Postharvest Decay Control of Apple by Acetaldehyde Vapor[1]

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Abstract. Decay of apples (*Malus sylvestris* Mill.) inoculated with *Penicillium expansum* was controlled by acetaldehyde vapor concentrations (v/v) of 0.5% for 180 min, 1% for 120 min, 2% for 60 min, and 3% for 30 min. The above treatments did not produce lenticel or skin injury. Fumigated conidia did not germinate in 21 days at 21°C on artificial media and failed to induce decay in stem-punctured apples. The pathogen could not be re-isolated from fumigated inoculated punctures, however, the pathogen was obtained from inoculated punctures not exposed to acetaldehyde vapor. Fungicidal action of acetaldehyde vapor was a function of concentration and exposure period. Objectional off-flavors were not detected in fumigated apples, although appreciable amounts of acetaldehyde vapor were absorbed.

Postharvest decay losses of apples during transit and unloading have been estimated at over 7 million dollars annually (18). The major proportion of these losses has been attributed to storage pathogens (5). Blue mold, caused by *Penicillium expansum* (Link) Thom, is an important storage disease. It is of minor importance in orchards except as a source of inoculum for later storage decay. Cost reduction handling practices tend to increase stem-punctures as well as minor cuts and bruises. Since *P. expansum* in water dump tanks readily enters cuts, bruises, and lenticels, an effective nontoxic fumigation treatment for decay control after apples are packed is highly desirable.

The inability to effectively control postharvest decay of fruits and vegetables by nontoxic chemical methods and the refusal to accept commodities treated with toxic fungicides have stimulated interest in biological methods for decay control. Acetaldehyde (Aa) is a natural volatile compound of fruits and vegetables (10). It is used as flavoring agent and is one chemical index of fruit ripening (8). It also has EPA clearance as a food additive under section 121:101 (19). The fungicidal property of Aa vapor has been reported (1, 4, 11, 12, 13, 14, 15, 16, 17). We undertook to determine if the fungicidal property of Aa vapor could be utilized commercially as a postharvest fumigant in controlling storage decay of apples.

Materials and Methods

Apples (cvs. Red Delicious and Golden Delicious) were obtained from a wholesale market and stored at 1°C until ready for use.

Inoculum. Penicillium expansum was isolated and maintained on apples. The pathogen was grown on potato-dextrose agar (PDA) for 7-9 days at 21°C. Conidia were harvested and suspended in sterile distilled water containing 1 drop per liter of polyoxyethylene sorbitan monolaurate and washed by 3 successive centrifugations and resuspended in distilled water. The concn was adjusted to 2.3 x 10^6 conidia/ml. A drop of spore solution was used for inoculation. Sterile distilled water was used as a control.

Inoculation. Apples were surface sterilized with 10% Roccal solution (10% dimethyl benzyl ammonium chloride) for 4 min and rinsed 4 times with sterile distilled water. Stem-punctures (3mm diam x 5mm deep) were made by breaking the apple skin with a sterile glass rod and inoculated with a drop of inoculum. Inoculated fruits were incubated for 8-10 hr at 21°C before fumigation. Apples fumigated in an 8-liter desiccator were incubated for 21 days at 24 ± 2°C and 65% relative humidity (rh) after exposure to Aa vapors. Decay readings were means of 2 experiments, each containing 100 fruits with 4 wounds per fruit. Three wounds in each fruit were inoculated.

Apples in the tray-pack carton tests were inoculated in a similar manner. The position of the inoculation sites were randomly located on the trays to represent commercial packing conditions. Each box contained 5 trays with 25 fruits per tray. The corrugated box with ten 10mm diam holes on each side was fumigated with the top open. This large-scale test was conducted with 2 boxes of apples per fumigation treatment and replicated 2 times. After fumigation, the boxes were covered and incubated for 30 days at 24 ± 2°C and 75% rh.

Treatment chambers. An 8-liter desiccator with a wire mesh shelf served as a small treatment chamber. The top of the chamber containing a gas-tight injection port was secured to withstand pressure produced by Aa. A gas-tight syringe kept on ice was used to inject accurate quantities of liquid Aa into the chamber. Acetaldehyde vapor equilibrium was quickly established and maintained with a magnetic stirrer. Chamber temperature was maintained at 21°C. Acetaldehyde vapor concn was expressed as percentage of atm by volume (v/v).

Large-scale tray-packed box tests were conducted in a 0.912m^3 gas-tight fumigation chamber. Liquid Aa was injected by gas-tight syringe previously kept on an ice bath. Atmosphere inside the chamber was agitated by an air-driven fan to establish equilibrium of Aa in the vapor phase. Chamber temp was maintained at 21 ± 2°C. Concn of Aa in the atm was monitored by Gas Liquid Chromatographic (GLC) techniques.

Inoculation and fumigation tests were at room temp. Storage trials under refrigeration have not been made. The possible rh effects were not considered in this investigation.

Gas chromatography. Direct vapor injection analyses were carried out with Areograph Hi-Fi Model 600-D gas chromatograph equipped with flame ionization detector, and a Texas Instruments strip chart recorder. The column, 10 ft x 1/8 in, was packed with 15% carbowax 600 on 800-100 mesh Chromasorb W. The GLC conditions were: N carrier gas, 95 ml/min; H_2, 60 ml/min; and air, 600 ml/min. Column temp was maintained at 80 ± 1°C.

Preparation of standard. Liquid Aa was injected into the exposure chamber by gas-tight syringe and allowed to equilibrate. A 20 µl samples of vapor was withdrawn and analyzed quantitatively with gas chromatography. Standard curve was prepared as percentage Aa in vapor phase versus detector response.

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[3]Shaw, G. W. 1969. The effect of controlled atmosphere storage on quality and shelf life of fresh strawberries with special reference to *Botrytis cinerea* and *Rhizopus nigrican.* Ph.D. Thesis. University of Maryland, College Park. 62 p.

Phytotoxicity. Apples were exposed to 5% Aa in the 8-liter treatment chamber. After the chamber was equilibrated, 20 µl vapor samples were withdrawn every 30 min and percentages of Aa were determined from the standard curve. A similar chamber containing Aa with apples was used as a control to study breakdown and disappearance of Aa.

In this investigation absorption was referred as the reduction of initial Aa concn in presence of apples from a given atm.

In another experiment, 4 apples weighing approximately 950 g were exposed to different concn of Aa. The exposed apples were incubated for 4 days at 21°C. Phytotoxicity of Aa was rated as follows: no injury, minor discoloration around artificial stem-puncture, minor stem-puncture and and lenticel injury, severe lenticel injury, and severe lenticel and skin injury.

Fungitoxicity. Conidia of *P. expansum* were dusted on sterilized glass rods and held in a water-saturated atm at 21°C for 8-10 hr before fumigation. Fumigated conidia were transferred to PDA and incubated at 21°C for 21 days to determine viability. In another experiment, culture slides (3 mm deep) containing PDA were seeded with conidia of *P. expansum* and incubated at 21°C for 8-10 hr prior to fumigation. The fumigated conidia on PDA were incubated at 21°C for 21 days to determine toxicity of Aa on germinating conidia.

Results

Decay control with Aa vapor. Decay of apples, inoculated with *P. expansum* was controlled by various concn of Aa vapor (Fig. 1). The plotted points represent control of decay in 4-fruit lots stored at 21°C for 21 days after fumigation. Since the design of the 8-liter exposure chamber permitted only 4 apples per Aa treatment, several experiments were conducted to determine Aa efficacy for decay control. The inoculated, untreated fruits showed more than 95% decay in every experiment. Acetaldehyde concn of 0.5% for 180 min was as effective in controlling decay of apples as 20% for 0.5 min. The effectiveness of Aa was evident in preventing decay and was related to concn and exposure (Fig. 1). At low concn, long Aa exposure was required for decay control. *Penicillium expansum* could not be reisolated from stem-punctures exposed to Aa. Additional experiments also showed that decay was prevented when inoculated apples in plastic bags were exposed to 0.5% Aa for 180 min.

Fungicidal action of Aa vapor. Germination of conidia of *P. expansum* held for 21 days at 21°C and 65% rh was inhibited after exposure to Aa (Fig. 2). Conidia not exposed to Aa germinated in 8-10 hr and the fungus sporulated in 7-10 days. Exposure to 20% Aa for 30 sec was as effective in preventing germination of *P. expansum* as was 0.5% for 180 min.

Penetration of Aa vapor into fruit containers. Exposure of apples in commercial tray-packed boxes to Aa concn ranging from 0.5% to 5% for various periods was effective in preventing decay of apples inoculated with *P. expansum* (Table 1). Decay of inoculated apples fumigated with Aa was reduced 94 to 99%. Inoculated fruits, without fumigation, decayed in 10-14 days at 24°C. Decay of some fumigated fruits developed when inoculated stem-punctures were oriented against the side of tray cups. In this position, contact of Aa with some punctures was prevented.

Penicillium expansum failed to grow on PDA when the germinating conidia were exposed to 0.25% Aa for 24 hr (Fig. 3). Viability tests showed that fumigated conidia failed to induce apple decay. Acetaldehyde concn of 0.5% for 180 min, which was highly effective in controlling decay of inoculated fruits also killed germinating conidia.

Phytotoxicity of apples to Aa vapor. Apples exposed to 0.25% or 0.5% Aa for 1440 min were indistinguishable from untreated fruits (Table 2). Fruit exposed to Aa at 1% for 180 min or longer showed skin discoloration around stem-punctures. Phytotoxicity is due to concn plus exposure period (Table 2).

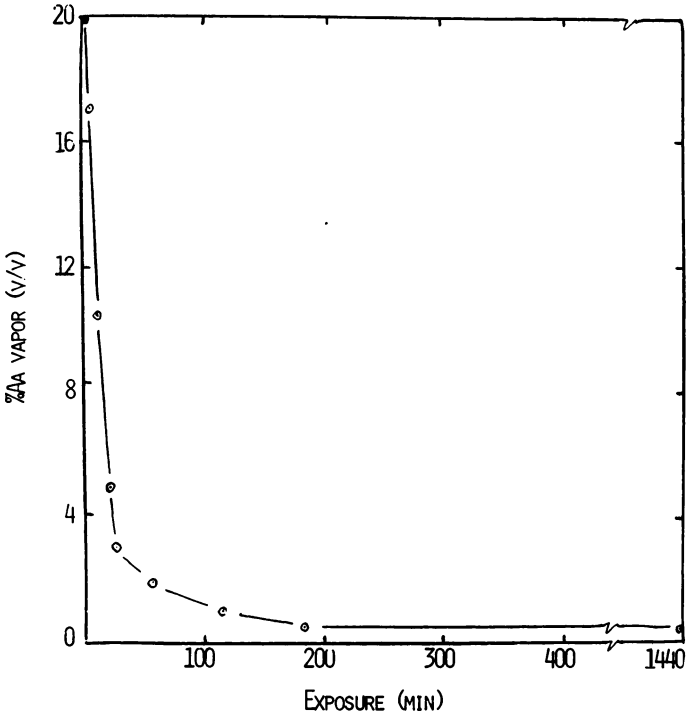

Fig. 1. The concn of Aa vapor (v/v) and exposure (min) required to control decay of apples inoculated with *P. expansum.* The plotted points indicate combination of conditions giving 100% decay control at 24°C.

The same is true for decay control. Minor lenticel injury became apparent when apples were exposed to 4% Aa for 180 min or longer. Lenticel and apple skin injuries were severe at 15% for 240 min and 20% for 180 min or longer. The injured areas

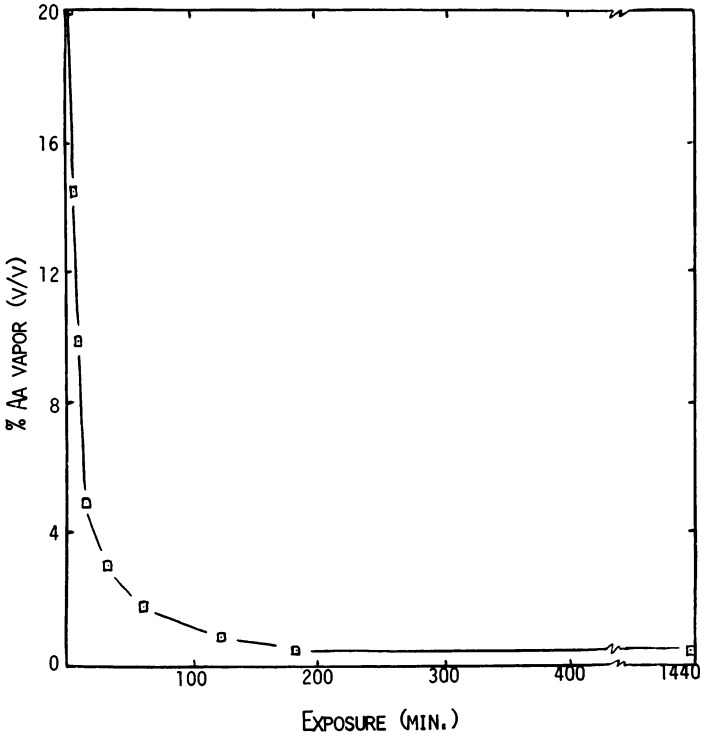

Fig. 2. The effect of Aa vapor concn (v/v) and exposure (min) on 100% inhibition of *P. expansum* conidia germination on PDA incubated at 21°C for 21 days.

Table 1. Effectiveness of acetaldehyde on decay caused by blue mold *(Pennicillium expansum)* on 'Red Delicious' and 'Golden Delicious' apples in tray packed boxes.

Acetaldehyde treatment		Total inoculated stem punctures (No)	Number of infections[z] Puncture orientation on tray		Total infections (No)	Decay reduction %
% (v/v)	Exposure (min)		Upright	Down		
0	0	172	83	89	172	0
0.5	240	140	1	7	8	94
1.0	120	136	2	6	8	94
1.0	240	176	0	2	2	99
2.0	60	100	0	2	2	98
5.0	30	160	0	1	1	99

[z]Incubated for 30 days at 24°C and 75% relative humidity after treatment with acetaldehyde.

frequently coalesced at these high Aa concn to resemble storage scald and the flesh under the injured skin turned brown. However, tolerance of apples to Aa was evident at concn that were highly effective in preventing decay of inoculated fruits. Effective concn of Aa exposure were 0.5% for 180 min or longer, 1.0% for 120 min, 2% for 60 min, 3% for 30 min, and 4% for 30 min.

The absorption of Aa by apples was linear in 24 hr and was directly related to wt of fruits confined in the 8-liter atm (Fig. 4). Apples weighing 1320g absorbed approximately 5% Aa in 28-30 hr from an 8-liter atm, and less than 1% was absorbed by 150-350g apples during the same period (Fig. 5). The absorption or disappearance of Aa from a given atm depended on apple volume as indicated by the wt of apples present.

The concn of Aa in 8-liter atm without apples changed only slightly from 0-24 hr but declined gradually between 24-72 hr (Fig. 6). There was an increase in concn of acetic acid vapor during 0-24 hr with a maximum at a time when the Aa concn decline was the most rapid.

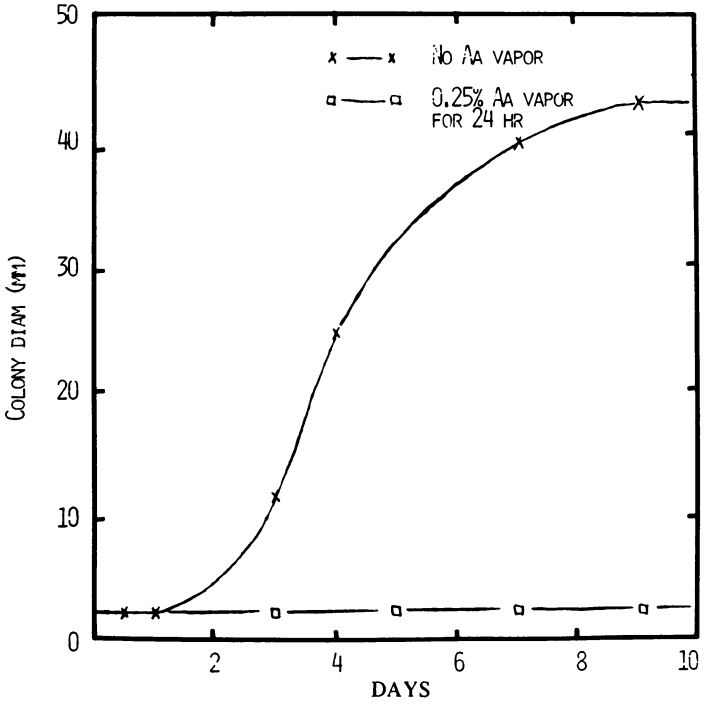

Fig. 3. Effect of 0.25% acetaldehyde vapor for 24 hr on growth of germinating conidia of *P. expansum* on PDA at 21°C.

Discussion

There is no hazard in handling Aa under normal operational procedures provided elementary precautions are taken (8). Our results show that *P. expansum* on apple fruits can be controlled by exposure to Aa concn which are neither flamable nor phytotoxic to fruits when applied for recommended periods. Redesigning tray cups, however, to provide a hole in the bottom of the cup may be required to permit complete exposure of the apple surface to Aa vapor in order to prevent fruit decay.

The failure of fumigated conidia to grow on PDA indicated that conidia on the surface of apple skin would be killed by Aa vapor at concn that are highly effective in preventing decay of inoculated fruits. Failure to re-isolate *P. expansum* from inoculated fruits after fumigation plus the decay of inoculated but non-fumigated controls also indicate the fungicidal action of

Table 2. Phytotoxicity of 'Red Delicious' and 'Golden Delicious' apples to acetaldehyde vapor.[z]

Acetaldehyde % (v/v)	Exposure (min) 0.5	1	5	10	30	60	120	180	240	1440
0.25	–	–	–	–	–	–	–	–	–	–
0.50	–	–	–	–	–	–	–	–	–	–
1.0	–	–	–	–	–	–	–	+	+	+
2.0	–	–	–	–	–	–	+	+	+	+
3.0	–	–	–	–	–	+	+	+	+	+
4.0	–	–	–	–	–	+	+	++	++	++
5.0	–	–	–	–	+	+	++	++	++	++
6.0	–	–	–	–	+	+	++	++	+++	+++
10.0	–	–	–	+	+	+	++	+++	+++	+++
15.0	–	–	–	+	+	++	+++	+++	+++	++++
20.0	–	–	+	+	++	+++	+++	++++	++++	++++

[z]Fumigated apples held for 4 days at 21°C and then rated for injury:
– = no injury.
+ = minor discoloration around artificial stem-puncture.
++ = minor stem-puncture and lenticel injuries.
+++ = severe lenticel injury.
++++ = severe lenticel and apple skin injuries.

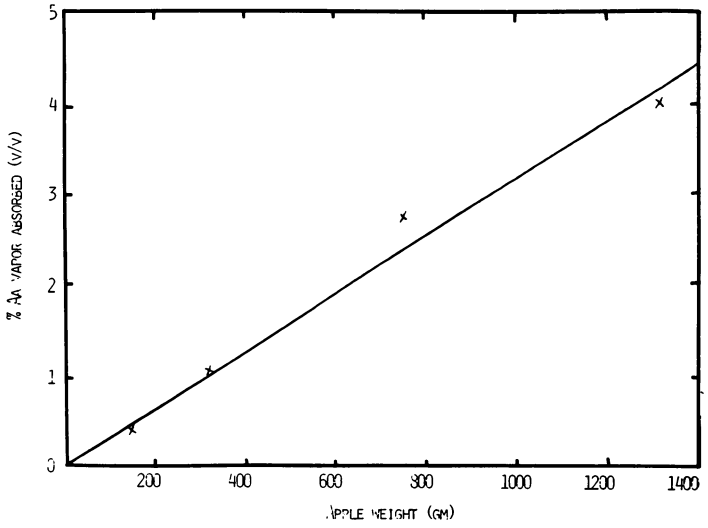

Fig. 4. Absorption of Aa vapor by apples in 24 hrs held at 21°C in 8-liter chamber.

Aa vapor.

Several fungi have been fumigated with various gases to inhibit spore germination, but growth resumed following aeration (5). In contrast, conidia of *P. expansum* fumigated with Aa did not induce apple decay following 21 days incubation. This difference in fungicidal activity offers a major advantage for the use of Aa vapor treatments over other fumigants for control of fruit decays.

Controlled atmospheres, within the limits of concn usable for fruit storage, are effective fungistatic agents (9). Acetaldehyde concn of 0.5mg/100g fresh wt occurs frequently in apples and may increase to 40mg/100g under high CO_2 storage atm (6, 16). Recent evidence indicates that Aa vapor production is stimulated in stored fruits, and it was suggested that Aa was mainly responsible for decay control[3]. Although apple tissue absorbs Aa, it disappears from the tissue due to certain metabolic processes (6).

The control of apple decay by Aa vapor is determined by

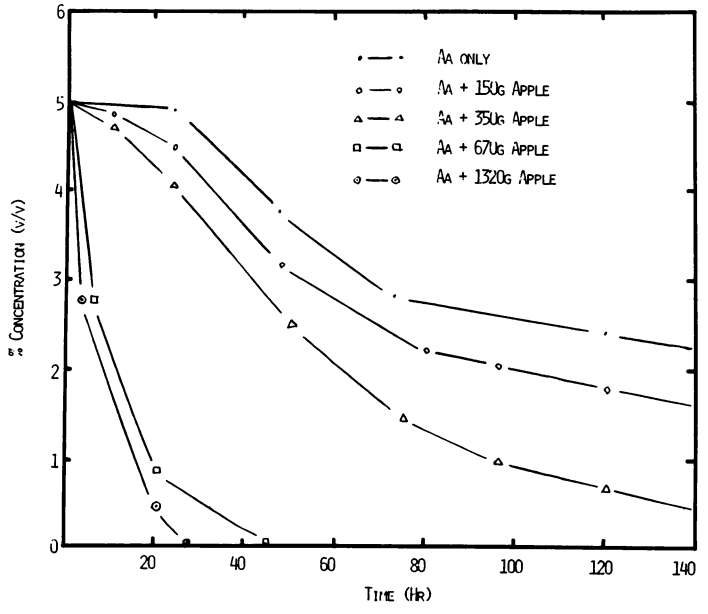

Fig. 5. Disappearance of Aa vapor in the presence of different quantities (wt) of apples held at 21°C in 8-liter chamber.

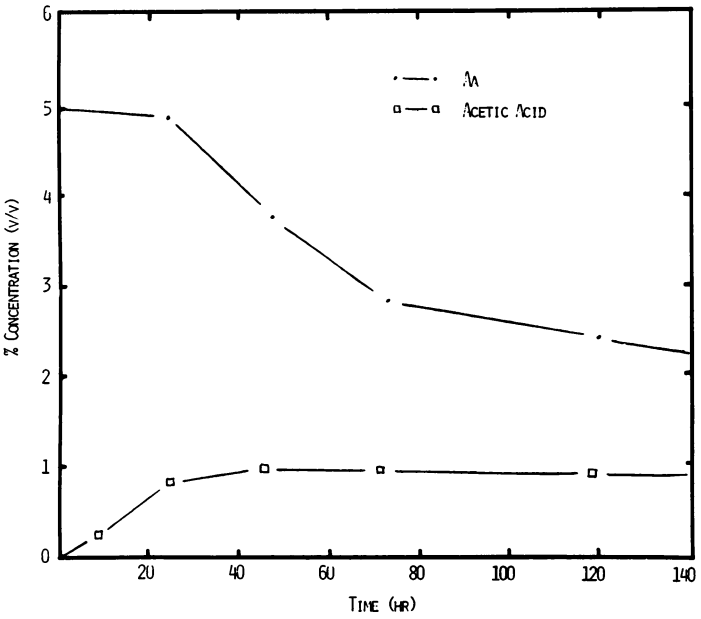

Fig. 6. Disappearance of Aa vapors and increase of acetic acid in 8-liter chamber containing no apple tissue at 21°C.

vapor concn and exposure period. Volatile aldehydes are effective inhibitors of fungal spore germination (1, 2, 3, 4, 7). Tolerance of apple tissue to Aa vapor and its disappearance without leaving objectional off-flavors suggest the potential value of Aa in preventing storage decay.

Effective control of *P. expansum* on inoculated apples with Aa vapors, freedom from injury at effective control rates, complete kill of *P. expansum* conidia and mycelium on artificial media, maintenance of effective treatment concn with high fruit volume, the absence of a toxic residue on the fruit (data to be presented in a later publication) and EPA registration of Aa as a food additive, indicates that Aa would be an ideal postharvest decay-control treatment for the apple cultivars tested.

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Precooling of Citrus Fruits Prior to Simulated Transport in Ventilated Ships[1]

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Abstract. Precooling at -2°C for 6 - 24 hrs prior to simulated ventilated shipment reduced weight loss of citrus fruit. Weight loss from the fruit was reduced as cooling rate increased. The difference in weight loss between precooled and control fruit was maintained during simulated shipment and after storage. Precooling the fruit to temp below 0°C could adversely affect its quality and should be avoided.

Benefits of precooling, the rapid removal of "field heat" from fruits and vegetables prior to refrigerated shipment or storage, are well established for highly perishable commodities, but less obvious in fruits, such as citrus which have a relatively long postharvest life. Recent studies in Florida indicate that rapid cooling of citrus fruits is possible both from engineering and physiological standpoints. Use of high air velocities (300 ft/min) in the range of -8°C reduced fruit temp from 20° to 5°C within 1-1½ hrs (2, 7). Weight loss was less than 1% and no injury occurred if surface temp was not below -2°C. In experiments with tangerines (8), oranges and grapefruit (3), precooling reduced incidence of rots during refrigerated shipment but had no effect when the fruit was transferred to shelf-life conditions. Hall (4) reported that for export of citrus fruits from Australia, precooling may not be required early in the season but is beneficial for summer shipment of 'Valencia' oranges.

Israel-grown citrus fruits are shipped to Europe mostly in non-refrigerated, ventilated ships. During transport to Northern Europe, which requires from 8 to 14 days, the temp of the fruit gradually decreases from about 20°C at loading to about 8°C upon arrival, due to decreasing air and sea temp along the voyage. One way by which quality of the fruit upon arrival at the markets can be improved is by refrigerated shipment (1, 5), however, excessive cost limits this practice. An alternative to refrigerated shipment, precooling the fruit prior to loading on ventilated ships, was suggested as a means of maintaining the temp of the fruit below ambient temp during the initial part of the shipment.

We evaluated the response of citrus fruits to forced-air precooling prior to simulated ventilated shipment, during a 3-year period. Specific objectives were to determine whether the fruit benefits from such a precooling. If so, to what extent does the fruit benefit and what are the optimum precooling conditions.

Materials and Methods

Medium-sized fruit of cv. Shamouti, Valencia, Navel, and Marsh were selected from the same orchards. The fruit was treated in a commercial packinghouse, disinfected with a 0.5% solution of sodium orthophenylphenate, and coated with a water emulsion wax which contained 0.4% active ingredient of thiabendazole. Most of the fruit was packed unwrapped in cartons. Some fruit was wrapped and packed in Bruce boxes.

The fruit was placed in cold storage rooms at temp 0, 3, 6, 9 and 17°C (± 0.5°C) for 36-40 hrs (air circulation was 20 - 30 changes/hr and relative humidity was kept at 80-90%). At the end of this period, center fruit temp of fruits in the center of containers was 2-4°C higher than that of the cooling air (Fig. 1). Such precooling rates can be considered relatively rapid (4). The temp of wrapped fruit packed in Bruce boxes at the end of precooling was similar to that of unwrapped fruit packed in cartons.

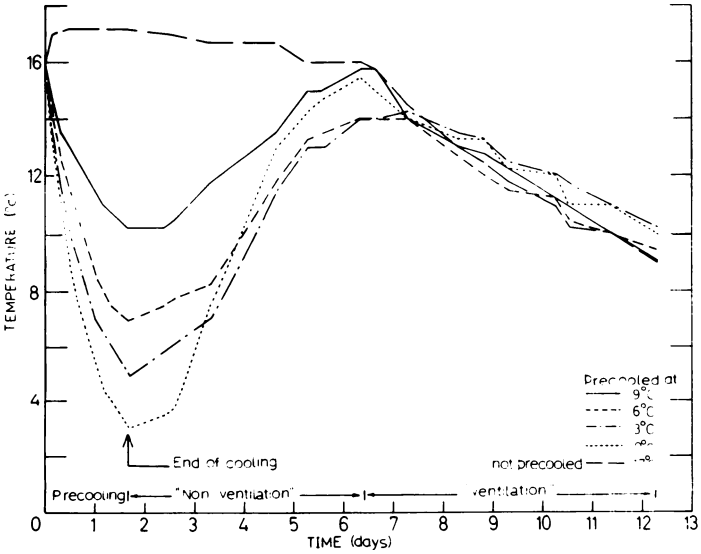

Fig. 1. Temperature of 'Shamouti' oranges packed in cartons during precooling and subsequent simulated ventilated shipment.

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