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Postharvest Heat Treatment of Apples to Control San Jose Scale (*Quadraspidiotus perniciosus* Comstock) and Blue Mold (*Penicillium expansum* Link) and Maintain Fruit Firmness

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Abstract. **Postharvest heat treatments were applied to three apple (*Malus domestica* Borkh.) cultivars: 'Anna', 'Golden Delicious', and 'Jonathan'. The temperatures ranged from 38 to 50 °C and from 5 to 96 hours. The temperatures of 50 °C for 5 or 10 hours and 46 °C for 10 hours controlled all developmental stages of San Jose scale on 'Golden Delicious' and 'Jonathan' fruit. Blue mold germination was prevented by 46, 42, and 38 °C after 28, 34, and 42 hours, respectively. The time needed to control the fungus was longer than that required to kill the insect. Apples were damaged by a 50 °C treatment but could withstand at least 12 hours at 46 °C and at least 24 hours at 42 °C. At 38 °C no damage was found on preclimacteric apples even after 96 hours, but if postclimacteric fruit were heated at 38 °C heat damage occurred. The treatments that did not cause damage maintained the fruit firmness during post storage ripening. The results are discussed in the context of developing integrated postharvest heat treatments.**

Apples are an important worldwide crop and, in contrast to most other fruit crops (excluding pears), can be stored for many months, ensuring a supply of fresh apples year-round. Stored fruit must be kept free of pathogen and insect pests, and for this pre- and postharvest chemicals are used. However, there are increasing problems to using postharvest chemicals, in that pathogens and insects are developing resistance to the compounds (Burton and Dewey, 1981; Eckert and Ogawa, 1988). In addition, consumers are increasingly requesting chemical-free produce (How, 1991).

Blue mold rot occurs in most areas of the world on apples and is one of the most important decay-causing fungi found on stored apples in Israel (Prusky et al., 1985). Infection can occur even at 0 °C and, although decay proceeds slowly at cold storage temperatures (Buchanan et al., 1974), rapid development ensues when the fruit are transferred to a warm environment. Although some chemicals are effective in retarding or preventing rots caused by blue mold, the use of pre- and postharvest chemical treatments is becoming limited due to consumer concerns (How, 1991).

Scale insects, such as the San Jose scale, are among the major insect pests of apples. Some countries are free of this insect and have restrictions against importing apples that may be contaminated. Fumigation by methyl bromide or hydrogen cyanide against scale insects has been used for a long time on nursery material and fruit (Mitsumi et al., 1994; Riehl, 1990). However, the use of these chemicals poses hazards and is restricted in many countries. A nonchemical method of control would be preferable, and hot-water immersion has been suggested for use against some scale insects (Hara et al., 1994).

Prestorage heat treatments have been used for many years to control fungal diseases and insect infestation of fruit (Couey, 1989). Porritt and Lidster (1978) showed that a prestorage exposure of apples to 38 °C for 4 d suppresses softening and naturally occurring decay, mostly due to *Penicillium* spp., after prolonged storage. Similar benefits on firmness are obtained with apples held at 38, 42, or 46 °C for 72, 24, or 12 h, respectively (Klein and Lurie, 1992). Sams et al. (1993) reported that heating 'Golden Delicious' apples for 4 d at 38 °C reduced decay caused by blue mold and maintained firmness during 6 months at 0 °C. The response of apples to heat treatment may vary according to cultivar, as 'Golden Delicious' and 'Delicious' show relatively strong tolerance to heat (Kim et al., 1993).

The goal of this work was to develop a postharvest heat treatment that would control blue mold and San Jose scale on stored apples without decreasing the fruit quality.

Materials and Methods

Heat treatment of fruit. Apples were obtained from different orchards at commercial harvest. Three cultivars were used in different experiments: 'Anna', 'Jonathan', and 'Golden Delicious'. The fruit were heated in a temperature-controlled chamber with the fruit in plastic trays inside nonsealed plastic bags to retard water loss. A water bath was placed inside the chamber to maintain

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relative humidity at 85% at the treatment temperature. Depending on the experiment, the fruit were inoculated with fungus, infected with insects, or not treated before the heat treatment. Following the heat treatment the fruit were either transferred to 0 °C for storage or removed to 20 °C to allow for decay or insect development.

Insect growth and infection of apples. A constant laboratory culture of San Jose scale was maintained on watermelon (*Citrulus vulgaris* Marsh) at 25 °C and a light–dark photoperiod of 18 h light and 6 h darkness. Insects were transferred by placing them in contact with the watermelon for a 3-d period. The heat treatments were conducted 14 or 21 d after the last day of exposure to scale for 'Jonathan' or 'Golden Delicious', respectively. At this point, as a check of the apples revealed, the San Jose scale population consisted of the first and second instar larva of both sexes and pronymphal and nymphal males. Experiments on female insects were carried out on 40- to 42-d-old insects (≈14-d-old females).

First instar diapausing larvae (black scale stage) were tested on naturally infested fruit collected from a 'Jonathan' orchard. The insects were in diapause for 3 months.

The fruit were held in the heat chamber for two exposure times—5 and 10 h—and three temperatures—50, 46, and 42 °C, as well as a control temperature of 20 °C. Following treatment the fruit were placed at 20 °C for 5 d and then the mortality of the insect populations was determined under a dissection microscope for each treatment. Five apples were used for each treatment, and the experiments were repeated twice.

Fungal culture and inoculation of fruit. Blue mold was isolated from a diseased 'Golden Delicious' apple and maintained as a single-spore culture on potato dextose agar (PDA) at 20 °C. For fruit inoculation spores from a 10-d-old culture were suspended in sterile distilled water and 0.03% Tween-20. Fruit were wounded on two sides to a depth of 1.5 mm by puncturing them with a pin. Each wound site was then inoculated with 40 µL of spore suspension (10^5 spore/mL) of blue mold. In some experiments, indicated in the text, spore concentration was greater or less than the normal concentration and the size of the wound was different. In addition, in one experiment the spores were held at 38 °C before fruit inoculation and in another the apples were heated before inoculation.

The apples were then held at three temperatures—46, 42, and 38 °C—for various times before being placed at 0 °C for storage or directly at 20 °C. 'Anna' apples were stored for 1 month and 'Golden Delicious' apples for 4 months. Control apples were inoculated and held at either 0 or 20 °C without heating. After 14 d at 20 °C, with or without a storage period, the apples were rated for decay severity by fixing the diameter of the decayed area as the mean of its width and length and then calculating the area of decay incidence as a circle. Each treatment was performed twice on 25 fruit each time.

Measurement of fruit softening. 'Anna' and 'Golden Delicious' apples were heated at three temperatures—46, 42, and 38 °C—for various times before being placed at 0 °C storage for 1 month ('Anna') or 4 months ('Golden Delicious'). At harvest, at the end of storage and following a 7-d shelf life at 20 °C, the unheated and heated fruit were measured for firmness. At each observation 10 fruit from each treatment were measured with a penetrometer (Hunter-Spring, Hatfield, Pa.) with an 11-mm tip on two peeled sides of each apple. The apples were also evaluated for heat damage by halving the fruit and examining them for skin scalding and flesh browning. In one experiment, 'Anna' apples were held at 20 °C and their respiration and ethylene were measured daily until peak ethylene production was reached, at which time they were heat treated. The fruit at harvest with very low ethylene production were termed preclimacteric, and following the ethylene peak they were postclimacteric.

Statistics. The effects of the independent variables of cultivar, time, temperature, and time × temperature on the dependent variables of insect mortality or fungal decay were analyzed using

Table 1. Effect of heat treatment on the survival of San Jose scale on 'Jonathan' and 'Golden Delicious' apples.

Treatment temp (°C)	Exposure time (h)	Cultivar	Female[z] insects (no.)	Mortality[y] (%)	Mixed insects (no.)	Mortality (%)	Diapausing (no.)	Mortality (%)
50	5	Jonathan	200	100	905	100	288	100
		Golden	301	100	1289	100		
	10	Jonathan	256	100	891	100	235	100
		Golden	252	100	1380	100		
46	5	Jonathan	226	100	621	88.0 ± 8.12	248	42.9 ± 7.88
		Golden	284	98.7 ± 1.02[x]	732	57.5 ± 11.70		
	10	Jonathan	219	100	608	99.5 ± 1.07	405	100
		Golden	295	100	768	100		
42	5	Jonathan	197	12.3 ± 5.12	596	59.7 ± 15.61	422	44.3 ± 2.47
		Golden	228	18.6 ± 4.64	613	45.6 ± 5.17		
	10	Jonathan	187	17.5 ± 7.05	680	87.9 ± 7.58	533	42.9 ± 7.88
		Golden	264	17.6 ± 5.94	622	62.6 ± 2.53		
Control		Jonathan	188	15.2 ± 4.59	447	42.2 ± 4.18	403	34.0 ± 6.94
		Golden	259	11.8 ± 9.45	508	45.6 ± 3.15		
Analysis of variance								
Temperature				***		***		***
Time				NS		***		***
Time × temperature				**		***		**
Cultivar				NS		***		---

[z]Female, mixed and diapausing stages are described in Materials and Methods.
[y]Mortality was determined 5 d after the heat treatment while the fruit were held at 20 °C.
[x]Standard deviation.
NS,**,***Nonsignificant or significant at $P = 0.01$ or 0.001, respectively.

Table 2. The effect of heat treatment on decay development in 'Golden Delicious' apples inoculated with different concentrations of blue mold spores, which had either been heated for 4 d at 38 °C, or not heated.

Treatment	Inoculum (spores/mL)	Decayed area (cm^2)[z]	
		Unheated spores	Heated spores[y]
Nonheated	10^3	0.75 ± 0.01[x]	0.002
Nonheated	10^5	18.8 ± 1.1	1.3 ± 0.3
Nonheated	10^7	25.9 ± 0.9	13.5 ± 0.8
38 °C for 3 d	10^5	0	0
38 °C for 4 d	10^5	0	0
Analysis of variance			
Spore concentration		***	
Heating of spores		***	
Heating of apples		***	

[z]Decay was determined after 14 d at 20 °C.
[y]Spores were heated for 4 d at 38 °C.
[x]Standard deviation.
***Significant at $p = 0.001$.

analysis of variance. Fruit firmness statistical analysis is given as least significant difference.

Results

Heat treatment on insect mortality. San Jose scale adult females were more sensitive to high temperature than a mixed population of different larval stages (Table 1). Exposure to 5 or 10 h at 46 °C killed the female insects, while in the mixed population only a 10-h exposure to 46 °C was effective. Exposure to 50 °C for either time exposure controlled both scale populations. For the female population the mortality at 42 °C was no different than control. The latter had a mortality of 15% on 'Jonathan' and 12% on 'Golden Delicious' (Table 1). This mortality was much lower than that of the mixed population, which in the control was 42% on 'Jonathan' and 46% on 'Golden Delicious'. In examining the mortality of the different instars, the effect of the heat treatment was found to be similar (data not shown). There was a significant interaction between time and temperature on the female and the mixed populations. Apple cultivar did not affect the mortality of the female insects, but in the mixed population there was greater mortality at lower temperatures on 'Jonathan' than on 'Golden Delicious' apples.

The effect of heat on diapausing first instar of San Jose scale was tested on naturally infested 'Jonathan' fruit (Table 1). In this developmental stage, temperature and time of exposure showed a significance of at least $p = 0.001$. When the naturally infested fruit were heat treated, only 50 °C for 5 or 10 h and 10 h at 46 °C controlled scale. Unacceptably high rates of insect survival occurred after 5 h at 46 °C or exposure to 42 °C.

Effect of heat treatment on blue mold development. As found previously (Fallik et al., 1995), spore germination and mycelial growth of blue mold was found to be sensitive to elevated temperature. Percentage spore germination and mycelial growth were inversely proportional to length of exposure to 38, 42, and 46 °C. Spore germination was more sensitive than mycelial growth at various temperatures. The temperature for 50% inhibition of spore germination was 42, 34, and 28 h at 38, 42, and 46 °C, respectively, whereas for mycelial growth it was 48, 44, and 36 h at those temperatures.

When apples were inoculated with spores that had previously been heated for 4 d at 38 °C, the severity of the infection was much less than for unheated spores (Table 2). The difference between decay development of heated and unheated spores increased as the concentration of the spores used for inoculation decreased. The

Table 3. The effect of heat treatment and storage on decay development in 'Anna' and 'Golden Delicious' apples inoculated with blue mold after 14 d at 20 °C, or 1 month ('Anna') or 4 months at 0 °C ('Golden Delicious'), and an additional 14 d at 20 °C.

Treatment temp (°C)	Time (h)	Cultivar	Decayed area (cm^2)	
			Post treatment	Post storage
46	12	Anna	1.7 ± 0.02[z]	1.4 ± 0.01
		Golden Delicious	3.1 ± 0.02	1.3 ± 0.01
42	24	Anna	0.3 ± 0.01	0.1 ± 0.005
		Golden Delicious	---	---
38	72	Anna	---	---
		Golden Delicious	0	0
	96	Anna	0	0
		Golden Delicious	0	0
Control		Anna	28.8 ± 1.3	14.0 ± 0.9
		Golden Delicious	20.8 ± 0.8	25.9 ± 1.1
Analysis of variance				
Temperature			***	
Cultivar			NS	

[z]Standard deviation.
NS,***Nonsignificant or significant at $p = 0.001$, respectively.

Table 4. The effect of the wound size on decay development in 'Golden Delicious' apples infected with blue mold.

Treatment	Decayed area (cm^2)[z]	
	Pin wound[y]	Peeling wound
Inoculated→20 °C	28.1 ± 0.8[x]	35.2 ± 1.1
Inoculated→38 °C 96 h	0	0
Wounded→38 °C 96 h then inoculated	0.1 ± 0.9	5.8 ± 0.01
38 °C for 96 h then inoculated	25.5 ± 0.7	28.1 ± 0.1

[z]Decay was determined after 14 d at 20 °C.
[y]Wounds were made by a 1.5-mm pin to a 1.5-mm depth, or by removing a 10-mm^2 section of peel to a depth of 3 mm.
[x]Standard deviation.

normal inoculation concentration of 10^5 spores/mL showed a 12-fold difference in the decayed area on the fruit if the spores were previously heated (1.3 vs. 18.8 cm^2 decay). If the apples were heated at 38 °C after inoculation with either preheated or nonheated spores, decay was prevented in both cases. All three variables examined, spore concentration, heating of spores, and heating of fruit significantly affected the amount of decay developing.

Development of decay on two apple cultivars inoculated before heating at different temperatures was measured immediately after treatment or after storing the apples (Table 3). In both cultivars tested, storage did not reduce the efficiency of the heat treatment. Heating at 50 °C damaged the apples and so was not tested (data not shown). However, 12 h at 46 °C or 24 h at 42 °C almost entirely prevented decay development in either stored or unstored 'Anna' and 'Golden Delicious' apples. Exposing fruit to 38 °C for either 72 or 96 h was the most effective treatment in that no decay at all resulted from inoculating the fruit.

Blue mold is a wound pathogen and the size of the wound affected the speed of fungal development and the extent of decay on unheated apples (Table 4). If a 10-mm section of the peel was removed for inoculation the area of decay was greater than if the spores were inoculated in a small puncture wound. However, heating the inoculated apples for 4 d at 38 °C prevented decay development both types of wounds (Table 4). If the apples were first heated and then inoculated, decay development was less than in unheated inoculated apples, but nonetheless extensive. However, if the apples were wounded before heating and inoculated at the end of heat treatment, then almost no decay developed.

Effect of heat treatment on fruit physiology. Holding the fruit at too high a temperature caused heat injury. Injury occurred at 50 °C even after 5 h (data not shown). However, the apple cultivars were able to withstand 12 h at 46 °C with no development of injury. If 46 °C was maintained for 24 h or 42 °C for 48 h, heat injury occurred (Table 5). At 38 °C apples were heated for up to 96 h with no injury.

Apples are generally stored when they are preclimateric as measured by ethylene production, and this ripeness stage may be more or less sensitive to high temperatures than apples more advanced in ripening. To check this, 'Anna' apples at defined maturity stages, determined by following their ethylene and respiration after harvest, were heat treated (Fig. 1). It was found that postclimacteric fruit were more sensitive than preclimacteric fruit to heat damage. Even at 38 °C the postclimacteric fruit had heat damage, which was evidenced by browning of the flesh at the distal end of the fruit. However, heating fruits of both maturity stages prevented blue mold from developing while decay occurred in nonheated inoculated fruit.

Heated apples maintained firmness during storage and shelf life better than unheated fruit (Table 5). Exposure to 46 °C resulted in the least softening in the apples during shelf life, while the effects of 42 and 38 °C were similar. All treatment temperatures caused the apples to be firmer than control apples at removal and after shelf life.

Discussion

In this study there was a range of temperatures that controlled San Jose scale and blue mold without damaging apples. San Jose scale insects were more sensitive to high temperature than blue mold, perhaps because they are more complicated organisms (Armstrong, 1994). A treatment of 10 h at 46 °C controlled San Jose scale, while 28 or 36 h at this temperature was necessary for preventing blue spore germination or mycelial growth. The time needed at 46 °C to control San Jose scale did not cause damage to preclimacteric apples, but the longer times needed at this temperature for fungal control caused heat damage.

At the lower temperatures, 42 and 38 °C there was less danger of fruit damage. These temperatures also controlled blue mold on apples if given for 24 h (42 °C) or 72 h (38 °C). These temperatures were more effective when fruit were inoculated with the pathogen than if the pathogen was tested alone. A possible explanation is that the heat treatment enhanced antifungal defense reactions in the fruit tissue making the heat more effective against the fungus. This might explain the fact that, if the apple was heated before inoculation, the decay developed more slowly than on unheated fruit (Table 4). We have observed a compound with antifungal activity induced at 38 °C (Fallik et al., 1995), and attempts to identify it are in progress.

Heat treatment shows promise as a means of controlling insect and fungal pests, thereby reducing postharvest use of chemicals.

Table 5. Firmness and heat damage of 'Anna' apples after 1 month of 0 °C storage.

Treatment temp	Time	Firmness (N)[z]		Heat damage[y]
(°C)	(h)	0 d	7 d	(%)
46	12	78	65	0
	24	76	65	100
42	24	68	56	0
	48	70	57	83
38	72	71	58	0
	96	71	59	0
Control		65	48	0
$LSD_{0.05}$		5.3	5.0	---

[z]Firmness was measured after storage (day 0) and after 7 d at 20 °C.
[y]Heat damage was measured after 7 d at 20 °C.

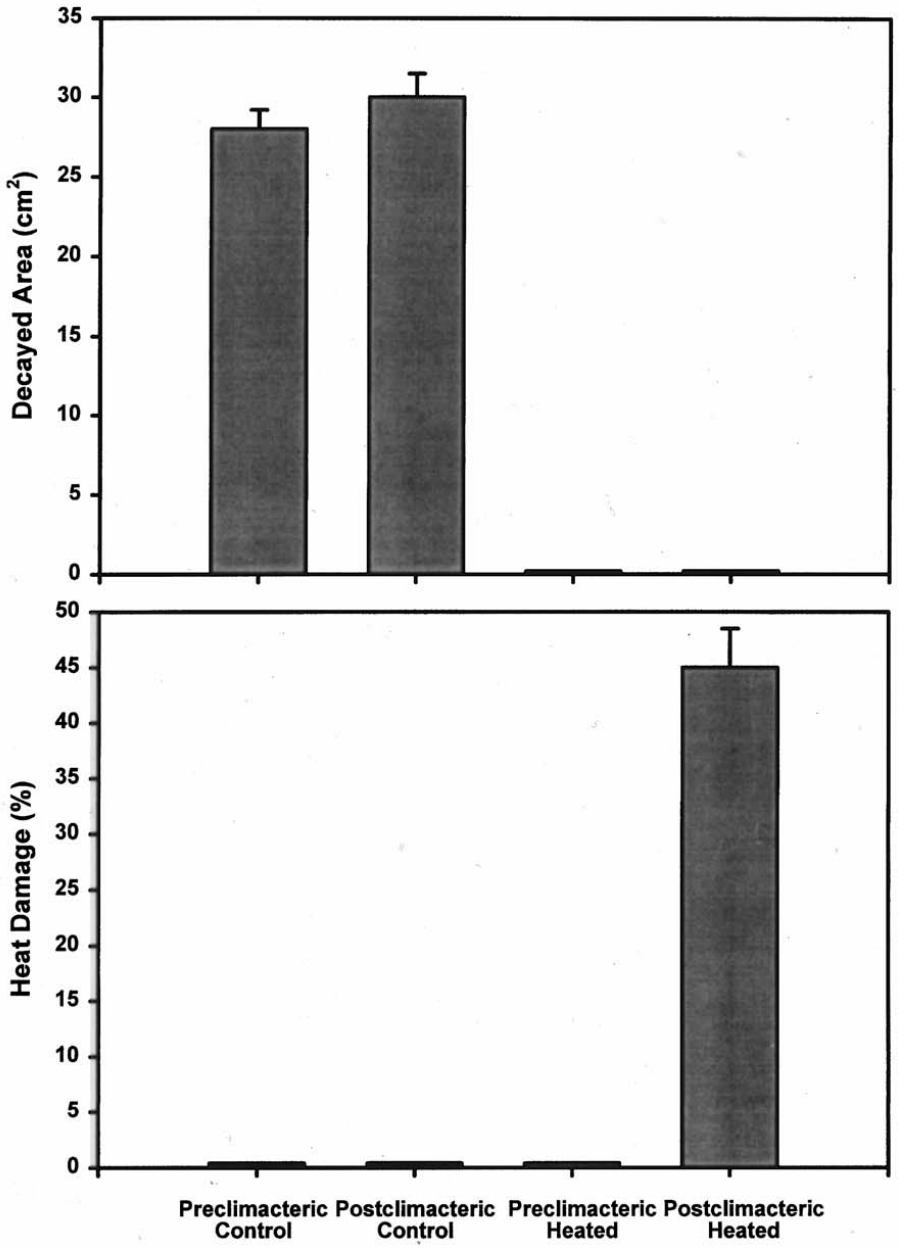

Fig. 1. The effect of a 3 d of 38 °C heat treatment on decay development and percentage of heat damage in 'Anna' apples inoculated with blue mold as affected by its stage of maturity (pre- or postclimacteric) after 14 d at 20 °C. The maturity stages were determined by holding freshly harvested apples at 20 °C and following their ethylene and respiration production. Control apples were inoculated at specific maturity stages and held at 20 °C for 14 d.

Unfortunately, unlike chemicals, the treatment must be individually tailored to each commodity. First, the limits of tolerance to the heat treatment at a range of temperatures must be determined for the commodity for the maturity stage at which it is harvested for marketing or storage. Then the particular pest to be controlled should be tested within the time and temperature constraints that do not damage the product. Different fungi and insects have different tolerances to temperature. For example, the postharvest pathogens blue mold (*Penicillium expansum* Link) and *Botrytis cinerea* (Pers.) are easily controlled by high temperatures, while *Alternaria alternata* (Fr.) Keissler is much more refractive (Barkai Golan et al., 1993; Fallik et al., 1993, 1995). For insects, different tolerances are also found. A study of heat treatment for persimmon (*Diospyros kaki* L.) disinfestation found that mealy bugs (*Pseudococcus longispinus* Targioni-Tozetti) were more tolerant than apple moth (*Epiphas postvittana* Walter) to hot-water and hot-air treatments (Dentener et al., 1996; Lester et al., 1995).

The tendency in heat research is to look for the shortest time of exposure needed to control a pest, which normally necessitates a higher temperature. We suggest that an equally valid approach is to concentrate on the temperatures that do not damage the commodity and perhaps invest them with beneficial attributes, such as slower softening or resistance to low temperature (Klein and Lurie, 1992). The time needed for pest control will be longer, but the fruit or vegetable will be of higher quality.

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