Non-Chemical Quarantine Treatment for Stone Fruit

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ABSTRACT

Three replicate tests were performed in which peaches were infested with Oriental Fruit Moth larvae and treated with forced hot air combined with a controlled atmosphere (CATTS) in a large chamber while packed into boxes that had been stacked onto a standard-sized pallet. In the first test in 18 °C per hour heating rate was used where the fruit seed surface temperatures were heated from 23 °C to 43.5 °C and the temperature held for 15 min. Complete insect kill was not achieved in this test as the overall mortality was 98.1%. In the final two tests the mortality was increased to 100% by lengthening the hold to 45 min. A total of 6391 larvae were killed in the final two tests. The need for extended treatment duration was likely due to the impeded air flow caused by the stone fruit boxes. Additional testing of CATTS tolerance under laboratory conditions was conducted for Wooly Apple Aphid, McDaniel Spider Mite, San Jose Scale and Oblique Banded Leaf Roller. Testing of the effect of CATTS treatment in boxes on fruit quality of four varieties of yellow nectarines was conducted to get a better idea of the range in tolerance of different varieties to this treatment. Three of the four showed no significant effect to the quality of the fruit, while one (Ruby Diamond) had a 15% decline in the percentage of marketable fruit due to treatment. Airflow measurements of boxes within the pallet stack indicated that the flow was around 1 cfm/lb., which was lower that the 2 to 3 cfm/lb needed to provide optimal heat transfer. Enhancing airflow by changing container type was found to improve fruit quality following CATTS treatment for one of the three varieties tested.

TESTS CONDUCTED

1. Insect Testing

Report on Efficacy Trials of CATTS Quarantine Treatments against 4th Instar Oriental Fruit Moth in Commercial Export Boxes of Peaches Stacked onto a Pallet (large-scale test)

A series of three replicates of CATTS efficacy trials were planned and run from July 22 through July 30, 2009. On July 20, 2009 24 pans of Oriental Fruit Moth larvae were transported by automobile from the USDA-ARS Yakima Agricultural Research Laboratory in Wapato, WA and arrived at the USDA-ARS San Joaquin Valley Agricultural Sciences Center in Parlier, CA on July 21, 2009. Organic peaches were pre-warmed for 2 days at 20°C prior to infesting with the first replicate of insects (July 22, 2009). On July 23, 2009 infested peaches were moved from infesting containers into commercial trays and enclosed in nylon organdy inside single layer commercial export boxes. A total of 3,750 larvae were individually applied to 625 peaches. During transfer, 487 insects were removed from the outside of the fruit, leaving an estimated 3263 larvae inside the fruit at the time of treatment for an infestation efficiency rate of 87%. The core temperatures of 2 fruit per box were recorded by a Hobo data logger. A CATTS treatment at a chamber heating rate of 18°C/hr from 23°C to 46°C with a core soak temperature of 43.5°C for 15 min was performed on July 23, 2009. During the CATTS treatment, the untreated infested control fruit were evaluated for survivorship. A total of 20 larvae were found outside of the fruit leaving an estimated 130 larvae inside the fruit. Upon evaluation, control mortality was calculated to be 9.9%. This high level of mortality was most likely due to the poor quality of the fruit (fruit showed excessive decay and were very soft). Following treatment, the fruit were removed from the CATTS chamber and stored overnight at 4°C. On July 24, 2009 the boxes of treated infested fruit were removed from the cold room and allowed to warm up to room temperature for three hours. Upon evaluation, 40 live larvae were found out of a total of 2,134 larvae found in 19 treated boxes for a mortality rate of 98.1%. Due to the high rate of survivorship, the remaining six boxes were not evaluated.

During CATTS treatment, only fruit in uninfested boxes were monitored for core temperatures. Hobos were used to record fruit core temperatures in infested fruits. Temperature probes from the CATTS chamber were used to determine the 15 min soak at 43.5°C. Upon downloading and examining the temperatures of the Hobo data loggers it was found that core fruit temperatures from all but one of the boxes were sufficient to meet the temperature/time requirement. It is possible that fruit in the boxes that were not probed were cooler, leading to the survivors. It is also likely that the nylon organdy surrounding the fruit in the infested boxes impeded the air flow through the box, thus reducing heat transfer to the fruit.

The decision was made to increase the minimum soak time to 45 minutes at 43.5°C for runs 2 and 3 of the test. Fruit used in these tests were only warmed overnight prior to infesting with larvae. All other aspects of the treatment remained the same. Replicates 2 and 3 were infested with a total of 3,750 larvae each on July 24 and July 27, respectively. In replicate #2 a total of 394 larvae were removed from the outside of the fruit prior to treatment, giving an estimated treatment number of 3356 larvae and an infestation efficiency rate of 89.5%. Control mortality for replicate #2 was 1.7%. Upon evaluation, a total of 3,073 larvae were recovered with zero larvae surviving the treatment. In replicate #3 a total of 353 larvae were removed from the outside of the fruit prior to treatment, giving an estimated treatment number of 3,397 and an infestation efficiency rate of 90.6%. Control mortality for replicate #3 was 3.7%. Upon evaluation, a total of 3,319 larvae were recovered with zero larvae surviving the treatment. Combining treatment numbers for replicates 2 and 3, an estimated total of 6,753 larvae were treated, with 6,391 larvae recovered and zero survivors. This number exceeded our target of 5,000 larvae killed with zero survivors. Upon evaluation of the Hobo data loggers, the minimum core target temperature of 43.5°C was maintained for well over 15 minutes

Conclusions from the Large-Scale Efficacy Test

The failure of the first trial may have been due to insufficient heating in infested boxes due to the presence of the nylon organdy and the generally poor airflow through the boxes. The target values for time and temperature obtained from this test exceed those used in the original laboratory testing and cause concern about whether the fruit will tolerate the additional heat dose needed for in-box treatments. Improvements to the packing boxes, such as increasing the height and width of the ventilation holes, and increasing the porosity of the packing trays, may increase airflow through the chamber and provide more uniform fruit heating, thus providing a means to reduce the treatment parameters to values closer to those obtained in previous laboratory testing. Another issue noted was that the use of plastic trays traps some moisture and provides a more optimal environment for the development of decay. Consideration should also be given to treating pre-approved export fruit in field bins and/or picking lugs within 2 days after harvest.

2. Additional Insect Testing

In this portion of the work testing was continued to extend the list of pests of interest to the stone fruit industry that could be treated using CATTS treatment. Some of the testing, such as that with Oblique Banded Leaf Roller (OBLR) is in its final stages, while for some of the other insect pests the research was being just initiated.

The first CATTS tests were initiated on Wooly Apple Aphid, McDaniel Spider Mite, and San Jose Scale using apples, leaves, and stems in the research-scale CATTS chamber. We have to store the diapausing stages for 3 months at 0 to 10°C before we can assess mortality. Also, we are collecting winter forms of Spider Mites, European Red Mite diapausing eggs, and Grape Mealy Bug from the field. Naked insects will be subjected to the controlled atmosphere heating block. Samples on hard materials (stems, leaves, fruits) will be treated in the research scale CATTS chamber. Work is continuing on building up a colony of Peach Twig Borers for use in CATTS testing. The colony is not yet robust enough for tolerance testing but is nearing the stage at which it will be useful for this. Adjustments to the growth room to optimize colony conditions are currently underway.

We are continuing running OBLR in the heat block. Right now, the eggs appear to be more CATTS tolerant than all the other stages of OBLR, but are more sensitive than codling moth (CM) and Oriental Fruit Moth (OFM). We were using the 24°C/hr treatment from 23 to 44°C for 1, 1.25, 1.5, 1.75 and 2 hr. All but the egg stages were killed at the 1.25 hr time point. The eggs were killed at the 1.5 hr time point. We are finishing off the dataset for a publication. We were just running the 1.25 and 1.5 hr time points to compare to OFM and CM, where the 4^{th} instar is controlled at the 2.5 hr time point (LT_{95} is at about 2 hr.).

We are assisting Peter Follett (USDA/ARS-Hilo, HI) in running heat block treatments on the Light Brown Apple Moth (LBAM) this coming winter. Help was provided to his technician on setting up the controlled atmosphere part of the system and he is expected to soon complete a few preliminary runs. Based upon data received from New Zealand, it was suggested to him that 3rd and 4th instars be tested with a 24 °C/hr heating rate up to 44 °C, using 1.25 and 1.5 hr time points. It is believed that this treatment will be successful since LBAM is much less CATTS-tolerant than is CM and OFM. As soon as the preliminary data is available it will be shared with CTFA.

3. Fruit Quality Testing

Work Done Prior to the Large Insect Efficacy Test

During this portion of the 2009 season, work was undertaken to provide information regarding the tolerance of a range of stone fruit varieties to CATTS treatment in the large chamber system that utilized boxed/palletized fruit. The results presented were completed prior to the efficacy test in late July where it was discovered that the run times that were utilized for this quality work may not be sufficient to achieve 100% mortality with this treatment system.

Testing was conducted following the protocol for CATTS treatment (12 °C/h rate, T601-a-1) in the APHIS Treatment Manual. The only alteration was that the chamber heating rate was increased to 18 °C/h in order to compensate for the slower heating of the boxed fruit. To save on the amount of fruit needed all 4 varieties were simultaneously run in each of the 3 replications using only a quarter of a pallet. To achieve the same airflow as was obtained using an entire pallet of fruit the fan speed was lowered to 70% of maximum as suggested by airflow calculations. All of the fruit were size 50 yellow nectarines that were warmed to 23 °C prior to beginning the treatment. Treatment times were rapid with all three replications being completed in less than 3 hours. Following treatment all of the fruit were placed at 1 °C for 2 weeks, followed by ripening at 23 °C. Color and weight loss measurements were taken immediately prior to treatment and after 2 days of ripening following cold storage. Firmness, % marketability, color and weight loss were determined after two days while brix and internal weight loss were measured when the fruit had softened to three pounds or less.

In Table 2 are presented the fruit quality results. Surface injury, as expressed as the percentage of marketable fruit, was not affected by treatment in Grand Sweet (GS), Diamond Ray (DR), or Spring Bright (SB). A large portion of the SB fruit decayed in both control and CATTS-treated lots. Surface injury in the form of brown lesions was enhanced in Ruby Diamond, with there being about a 15% loss in the marketable fruit due to treatment. Firmness was significantly enhanced in all treated fruit. Color was only altered in the case of DR, although the overall color differences were very slight and probably not that important. Slightly more weight loss occurred in the CATTS-treated fruit of GS, SB and RD but wrinkling in either the control or CATTS-treated fruit was only rarely noticed. In contrast to last season there was no browning around the pit noted in any of the 4 varieties tested this season. It is unclear if this is due to the yellow coloration of this season's fruit or that the treatments were conducted faster due to the difference in box configuration.

As noted in prior tests with boxed/palletized fruit, the boxes on the outside heated more rapidly than those on the inside. Heating to a core temperature of 43 °C required an average of 11 minutes longer for boxes on the inside of the stack. The height position of the boxes on the pallet did not matter. Greater heat in the outside of the stack caused the fruit from this position to be firmer and lose more weight, but there was no clear relationship with injury (data not shown).

Table 2. Fruit quality following semi-commercial CATTS treatment using packed fruit in boxes stacked onto pallets. Evaluation was conducted following 2 weeks of storage at 1 °C and subsequent ripening. Numbers with different letters within a variety are significantly different from each other at P ≤ 0.05ND = not detectable.

Variety	Treatment	% Marketable[z]	Firmness (lbs)	Brix	Color[y] L	C	h	Weight Loss (g)[x]	Internal Browning
Grand Sweet	Control	95.9a	2.1a	12.6a	2.05a	-1.68a	5.03a	8.06a	ND
	CATTS	91.5a	4.7b	13.4a	1.85a	-0.92a	4.41a	11.1b	ND
Diamond Ray	Control	97.2a	5.5a	13.0a	-0.04a	-2.46a	2.53a	10.2a	ND
	CATTS	95.5a	8.8b	13.0a	1.47b	-0.77b	4.25a	13.4a	ND
Spring Bright	Control	69.4a	2.1a	10.5a	3.38a	1.69a	4.55a	8.8a	ND
	CATTS	51.9a	4.3b	10.6a	2.67a	0.85a	3.82a	10.8b	ND
Ruby Diamond	Control	94.4a	2.5a	11.5a	2.24a	-1.20a	3.11a	7.5a	ND
	CATTS	78.5b	6.0b	12.4a	2.00a	-0.42a	2.20a	10.6b	ND

[z]Based upon surface injury only.
[y]Color change from prior to treatment to after storage in L, C or h color units.
[x]Weight loss from prior to treatment to after storage.

Air Flow Calculations

Airflow through the boxed fruit is a very critical aspect of the treatment as it helps determine both heating rate and the evenness of heating. With the assistance of Jim Thompson (Agricultural Engineer, UC Davis) estimates were made of the amount of airflow occurring through the loaded boxes in the large chamber. This was done by the use of “dummy” boxes within the pallet stack that contained a steel plate with holes of specified dimensions, across which the pressure drop was measured when the fans were started. Calculations using the pressure drop values indicated that the airflow through one loaded pallet was one cfm/lb. A better value according to Jim Thompson would be two to three cfm. The low airflow is due to the difficulty in pushing air through the vents, trays and fruit of four boxes. Increasing this airflow through box or chamber redesign should lead to more even heating, faster treatments and better fruit quality.

Comparison of Treatment in Boxes Versus Picking Lugs

An experiment was conducted to determine if fruit quality would be better following treatment in picking lugs (great ventilation, good airflow) versus treatment in standard one-layer boxes (marginal ventilation, relatively poor airflow). Two white-flesh peaches were included among the varieties tested because fruit of this flesh color have been found to be susceptible to the development of internal browning as a result of CATTS treatment. A final core temperature of 43.5 °C and hold time of 45 min were utilized to take into account the findings of the large-scale efficacy tests in terms of efficacious temperature and time in the large-scale CATTS chamber. The very different dimensions of the two container types meant that the same number of containers and the same amount of fruit per test could not be utilized in a comparison of container types. These differences meant that the comparison of the two container types was not exactly “fair”, but it still is an instructive comparison of the current situation (single-layer boxes) with a much more optimal situation in terms of airflow. Fruit were stored for two weeks at 1 °C followed by ripening at 23 °C prior to evaluation for surface injury and internal browning.

Although it was hoped that there would be some visible damage due to treatment in the single layer boxes, neither ‘September Snow’ nor September Sun’ showed any adverse effects externally or internally as a result of treatment in either of the container types (Table 3). This tolerance of the extended treatment was encouraging for the ability to treat boxed or containerized stone fruit but made it difficult to assess the effect of the increased airflow. In ‘Snow Giant’, although there was no effect due to treatment on surface injury (%marketable), there was a definite increase in internal browning as a result of treatment that was significantly less in the lugs than in the single-layer boxes. The total duration of the treatment in picking lugs was only 15 minutes shorter than in the single-layer boxes, so it is somewhat surprising that this difference was observed. Still, the added heating may have acted to enhance internal browning. This test was conducted using only a partial pallet stack to simplify the experimental design of the test which means that the single-layer boxes likely had greater air flow through them than would have occurred with an entire pallet. Comparisons from a whole pallet test may have yielded even greater differences due to container types.

Table 3. Surface damage and internal browning in peaches heated to and held at a core temperature of 43.5 °C for 45 min in the large CATTS chamber in either one-layer boxes or picking lugs. Numbers with different letters within a variety and within a container type or stack position are significantly different from each other at P ≤ 0.05.

[z]Percentage of fruit having either slight or no surface damage, [y]percentage of fruit having browning immediately surrounding the pit.

Variety	Flesh Color	Treatment	% Marketable[z]	% Internal Browning[y]
Snow Giant	white	none	86.4a	2.6b
		heated	76.0a	29.2a
			Container	
		none	Box	35.9a
		heated	Lug	13.5b
			Stack Position	
		none	Inner	26.9a
		heated	Outer	22.5a
September Snow	white	none	86.8a	2.0a
		heated	88.2a	11.4a
September Sun	yellow	none	71.5a	0a
		heated	70.8a	3.4a

Final Project Summary – Key Points Learned During the Duration of the Project

Fruit Quality

1. To lessen the possibility of treatment-induced damage CATTS treatment must only be applied to fruit of good quality with a minimum amount of surface blemish.

2. Ripening is inhibited by treatment by an average of 2 to 3 days, but it will satisfactorily occur except in the case of a very extended treatment well in excess of what is needed for insect efficacy.

3. CATTS treatment can inhibit mealiness development to a similar degree as preconditioning does as long as the proper heat dose is given. Heat treatments that are given short of the needed intensity for quarantine efficacy can actually enhance mealiness.

4. Brown rot is completely controlled by CATTS treatment but other decay organisms are not. Decay is sometimes enhanced by CATTS treatment due to what is believed to be due to an enhancement in sensitivity of the fruit to the initiation of decay. Proper decay control procedures such as sanitation and effective fungicide use are important to successful use of the treatment.

5. Maintain fruit core heating rates at or below 12 °C per hour to better ensure fruit quality. Faster heating rates can have adverse effects on flavor.

6. The primary fruit quality problems that have been observed are the initiation of brown lesions on the fruit surface, increase in decay incidence and the formation of internal browning around the pit. White varieties are more problematic for the browning since the disorder is much more visible in white versus yellow fruit. Skilled panelists can detect a flavor difference due to treatment but this difference is not thought to be noticeable to the average consumer.

7. The presence or absence of the controlled atmosphere has no influence on fruit quality following CATTS treatment. This means there would be no advantage to fruit quality in trying to tinker with the treatment by removing the controlled atmosphere portion of the protocol.

8. Forced air cooling following treatment does not appear to offer any benefit in terms of the mitigation of treatment damage.
9. There is a range in the tolerance of different varieties to CATTS but mid- and late-season varieties appear to be more tolerant that early-season varieties.

10. The smaller a fruit size is, the greater the likelihood is that the treatment will be successful in terms of fruit quality. This is due to the fact that large fruit require longer treatment times.

11. Treatment of fruit in boxes versus bulk treatment in bins tends to lessen the overall rate of heating and increase fruit-to-fruit variability due to there being a lesser amount of airflow through the fruit. If airflow is not improved the overall impact is that the entire load needs to be heated longer to bring the colder fruit up to temperature, increasing the potential for quality damage.

Insect Mortality and Treatment Efficacy

1. Codling Moth and Oriental Fruit Moth are equally tolerant to CATTS treatments. The 4th instar of both species is the most tolerant immature life stage to the CATTS treatment.

2. It is essential to keep oxygen levels below 2% and carbon dioxide levels between 10 and 20%. Lower or higher levels of CO_2 will result in survivors. Higher levels of O_2 will result in survivors. Levels of oxygen below 1% are usually better for optimal insect kill, but less cost effective to the operator since it requires more nitrogen to be used and more effort to build and maintain a tight seal on the CATTS chamber.

3. It is essential to establish the controlled atmospheres before increasing the temperature during a CATTS treatment. This prevents the insects from acclimating to the heat load.

4. It is important to keep high air flow over the fruit to ensure uniform heat distribution. Insects have the uncanny ability to locate 'cool spots', which can result in survivors.

5. It is important to keep humidity high, but not condensing on the fruit. This helps ensure a more uniform heat distribution in the load.

6. All life stages of Oblique banded leafroller are less tolerant to CATTS than CM or OFM.

7. It is recommended to bring harvested fruit core temperatures down to 20 to 23° (68 to 74°F) for at least 4 hr prior to CATTS treatment. This will allow for any heat shock proteins in the insect to turn over, and reduce thermo tolerance.

8. It is the combination of the low oxygen and high carbon dioxide that help kill CM, OFM, and OBLR faster than treatments under air, low oxygen only, or high carbon dioxide only. Low oxygen inhibits the production of ATP and heat shock proteins. High carbon dioxide impairs the ability of the insects to utilize ATP, which is essential for thermo tolerance.

9. It is better to treat fruit as close to harvest as possible. This helps maintain their natural thermotolerance obtained in the field. The longer the fruit have been removed from field temperature fluctuations, the greater the loss of their natural thermotolerance (diurnal production of heat shock and stress proteins).

10. In general, the faster the heating rate, the shorter the time the insects need to be held at the final treatment temperature to kill them.

11. Review of the literature from New Zealand indicates that Light Brown Apple Moth (LBAM) is less tolerant of high temperature, controlled atmosphere treatments than Codling Moth and Oriental Fruit Moth.

12. When performing large-scale efficacy trials of infested fruit in nylon organdy bags, it is important to have continuous reading probes in the fruit in the nylon organdy bags. There is a high probability that the bags impede airflow, and thereby impair even heat distribution, resulting in fruit not heating at appropriate rate or to the desired final treatment temperature and duration.

Conclusions

As long as the considerations listed above, such as the use of good quality fruit, are taken into account, then the treatment can be successfully completed without the loss of fruit quality. This is true even using treatments with boxed fruit, although treatments with boxed fruit are more prone to causing quality loss. There are, however, still a number of factors that influence the impact of CATTS treatment on fruit quality that are not fully defined, meaning that the treatment may negatively impact quality in some situations. Further work is needed to fully define what the all of the “boundaries” of a successful treatment are in term of maintaining fruit quality. CATTS can be a very effective treatment in terms of insect efficacy as long as the recommended levels of atmospheric gas levels are maintained, air flow is sufficient to maintain uniform heat distribution over the load, and target core temperatures are attained at the proper heat rate targets and durations. If the heating rate in the fruit is lower than 12°/hr, then the soak time will need to be greatly increased to ensure treatment efficacy. Treatment of fruit within boxes has proven to be challenging, but the work has also shown that it may be possible if sufficient effort is given to making CATTS a commercially-viable treatment. We will continue to aid and assist anyone that wishes to further develop this treatment.