Final Report

March 1993 to September 1996

Submitted to the Office of the Science Advisor
US Agency for International Development

Prevention of pathogen infection and insect infestation by a postharvest heat treatment

Principal Investigator: Susan Lurie
Grantee Institution: Volcani Center, Agricultural Research Organization

Collaborator: K. Kovacs
Institution: Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences

Project Number: C12-081

AID Grant Project Officer: Boaz Ayalon

Project Duration: March 1993 - September 1996

2. Table of Contents

Cover Page	1
Table of Contents	2
Executive Summary	3
Research Objectives	4
Methods and Results 1.	4
Methods and Results 2.	37
Methods and Results 3	50
Impact and Relevance	97
Project Activities	97
Project Productivity	98
Future Work	99

3. Executive Summary

The overall aims of the project were to develop a non-chemical method to achieve three objectives:

1. slow fruit ripening and extend postharvest storage and shelf life
2. prevent fungal infection of harvested fruit
3. prevent insect infestation of harvested fruit

The method chosen was a postharvest heat treatment; both hot water dips and a dry heat treatment. The Israeli scientists investigated the first two objectives and the Hungarian scientists were in charge of the third objective. There was good coordination between the parties in all phases of the research.

Hot water dips for control of insects were investigated using the fruit fly *Drosophila melanogaster* as a model. Once conditions were established which could control the insect, hot water dips were used on sweet pepper and the control of grey and black mould, the two major causes of pepper decay, were examined.

Dry heat treatments were used on tomatoes and apples, and conditions were found which could control insects and fungal decay on both these commodities. Both tomatoes and apples are climacteric, and therefore, unlike pepper, continue to ripen after harvest. The effect of heat treatments on tomatoes was examined and it was found that ripening was reversibly inhibited by this treatment. A result of this reversible inhibition was a longer postharvest life with no loss of quality.

4. Research Objectives

The three objectives of the project were to develop a heat treatment to:

1. slow fruit ripening and extend postharvest storage and shelf life.
2. prevent fungal infection of harvested fruit.
3. prevent insect infestation of harvested fruit.

We will present the results in three sections, each covering the work conducted on one of the objectives.

5. Methods and Results

1. Slow fruit ripening and extend postharvest storage and shelf life. We observed early on that a heat treatment not only reversibly inhibited fruit softening, but also prevented chilling injury in commodities sensitive to low temperatures. This was investigated further using various time-temperature manipulations of tomato fruit and the results are presented in the paper: S. Lurie and A. Sabehat. 1997. Prestorage temperature manipulations to reduce chilling injury in tomatoes. Postharvest Biology and Technology, in press. The heat treatments in this paper were dry heat. However, since hot water dips show promise of fungal and insect control, we also compared dry heat to hot water dips and their effect on preventing chilling injury in tomatoes. Chilling injury is usually thought to originate in membrane damage, so in this work we examined the lipid components of the fruit after the storage period. The results are presented in: S. Lurie, M. Laamim, Z. Lapsker and E. Fallik. 1997. Heat

treatments to decrease chilling injury in tomato fruit. Effects on lipids, pericarp lesions and fungal growth. Physiologia Plantarum, 99: in press.

The inhibition of ripening, we felt, originated at the level of gene expression. At high temperatures normal protein synthesis is inhibited and the synthesis of heat shock proteins is activated. It was possible that ripening related processes such as ethylene production, fruit color development and softening were inhibited because the enzymes which were involved in these processes were not synthesized at high temperature. This hypothesis was examined and found to be correct, and the results are presented in: S. Lurie, A. Handros, E. Fallik and R. Shapira. 1996. Reversible inhibition of tomato gene expression at high temperature. Plant Physiology 110:1207-1214, and in S. Lurie, A. Handros, A. Sabahat, J.D. Klein, E. Fallik and R. Shapira. 1995. The effect of high temperature on maintaining quality of harvested tomatoes. In: Postharvest Physiology, Pathology and Technologies for Horticultural Commodities (eds. A. Ait-Oubahou and M. El-Otmani), Institut Agronomique et Veterinaire Hassan II, Agadir, Morocco, pp. 234-240.

Prestorage Temperature Manipulations to Reduce Chilling Injury in Tomatoes

Susan Lurie and Adnan Sabehat, Department of Postharvest Science, Volcani Center, ARO, Bet Dagan 50250, Israel

Correspondence to:

Susan Lurie

Department of Postharvest Science

Volcani Center, ARO

Bet Dagan 50250, Israel

FAX: 972-3-9683622

e-mail: VTFRST@VOLCANI.agri.gov.il

ABSTRACT

Temperature manipulations of harvested mature green tomatoes (*Lycopersicon esculentum* cv Daniella) were found to decrease chilling injury (CI) development if applied before 2°C storage. The tomatoes developed CI after transfer to 20°C if held for longer than one week at 2°C. Gradual lowering of the storage temperature prevented injury from developing. Holding the tomatoes for various times at 38°C before storage also prevented CI for up to 4 weeks of storage at 2°C, while this protection disappeared if the tomatoes were transferred from 38°C to 20°C for 4 days before 2°C storage. Holding tomatoes at 20°C before storage did not affect CI development. Temperatures higher than 38°C were not generally as effective as 38°C, and 24 hours at 42 or 46°C caused heat damage. The ability of mature green tomatoes to ripen after 3 weeks at 2°C increased as the growing season advanced, but there was no similar change in susceptibility to CI. The effect of chilling temperatures on ripening and on the development of CI does not appear to be connected, but a prestorage heat treatment negated the effect of chilling on both processes.

Keywords: decay, *Lycopersicon esculentum,* ripening, storage

Abbreviations: HSP=heat shock proteins; SSC= soluble solids content; TA= titratable acidity.

INTRODUCTION

The storage temperatures of subtropical fruits such as tomatoes are arrived at by a compromise between temperatures which are low enough to inhibit ripening processes, but cause chilling injury, and those which are high enough to avoid chilling injury, but do not prevent the continuation of ripening (Couey, 1982). The compromise temperature for most varieties of tomatoes is 12°C, but since ripening is not prevented at this temperature, most tomatoes can be stored for only 2 weeks (Hobson, 1981). Temperatures above 35°C have been found to inhibit fruit ripening (Biggs, et al., 1988; Klein and Lurie, 1992). Storage of fruit at elevated temperatures is not feasible, as there is excessive weight loss and the ability to ripen normally is lost after extended periods at high temperatures (Eaks, 1978; Tsuji et al., 1984). In a previous study, mature green tomatoes were held at 38°C for 3 days, before storing them at 12°C or 2°C (Lurie and Klein, 1992). The prestorage heat treatment caused faster ripening in tomatoes held at 12°C than control tomatoes, but prevented chilling injury in tomatoes held at 2°C. The present study explores in greater depth the response of mature green tomatoes harvested at different times during the season to temperature manipulations before or during storage.

MATERIALS AND METHODS

Mature green tomatoes (*Lycopersicon esculentum* cv Daniella [also known as Rehovot 144]) were harvested from plants growing in a greenhouse. The fruits were randomly divided into replicates of five fruit, and five replicates

(25 fruit) were used for each treatment. For heat treatments the fruits were held in a tray covered with a polyethylene bag with damp filter paper on the sides of the tray. The tray was put in a heating chamber for the time and temperature indicated in the experiments, and then transferred to storage at 2°C. Relative humidity of all storage and treatment chambers was maintained at 85-90%, with one or two atmosphere changes each hour. After the appropriate time in storage, the fruits were removed to 20°C and monitored over 10 days.

Immediately after harvest 10 fruits in each treatment (2 replicates) were weighed. These 10 fruits were monitored throughout the length of the experiment for weight loss, firmness, and color change. Firmness was measured nondestructively with a Durometer (Shore Manufacturing, NY). Color development was measured with a chromometer (Minolta, Japan) using the L, a, b scale. The 'a' value on green fruit is negative; when the 'a' value changed from negative to positive the fruit had developed red color. Chilling injury, measured visually as surface lesions on the fruit, decay and fruit color from green to red, were determined at each observation time on all fruits in the five replicates of each treatment. Decay was not classified by pathogenic organisms, but over 90% of the decay was caused by *Botrytus cinarea* and *Alternaria alternata*. At the end of shelf life, slices from each fruit in a replicate were combined for juicing, and soluble solids content (SSC) and titratable acidity (TA) measured. SSC was determined on a hand held digital refractometer (Atago, Japan), and TA by titrating 2 ml juice to pH 8.2 with 0.1N NaOH and expressing the result as citric acid.

Analysis of variance was performed on the results. Based on significant F-test, means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

Mature green tomatoes were held at 2°C for varying lengths of time to determine when they became sensitive to CI. One week at 2°C was not harmful, and tomatoes developed color normally with no fungal rots (Table 1). After 11 days at 2°C, rots began to appear in after-storage ripening, and color development was impaired. After 3 weeks at 2°C the tomatoes no longer developed a red color.

As the fruits were held longer at 2°C weight loss increased, but the rate of weight loss at 20°C was similar (Fig. 1A). Firmness also was lower in 14 days stored fruit compared to 7 or 11 days, but the rate of softening at 20°C was similar in all three cases (Fig. 1B).

Mature green tomatoes were held for three weeks at 4 different storage temperatures before ripening at 20°C (Table 2). At 12°C all the fruit ripened normally, but some rots developed. At 8°C there were no rots, and the tomatoes developed red color to the same extend as fruit held at 12°C. At 5° and 2°C CI and rots were apparent, and the color distribution of the fruit showed impairment of ripening. At removal from storage the fruits stored at 8 or 12°C were softer than those from 5 or 2°C storage. Fruit removed from 2, 5, 8, or 12°C measured 62, 62, 58 and 51 Durometer units firmness, respectively. After 7 days at 20°C

all the fruit had softened an average of 33 Durometer units. Weight loss was approximately 2% at removal, and 5% after shelf life, regardless of storage temperature.

Since holding the fruit at 12 or 8°C did not cause the development of CI, it was thought that decreasing the temperature gradually might allow the fruit tissue to adapt and thus prevent damage at the final storage temperature of 2°C. Holding the tomatoes for 2 days at 12, 8 and 4°C successively before 3 weeks storage at 2°C was compared with immediate 2°C storage with gradual rewarming. This is a treatment which has been effective on citrus fruits which are also susceptible to low temperature damage (Cohen et al., 1983). Disinfestation treatments require holding the fruit at 1°C, and to prevent CI, the fruits are given an acclimization period before reaching the final temperature (Couey, 1989).

Gradual reduction of temperature enabled the tomatoes to develop red color after removal from 3 weeks at 2°C and prevented the development of CI and rots (Table 3). In contrast, gradual warming did not have the same beneficial effect. The fruits remained green after 7 days at 20°C following storage (as indicated by a negative 'a' value), and there was some development of CI and decay, although less than fruits placed immediately at 2°C. There were no differences among the treatments regarding fruit firmness and weight loss. The former averaged 40 Durometer units and the latter 5% in all treatments after 7 days at 20°C.

The ability of mature green tomatoes to ripen after low temperature storage changed during the harvest season (Table 4). Fruits harvested during February ripened if held at 20°C (data not shown), but did not develop red color if first stored at 2°C and then held for ripening at 20°C. However, later harvests of mature green fruits did allow some ripening to proceed following storage. It is interesting that there was no trend observed with these fruits for susceptibility to CI. The percentage of fruits which suffered from CI after 2°C storage varied from 30 to 60% during the season, with no perceivable pattern. It appears that the ability to ripen after low temperature storage is not tied to the development or nondevelopment of CI lesions.

It has previously been found that holding tomato fruit for 3 days at 38°C prevented CI from developing after 3 weeks 2°C storage (Lurie and Klein, 1991). It is possible that shorter times of prestorage heat treatment might be equally effective, as was found with apples (Klein and Lurie, 1992). Six hours of heat treatment did not prevent the effects of CI following storage (Table 5). Twelve hours decreased the amount of CI to 50% of the unheated fruits, while 1 or 2 days at 38°C almost totally eliminated CI symptoms. The unheated fruits reached the pink stage of color development after 10 days at 20°C following storage, while all the heat treated fruit, except those from 6 hours heat, achieved full color. Two days at 38°C was effective in preventing CI for up to 4 weeks storage at 2°C. However, after 5 weeks the protection afforded by the heat was no longer apparent. Heating the fruit before storage caused greater weight loss at removal from storage, but during the ripening period at 20°C the unheated

control fruits had a greater rate of weight loss, probably due to the development of CI. Consequently, at the end of shelf life fruit from all treatments had lost between 6.2 to 7.7% of their weight.

The protective effect of heat treatment against CI in tomatoes has been correlated with the accumulation of heat shock proteins (HSP) in the fruit tissue (Sabehat et al., 1996). The elevated temperature initiates synthesis of these proteins, and mRNA of the HSPs was found to be most abundant after 24 h at 38°C (Lurie et al., 1996). The protein continued to accumulate throughout the heating period and remained present during storage for 3 weeks at 2°C (Lurie et al., 1996). However, after more than 4 weeks storage the protein as measured by Western blot disappeared (unpublished). This may explain the dissipation of the protection afforded by prestorage heat at longer times of storage.

Although HSPs remain present if fruits are transferred from 38°C to 2°C, if transferred to 20°C the proteins disappear over the course of a few days (Sabehat et al., 1996; Vierling, 1991). To examine if the protection to CI conferred by heating the tomatoes before storage would disappear, the fruit were held for varying periods of time at 20°C before being placed at 2°C storage (Table 6). Unheated fruit after storage did not develop a red color, but did develop high levels of CI and decay. Two days heating at 38°C before storage allowed the fruit to develop full red color, but if held at 20°C following the heat treatment for 4 or more days before storage, the fruits also developed CI and decay. Holding the tomatoes at 20°C for 4 days before storage to allow ripening to proceed allowed for color development after storage, but did not prevent the

fruits' susceptibility to CI or rots. This is in contrast to a study with Rutgers tomatoes which found that holding them at 20°C before low temperature storage prevented CI better than holding them at 38°C (Whittaker, 1994).

Two temperatures higher than 38°C, 42 and 46°C, were investigated to see if a shorter time at a higher temperature would incur the same benefit seen in tomatoes after 1 or 2 days at 38°C (Table 7). Twenty four hours heating at 42 or 46°C caused heat damage to the fruits (data not shown). None of the heating times at 46°C prevented the development of high levels of CI and decay, though 6 h at 46°C accelerated peel color development. Six hours at 42°C controlled CI and rots as effectively as 2 days at 38°C. Weight loss in this experiment was higher than in other experiments, and 46°C caused greater weight loss than lower temperatures. However, the firmness of the heated fruit was in all cases higher than the control fruit. Fruit held two days at 38°C or 6 h at 42°C had similar firmness which was intermediate between control fruit and fruit heated at 46°C or at 42°C for longer than 6 h.

SSC and TA were not affected by heat treatments. In all the experiments SSC averaged 4.3%, while TA averaged 0.71%. There was no trend of increasing SSC or decreasing TA as the season advanced (data not shown). This finding is in contrast to a previous study on a different tomato cultivar where 3 days at 38°C increased SSC over control tomatoes (Lurie and Klein, 1992).

In summary, temperature manipulations before storage can prevent the development of CI on mature green tomatoes. However, it appears that there

may be cultivar differences which will determine whether a heat treatment will be effective. 'Rutgers' did not appear to respond favorably to a prestorage heat treatment (Whittaker, 1994). A study with 'Rehovot 121' showed quality improvement in that better color and higher SSC were found in heated tomatoes (Lurie and Klein, 1992). The cultivar in the present study, 'Daniella', is a slow ripening variety, and the heat treatment allowed storage for up to 4 weeks with a possible shelf life of over 10 days.

ACKNOWLEDGEMENTS

This is a contribution fro the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel No. ICOI-E, 1996. This research was partially funded by the United States-Israel Binational Agricultural Research Foundation and the Ministry for Economic Cooperation of the Federal Republic of Germany under the German-Iraeli Agricultural Research Agreement.

REFERENCES

Biggs, M.S., Woodson, W. R. and Handa, A.K., 1988. Biochemical basis of high-temperature inhibition of ethylene biosynthesis in ripening tomato fruits. Physiol. Plant., 72:572-578.

Cohen, E., Shuali, M. and Shalom, Y., 1983. Effect of temperature manipulation on the reduction of chilling injury of Villa Franka lemon fruits stored at cold temperature. J. Hort. Sci., 58:593-598.

Couey, H.M., 1982. Chilling injury of crops of tropical and subtropical origin. HortSci., 17:162-165.

Couey, H.M., 1989. Heat treatment for control of postharvest diseases and insect pests of fruits. HortSci., 24:198-202.

Eaks, I., 1978. Ripening, respiration, and ethylene production of "Haas" avocado fruits at 30 to 40°C. J. Amer. Soc. Hort. Sci., 103:576-578.

Hobson, G.E., 1981. The short term storage of tomato fruit. J. Hort. Sci., 56:363-368.

Klein, J.D. and Lurie, S., 1992. Heat treatments for improved postharvest quality of horticultural crops. HortTechnology, 2:316-320.

Klein, J.D. and Lurie, S., 1992. Prestorage heating of apple fruit for enhanced postharvest quality; interaction of time and heating temperature. HortSci., 27:326-328.

Lurie, S. and Klein, J.D., 1991. Acquisition of low temperature tolerance in tomatoes by exposure to high temperature stress. J. Amer. Soc. Hort. Sci., 116:1007-1012.

Lurie, S. and Klein, J.D., 1992. Ripening characteristics of tomatoes stored at 12°C and 2°C following a prestorage heat treatment. Scient. Hort., 51:55-64.

Lurie, S., Handros, A. and Shapira, R., 1996. Reversible inhibition of tomato fruit gene expression at high temperature: effects on tomato fruit ripening. Plant Physiol., 110:1207-1214.

Sabehat, A., Weiss, D. and Lurie, S., 1996. The correlation between heat shock protein accumulation and persistence and chilling tolerance in tomato fruit. Plant Physiol., 110: 536-541.

Tsuji, M., Harakawa, H. and Komiyama, Y., 1984. Changes in shelf life and quality of plum fruit during storage at high temperature. J. Jpn. Soc. Hort. Sci., 52:469-473.

Vierling, E., 1991. The roles of heat shock proteins in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol., 42:579-620

Whitaker, B., 1994. A reassessment of heat treatment as a means of reducing chilling injury in tomato fruit. Postharv. Biol. and Technol., 4:75-83.

Table 1. Fungal rots, CI and color development of fruits stored for varying times at 2°C before transfer to 20°C for 10 days.

Time at	Rots (%)	CI (%)	Fruit Color (%)		
2°C			Green	Turning	Red
7 d	0d	0b	0b	20b	70a
11 d	30c	15b	15b	40a	45b
14 d	75b	60a	0b	40a	60ab
21 d	100a	75a	100a	0c	0c

Different letters in the same column denote significant difference at P = 0.05.

Table 2. Fungal rots, chilling injury and fruit color development after 10 days at 20°C following 3 weeks storage at different temperatures.

Storage	Rots (%)	CI (%)	Fruit Color (%)		
Temperature			Green	Turning	Red
12°	20b	0b	0c	10b	90a
8°	0c	0b	0c	20ab	80a
5°	30ab	40a	25b	25a	50b
2°	50a	50a	45a	25a	30c

Different letters in the same column denote significant difference at P = 0.05

Table 3. The effect of gradual cooling (2 days at 12°, 9° and 5°) or gradual rewarming (2 days at 5°, 8° and 12°C) on color development, CI and decay of mature green tomatoes held for 3 weeks at 2°C and then 7 days at 20°C.

Treatment	Peel Color ('a" value)	CI (%)	Decay (%)
Gradual cooling	6.8a	0c	0b
Gradual rewarming	-4.7b	15b	15a
Control	-2.5b	35a	15a

Different letters in the same column denote significant difference at P = 0.05.

Table 4. Color development and CI of unheated fruit harvested during a 3 month period, held for 3 weeks at 2°C and then 10 days at 20°C.

Date of Harvest	Peel Color ('a' value)	CI (%)
February 9	-6.2a	60
February 13	-4.1a	40
February 27	-1.4b	60
March 15	5.8c	35
March 20	6.1c	50
April 10	8.4d	30
		N.S

Different letters in the same column denote significant difference at P = 0.05.

N.S. = non significant.

Table 5. Red color development, CI and rots on mature green tomatoes held at 38°C before 2°C storage and then ripened for 10 days at 20°C.

t	Time of storage (wks)	Color ('a' value)	Chilling Injury (%)	Decay (%)	Weight Loss (%) Removal	Shelf life
Control	3	6.1b	65a	20a	1.7b	7.0
6 h 38	3	9.5b	55a	10b	2.3a	7.7
12 h 38	3	13.8a	30b	10b	2.6a	7.5
1 d 38	3	14.1a	10c	0c	2.4a	6.2
2 d 38	3	13.8a	10c	5c	2.5a	6.6
2 d 38	4	15.5a	20bc	15ab	-	-
2 d 38	5	8.7b	50a	25a	-	-
						N.S.

Different letters in the same column denote significant difference at P = 0.05.

N.S. = non significant.

Table 6. The effect of holding tomatoes at 20°C following heating and before storage, on the development of peel color, CI and decay during 7 days at 20°C after 3 weeks 2°C storage.

Treatment	Color ('a' value)	CI (%)	Decay (%)
Control	-1.2d	50a	30b
2 d 38	3.7c	0c	10c
2 d 38, 1 d 20	8.0b	10c	0c
2d 38, 2 d 20	11.6a	10c	10c
2 d 38, 4 d 20	7.7b	30b	15c
2 d 38, 6 d 20	6.3b	50a	60a
4 d 20	4.1c	35b	50a

Different letters in the same column denote significant difference at P = 0.05.

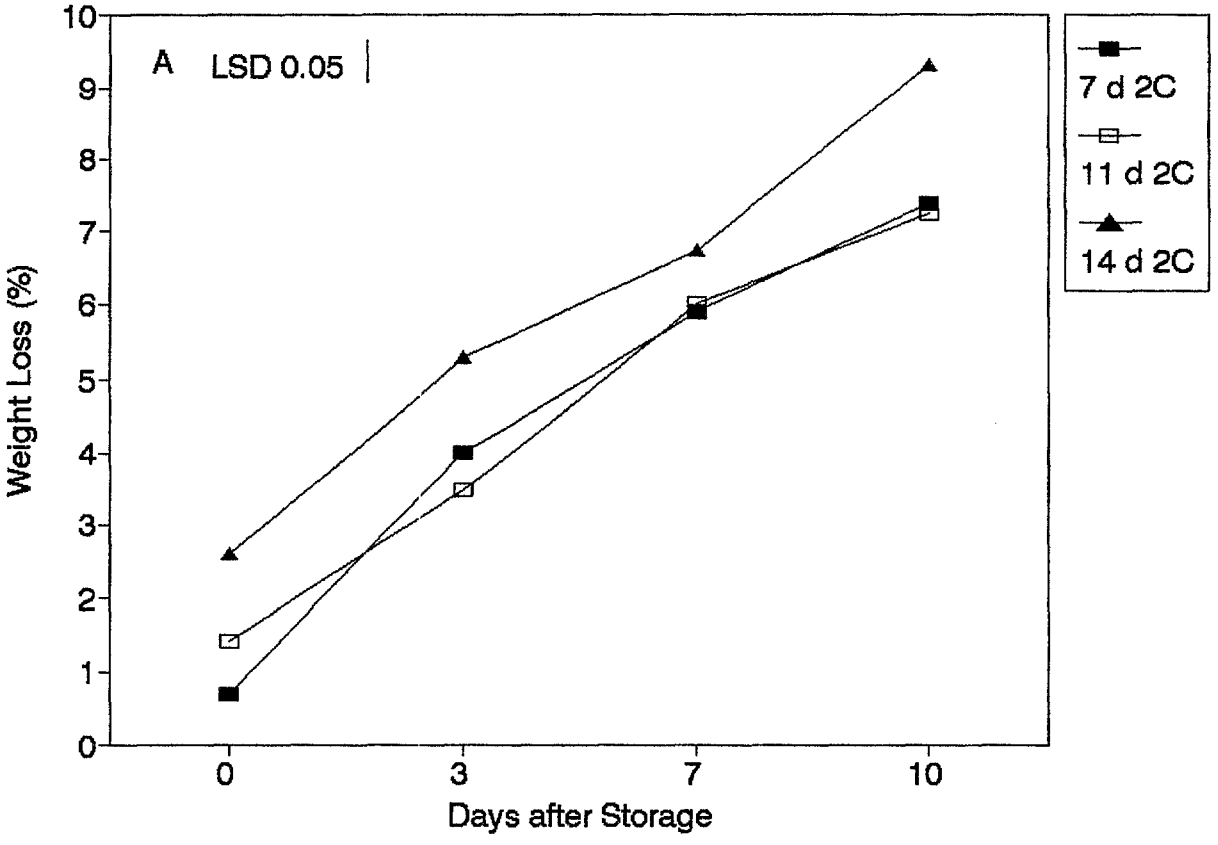
Table 7. Color development, CI, rots, firmness, and weight loss of tomatoes heated at 38, 42 or 46°C for various times before 2°C storage for 3 weeks. Measurements were made after 10 days at 20°C following removal from storage.

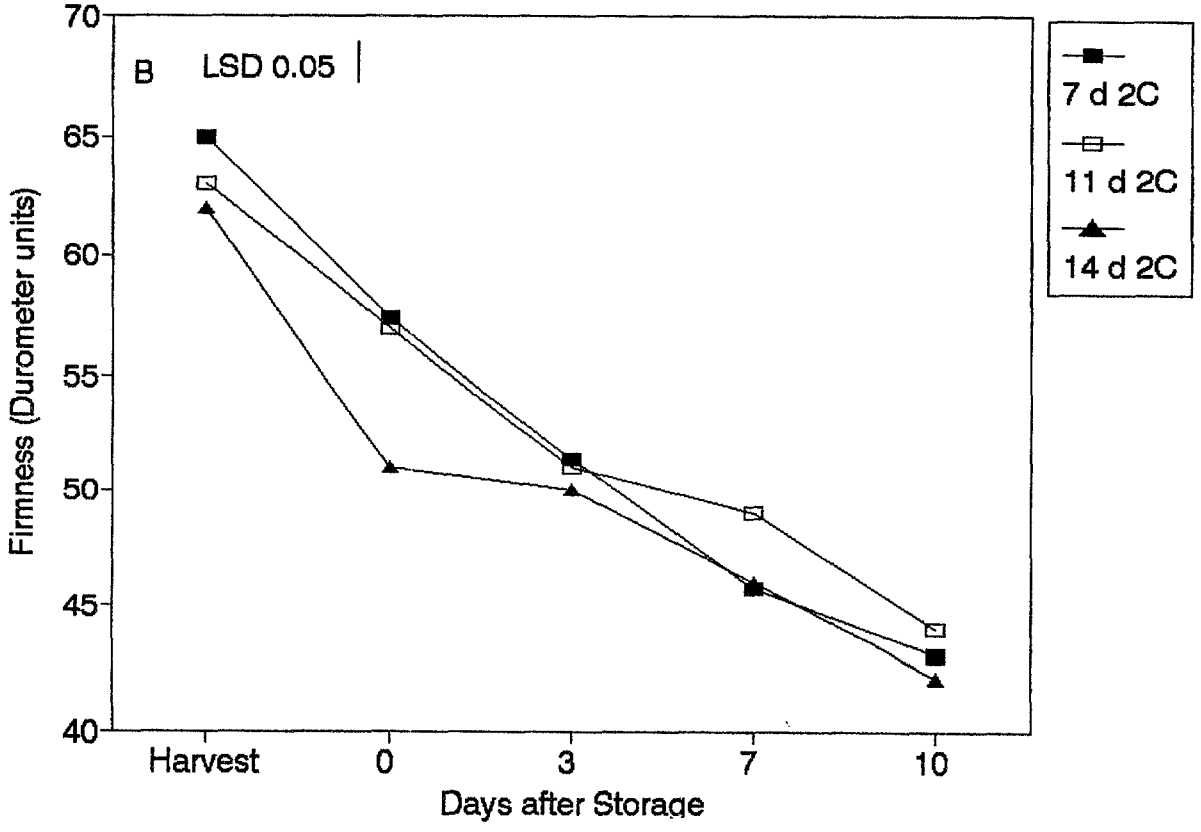
Treatment	Color ('a' value)	CI (%)	Rots (%)	Firmness (Durometer units)	Weight Loss (%)
Control	9.6bc	50a	45a	33.6c	7.4bc
2 d 38	17.8a	0d	20b	37.7b	8.0b
6 h 42	11.2b	10d	10b	38.8b	6.0c
12 h 42	15.0a	20c	20b	42.6a	7.3bc
24 h 42	7.8c	35b	20b	47.0a	8.9b
6 h 46	18.5a	50a	30ab	44.0a	8.5b
12 h 46	10.1b	50a	55a	45.1a	11.0a
24 h 46	10.7b	50a	40a	46.2a	11.6a

Different letters in the same column denote significant difference at P = 0.05

Figure Legend

Figure 1. Weight loss and decrease in firmness of tomatoes held at 20°C after 7, 11 or 14 days storage at 2°C.


A
LSD 0.05
Weight Loss (%)
0
1
2
3
4
5
6
7
8
9
10
0
3
7
10
Days after Storage
7 d 2C
11 d 2C
14 d 2C


B
LSD 0.05
Firmness (Durometer units)
40
45
50
55
60
65
70
Harvest
0
3
7
10
Days after Storage
7 d 2C
11 d 2C
14 d 2C

AKA A/S - 1. korr - bia - 02-01-97 - 60495.fm - Physiologia Plantarum - 96:344

PHYSIOLOGIA PLANTARUM 99: xxx–xxx. 1997
Printed in Denmark – all rights reserved

Copyright © Physiologia Plantarum 1997
ISSN 0031-9317

Heat treatments to decrease chilling injury in tomato fruit. Effects on lipids, pericarp lesions and fungal growth

Susan Lurie, Mostafa Laamim, Zoria Lapsker and Elazar Fallik

Lurie, S., Laamim, M., Lapsker, Z. and Fallik, E. 1997. Heat treatments to decrease chilling injury in tomato fruit. Effects on lipids, pericarp lesions and fungal growth. – Physiol. Plant. 99: xxx–xxx.

Tomato fruits are sensitive to low temperature and develop chilling injury, while at nonchilling temperatures they ripen rapidly. Previously, a 6° air treatment was found to reduce the sensitivity of the fruit to low temperatures. In the present study hot air was compared to hot water and their effects on reducing chilling injury and fungal decay were investigated. Tomatoes (Lycopersicon esculentum cv. Daniella) at the breaker stage were subjected to hot air, 48 h at 38°C, or various hot water dips, 30 min at 40°C or 2 min at 46, 48 or 50°C, before holding at 2°C. The unheated tomatoes developed chilling injury and fungal infections at 2°C, but not at 12°C. All the heat treatments reduced chilling injury and decay in tomatoes held for 3 weeks at 2°C. The outer pericarp tissue of heated tomatoes had higher phospholipid and lower sterol contents than unheated tomatoes. Heated tomatoes also had less saturated fatty acids than unheated tomatoes held at 2°C, but not at 12°C. Scanning electron micrograph observations showed that all the fruits had microcracks in their surface, but the unheated chilled tomatoes had also fungal growth in the cracks, while those of the heated tomato fruit did not. In the areas of chilling injury collapsed cells were present under the peel and could also support pathogen development. It is suggested that the heat treatment institutes a response to high temperature stress in the fruit tissue that leads to strengthened membranes. This prevents the loss of function and cell collapse which was found in the chilling-injured areas of affected fruit.

Key words – Chilling injury, heat shock, lipids, Lycopersicon esculentum, tomato.

S. Lurie (corresponding author, e-mail vfrst@volcani.agri.gov.il), Z. Lapsker and E. Fallik, Dept of Postharvest Science, Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel; M. Laamim, Inst. National de la Recherche, B. P. 124, Inezgane, Morocco.

Introduction

Tomato fruits are susceptible to chilling injury when exposed to temperatures below 10°C for a week or more (Hobson 1981). Two main consequences of chilling injury are the failure to ripen properly and increased susceptibility to decay (Autio and Bramlage 1986, Cheng and Shewfelt 1988). In addition, lesions on the peel can appear that may develop into necrotic areas (Lurie and Klein 1991). Dysfunction of one or more cell membranes (loss of semipermeability or enzyme activity) at chilling temperatures is thought to be the primary event that ultimately leads to injury (Lyons 1973). Tomato fruits are particularly susceptible to chilling injury at the mature green and breaker stages of development (King and Ludford 1983), and partial ripening of tomatoes and other fruits of subtropical or tropical origin reduces their sensitivity to chilling temperatures (Autio and Bramlage 1986, Lipton 1978). Since ripening involves changes in the lipid components of the membranes (Lurie and Ben Arie 1983), this may be one reason riper fruit are less susceptible to low temperature injury.

Ultrastructural studies of tomato fruit pericarp have shown that the internal lamellae of chloroplasts become disorganized after 10 days of chilling (Maragoni et al. 1989, Moline 1976). The tonoplast membrane is also affected (Maragoni and Stanley 1989, Maragoni et al. 1989). However, the fruit surface where the lesions occur has not been examined.

A high temperature stress before low temperature storage has been found to prevent chilling injury in tomatoes (Lurie and Klein 1991). The treatment used was

Received 26 August, 1996; revised 28 November, 1996

AKA A/S - 1. korr - bia - 02-01-97 - 60495.fm - Physiologia Plantarum - 96:344

a dry heat for two or three days. This treatment led to the accumulation of heat shock proteins and to membrane changes which affected tissue electrolyte leakage (Lurie and Klein 1991, Sabehat et al. 1996). However, changes in lipid components of the tissue were not investigated.

Other heat treatments, such as hot water dips, have recently been shown to decrease chilling injury on citrus fruits (Rogov et al. 1995). The present study compares various methods of heat treatment of tomato fruit, and how these affect fruit lipids, chilling injury and tissue cell structure.

Materials and methods

Plant material

Breaker stage tomatoes (*Lycopersicon esculentum* cv. Daniella, also known as Rehovot 144) were harvested from plants growing in a greenhouse. The fruits were randomly divided into replicates of five, and five replicates (25 fruit) were used for each treatment. For dry heat treatments the fruits were held in a tray covered with a polyethylene bag with damp filter paper on the sides of the tray. The tray was put in a thermostated heating chamber for 48 h at 38°C, and then transferred to storage at 2°C. For hot water treatment, the tomatoes were placed in a mesh bag and submerged in a jacketed water bath with a circulating heater for the indicated times and temperatures, then allowed to air dry and placed at 2°C. Unheated control fruits were either placed immediately at 2 or at 12°C. After three weeks the fruits were removed to 20°C and monitored either immediately or after 5 days. Chilling injury, measured visually as surface lesions on the fruit, and decay were determined after 5 days at 20°C.

Lipid extraction and analysis

At removal from 2 or 12°C, outer pericarp tissue was sampled for measurement of lipids. Samples from each treatment were bulked and frozen until lipid extraction. Five g of frozen tissue were ground in a blender in 100 ml of chloroform:methanol (8:5, v/v) according to Renkonen et al. (1963). The mixture was placed in a separatory funnel and allowed to settle for 5 min. An additional 50 ml of the chloroform:methanol were added and the funnel shaken for 2 min. Then 20 ml of 0.8% (w/v) NaCl were added and the mixture allowed to sit for 5 min. The chloroform phase was collected, dried in a rotovaporator, redissolved in 1 ml hexane, and stored under N_2 at −20°C.

Aliquots were taken from the hexane extract for determination of total phospholipid and sterol content. The content of phospholipids was determined by phosphorus analysis after conversion to phosphoric acid with 70% perchloric acid at 190°C for 60 min by the method of Bottcher et al. (1961). Total sterol in the lipid extract was determined colorimetrically by the method of Chiamori and Henry (1959), using stigmasterol as a standard.

A third aliquot of the total lipid fraction was prepared for fatty acid analysis, by methylation in 4 ml of 5% (v/v) H_2SO_4 in methanol held for 80°C for 1 h. The fatty acid methyl esters were partitioned into hexane and analyzed on a gas chromatograph with a flame ionization detector (FID). The column used was a 1.8 m packed column with GP10%SP-2330 on 100/120 Chromosorb W (Supelco, Belefonte, PA, USA). Reference methylated fatty acids were run for identification. Each assay was run in triplicate, and lipid extraction from tissue from each treatment was performed twice.

Scanning electron micrography (SEM)

After 5 days at 20°C, five fruits from each treatment had thin sections of tissue excised and placed on a flat specimen holder peel side up. The tissue was freeze dried and then coated with gold:palladium alloy. Samples were examined in a JEOL GSM-T300A scanning electron microscope. Triplicate samples from each fruit were examined at levels of magnification ranging from 35 to 3500×.

Statistical Analysis

Analysis of variance was performed on the results. Based on significant *F*-test, means were separated using Duncan's multiple range test.

Results and discussion

Tomato fruits, which were held for 3 weeks at 2°C, developed high levels of chilling injury and decay upon rewarming, while fruits held at 12°C did not (Tab. 1). All of the prechilling heat treatments decreased, but did not eliminate chilling injury and decay. The decrease in decay level was at least partially due to the heated fruit developing rot as a result of infection by *Alternaria alternata* exclusively, while the unheated 2°C fruits were infected by both *Alternaria alternata* and *Botrytis cinerea* (data not shown). It has been shown that *B. cinerea* is sensitive to high temperature and can be controlled more readily by a heat treatment than *A. alternara* (Barkai Golan and Phillips 1991). Both dry heat as well as a hot wa-

Tab. 1. Percentage fruits exhibiting chilling injury or decay after 3 weeks of holding at 2 or 12°C and 5 days at 20°C. Numbers in the same column with different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test.

Treatment	Chilling Injury (%)	Decay (%)
2°C control	80a	100a
38°C air 48 h	24b	20b
40°C water 30 min	20b	30b
46°C water 2 min	14bc	25b
48°C water 2 min	3c	25b
50°C water 2 min	10bc	30b
12°C control	0c	0c

AKA A/S - 1. korr - bia - 02-01-97 - 60495.fm - Physiologia Plantarum - 96:344

ter dip were effective in eliminating *Botrytis* (Elad and Volpin 1991, Fallik et al. 1993). *A. alternata* is a less virulent pathogen and develops more slowly on fruits than *Botrytis*, but is more resistant to high temperatures.

Both a dry heat treatment at 38°C for 48 h, and various regimes of hot water dips significantly decreased the extent of chilling injury on the tomato fruit (Tab. 1). The best preventive treatment was 2 min at 48°C. Previously, we found that dry heat could prevent chilling injury and decrease rot (Lurie and Klein 1991, Fallik et al. 1993), but the much shorter times used in hot water dips had not been investigated. However, Rogov et al. (1995) had shown that a 2- to 3-min dip at 53°C could decrease chilling injury in citrus. The symptoms of local necrotic areas in the citrus peel bear some resemblance to the affected areas in the outer pericarp of tomato fruit.

McDonald et al. (1996) compared a water dip for 60 min at 42°C to air at 38°C for 48 h on inducing chilling tolerance on mature green tomatoes when stored at 2°C. They found that fruit treated by either heating regime ripened normally, while control fruit decayed before ripening.

The phospholipid content was higher and sterol content lower in heated fruit, irrespective of heating regime, than in unheated tomato fruit held at either 2 or 12°C (Tab. 2). Consequently, the sterol to phospholipid ratio was lower in the heated than in the unheated fruit. The method employed to measure sterols will quantify all steryl lipids including esters which are generally excluded from cell membranes. However, these results are similar to those found in plasma membrane lipids of heated apple fruit, although after a longer time at low temperature (Lurie et al. 1995). In the latter case, it appeared that phospholipid synthesis at low temperature was greater in heated than in unheated fruit. An increase in phospholipids in cellular membranes is one of the adaptive changes occurring in plants acclimating to low temperatues (O'Neill et al. 1981, Yoshida 1984).

The higher sterol content of unheated fruit may reflect increases due to either ripening (at 12°C) or response to low temperature (at 2°C). During ripening there is an overall decline in phospholipids (Nguyen and Mazliak 1990, Whitaker 1991, 1994). In addition, there is a substantial increase in sterol synthesis accompanied by marked changes in sterol composition and conjugation (Whitaker 1988, 1994). At low temperatures, phospholipids have been found to decrease and sterol concentrations to increase, although this was not accompanied by fruit ripening (Whitaker 1993).

Tab. 2. Contents of phospholipids and sterols in the outer pericarp of tomato fruits after 3 weeks at 2 or 12°C. Numbers in the same column with different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test. sterol

Treatment	Phospholipid nmol g^{-1} FW	Sterol	Sterol/phospholipid
2°C control	350b	140a	0.40
38°C air 48 h	450a	123b	0.27
40°C water 30 min	456a	125b	0.27
46°C water 2 min	430a	129b	0.30
48°C water 2 min	440a	125b	0.28
50°C water 2 min	427a	131b	0.31
12°C control	390ab	138a	0.35

Tab. 3. ~~Fatty acid composition of total lipids from the outer pericarp of tomatoes held 3 weeks at 12°C (A), and~~ The saturation index of fatty acids from tomatoes held 3 weeks at 2 or 12°C (B). Numbers in the same column with different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test. Saturation index is (16:0+18:0)/(18:1 + 18:2 + 18:3).

Treatment	Saturation index
2°C control	0.77a
38°C air 48 h	0.38c
40°C water 30 min	0.41c
46°C water 2 min	0.52b
48°C water 2 min	0.43c
50°C water 2 min	0.52b
12°C control	0.57b

Two fatty acids, palmitic (16:0) and linoleic (18:2), make up over three fourths of the total fatty acids in the tomato fruits. Representative values of percent content of the fatty acids from control fruits are palmitic (16:0) 32%, stearic (18:0) 5%, oleic (18:1) 8%, linoleic (18:2) 47% and linolenic (18:3) 9%. The fruits which were heated before being held at 2°C had increased proportions of linoleic and linolenic acid in their fatty acids, and, therefore, had a lower saturation index than unheated tomatoes held at 2°C (Tab. 3). In the latter case, a drop in the percentage of linoleic acid resulted in an increase in the saturation index compared to the 12°C control. Lipids from tomatoes dipped for 2 min at 46 or 50°C were similar in their saturation index to those from unheated tomatoes held at 12°C, while the dry heat at 38°C and hot water at both 40°C for 30 min and 46°C for 2 min led to a decrease in palmitic and increases in linoleic and linolenic acids, yielding a low saturation index.

The heated fruits should have more fluid membranes due to both a higher proportion of unsaturated fatty acids in their lipid components (Tab. 3), and also a lower sterol to phospholipid ratio (Tab. 1). An increase in the latter ratio occurs with senescence and ripening and is associated with a loss of membrane function (Lees and Thompson 1980, Thompson et al. 1982, Lurie and Ben Arie 1983). Conversely, a lower ratio generally reflects membranes with better selective permeability and greater responsivness to environmental stress (Raison et al. 1980). During adaptation of apple fruit to low temperature it was found that first the fatty acid saturation and then the sterol to phospholipid ratio decreased due to increases in phospholipid (Lurie and Ben Arie 1987). Similar adaptive changes may be occurring in the heated tomato fruit allowing them to withstand low temperatures, while the unheated fruits are unable to adapt and therefore develop chilling injury.

AKA A/S - 1. korr - bia - 02-01-97 - 60495.fm - Physiologia Plantarum - 96:344

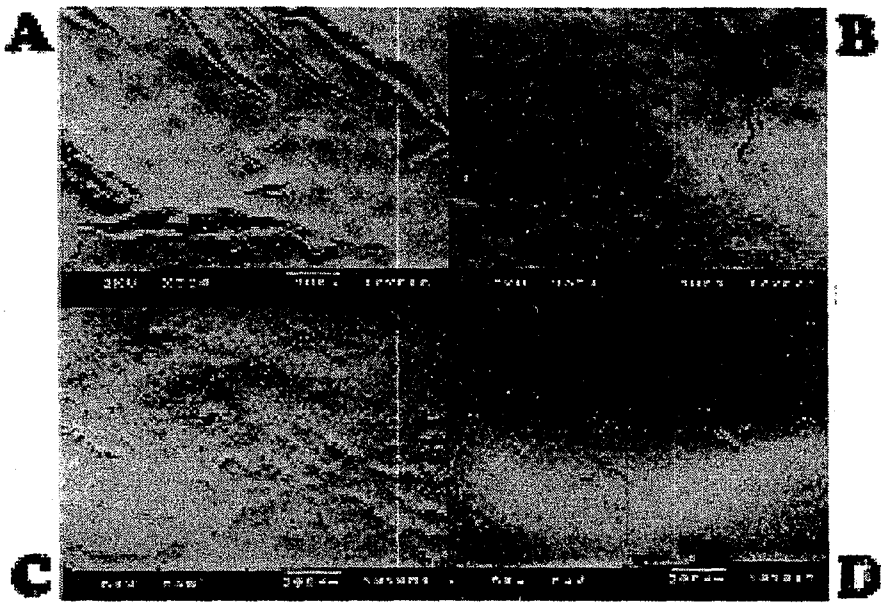


Fig. 1. Cracks on the surface of tomato fruits after 3 weeks at 2 or 12°C and 5 days at 20°C. A. Unheated 2°C fruit. B. Unheated 12°C fruit. C. Fruit heated for 48 h at 38°C. D. Fruit heated for 2 min at 48°C.

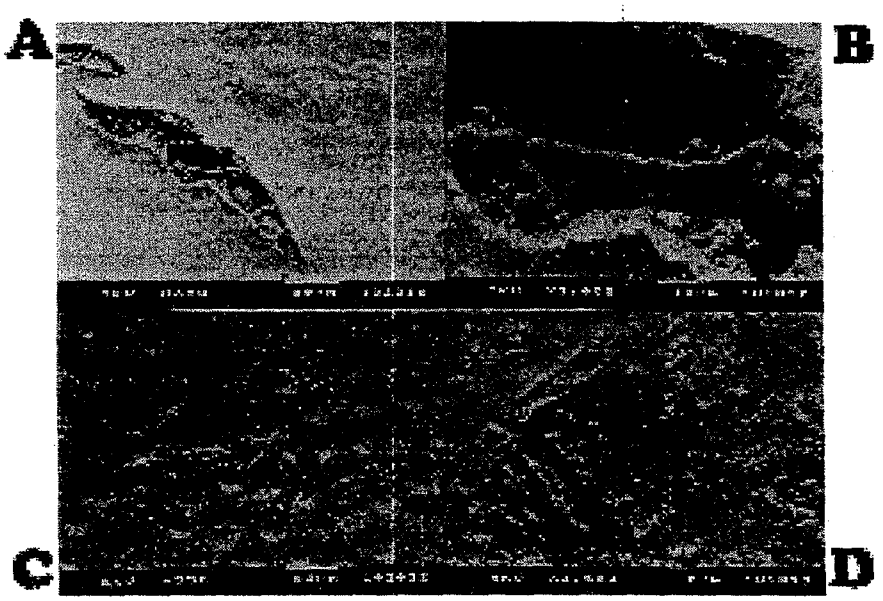


Fig. 2. Cracks on unheated fruit at 2°C (A,B) showing the development of fungal hyphae; (C, D) fruit heated for 30 min at 40°C ~~and~~ where cracks can be seen to be partially filled.

An examination of the tomato fruits by electron microscopy showed that all the fruits had surface cracks (Fig. 1). Cohen et al. (1994) had shown that surface cracks developed in chilled citrus fruit and that weight loss was greater in those fruits which developed chilling injury. Weight measurements of the tomato fruits did not show increased weight loss in the unheated fruit held at 2°C compared with heated fruit (data not shown). However, since all the fruits had cracks this is not surprising. In the unheated chilled fruit, the cracks were an environment for the development of fungal spores (Fig. 2A,B), while the cracks in the heated fruit were clean, and in some cases appeared to be filled by surface wax (Fig. 2C,D). In the hot water-dipped fruits, ungerminated fungal spores could be found on the fruit surface, and a cross section of the tissue showed cells of normal shape (Fig. 3A,B).

In addition to surface cracks, the sunken areas affected by chilling injury were examined. These areas in cross section showed collapsed cells under the epidermal cell layer (Fig. 4A), and sometimes also within the epidermal layer (Fig. 4B). These collapsed areas supported fungal growth (Fig. 4C), which could also be seen erupting through the epidermal surface (Fig. 4D).

AKA A/S - 1. korr - bia - 02-01-97 - 60495.fm - Physiologia Plantarum - 96:344

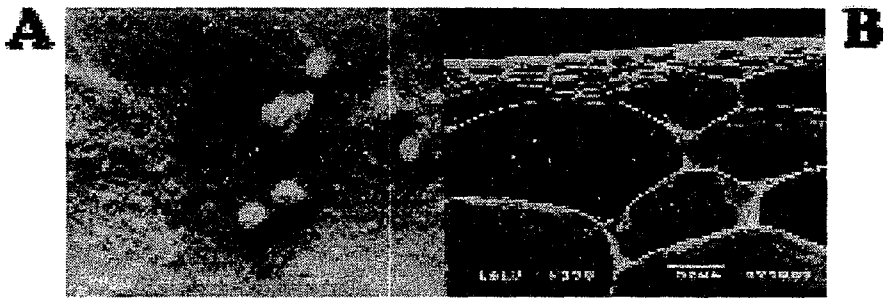


Fig. 3. A. Ungerminated spores on the surface of heated fruit. B. Cross-section of outer fruit tissue showing healthy cells.

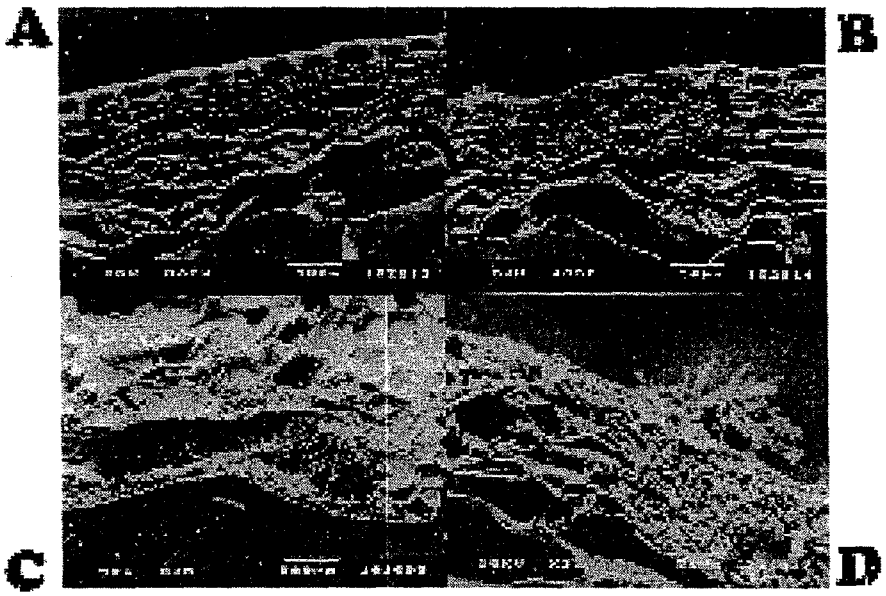


Fig. 4. Cross-sections of tomato fruit tissue showing chilling injury. A. The outer layer of cells is undamaged but layers beneath have collapsed. B. The injury affects all the cell layers. C. Under the epidermis cells have collapsed and fungus is developing. D. Fungal hyphae have grown through to the surface and sporulation is occurring.

In summary, a number of heat treatments were able to reduce chilling injury in tomatoes at the breaker stage. These treatments led to an increase in phospholipid content, a lower sterol to phospholipid ratio, and more unsaturated fatty acids, relative to the unheated fruits. These differences in lipid composition may have strengthened the membranes and prevented loss of membrane function and cellular collapse seen in the areas of fruit pericarp affected by chilling injury. The responses in the heated fruit may be due to tissue response to high temperature stress which also protects against low temperatures. The unheated fruit, not having been stimulated by a heat stress, failed to adapt to low temperature, and developed chilling injury.

Acknowledgments – This research was partially funded by the US Cooperative Development Foundation and the Minstry for Economic Cooperation of Germany. M. Laamim was supported by a fellowship from the Dept of International Cooperation of the Ministry of Foreign Affairs in Israel. This is a contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel No. 1952-E, 1996.

References

Autio, W. R. & Bramlage, W. J. 1986. Chilling sensitivity of tomato fruit in relation to ripening and senescence. – J. Am. Soc. Hortic. Sci. 111: 201–204.

Barkai-Golan, R. & Phillips, D. J. 1991. Postharvest heat treatment of fresh fruits and vegetables for decay control. – Plant Dis. 75: 1085–1089.

Bottcher, G., Van Gent, C. & Pries, C. 1961. A rapid and sensitive sub micro phosphorus determination. – Anal. Chim. Acta 24: 203–204.

Cheng, T. S. & Shewfelt, R. L. 1988. Effect of chilling exposure of tomatoes during subsequent ripening. – J. Food Sci. 53: 1160–1162.

Chiamori, B. S. & Henry, R. J. 1959. Study of the ferric method for determination of total cholesterol and cholesterol esters. – Am. J. Clin. Pathol. 31: 305–309.

Cohen, E., Shapiro, B., Shalom, Y. & Klein, J. D. 1994. Water loss: A nondestructive indicator of enhanced cell membrane permeability of chilling injury in citrus fruit. – J. Am. Soc. Hortic. Sci. 119: 983–986.

Elad, Y. O & Volpin, H. 1991. Heat treatment for the control of rose and carnation grey mould (*Botrytis cinerea*). – Plant Pathol. 40: 278–286.

Fallik, E., Klein, J. D., Grinberg, S., Leomniec, E. & Lurie, S. 1993. Postharvest heat treatment of tomatoes: Effect on *Botrytis cinerea* and fruit ripening. – Plant Dis. 77: 985–988.

AKA A/S - 1. korr - bia - 02-01-97 - 60495.fm - Physiologia Plantarum - 96:344

Hobson, G. 1981. The short term storage of tomato fruit. – J. Hortic. Sci. 56: 363–368.

King, M. M. & Ludford, P. M. 1983. Chilling injury and electrolyte leakage in fruit of different tomato cultivars. – J. Am. Soc. Hortic. Sci. 108: 74–77.

Lees, G. L. & Thompson, J. E. 1980. Lipid composition and molecular organization in plasma membrane enriched fractions from senescing cotyledons. – Physiol. Plant. 69: 859–863.

Lipton, W. J. 1978. Chilling injury of 'Honey Dew' muskmelons: Symptoms and relation to degree of ripeness at harvest. – HortScience 13: 45–46.

Lurie, S. & Ben Arie, R. 1983. Microsomal membrane changes during ripening of apple fruit. – Plant Physiol. 73: 636–638.

– & Ben Arie, R. 1987. Microsomal membrane changes of apples during storage. – Sci. Hortic. 32: 73–83.

– & Klein, J. D. 1991. Acquisition of low temperature tolerance in tomatoes by exposure to high temperature stress. – J. Am. Soc. Hortic. Sci. 116: 1007–1012.

– , Othman, S. & Borochov, A. 1995. Effect of heat treatment on plasma membrane of apple fruit. – Postharv. Biol. Technol. 5: 29–38.

Lyons, J. M. 1973. Chilling injury in plants. – Annu. Rev. Plant Physiol. 24: 245–261.

Maragoni, A. G. & Stanley, D. W. 1989. Phase transitions in microsomal membranes from chilling sensitive and chilling resistant tomato plants and fruit. – Phytochemistry 28: 2293–2301.

– , Smith, A. K., Yada, R. Y. & Stanley, D. W. 1989. Ultrastructural changes associated with chilling injury in mature green tomato fruit. – J. Am. Soc. Hortic. Sci. 114: 958–962.

McDonald, R. E., McCollum, T. G. & Baldwin, E. A. 1996. Prestorage heat treatments influence free sterols and flavor volatiles of tomatoes stored at chilling temperature. – J. Am. Soc. Hortic. Sci. 121: 531–536.

Moline, H. E. 1976. Ultrastructural changes associated with chilling of tomato fruit. – Phytopathology 66: 617–624.

Nguyen, X. V. & Mazliak, P. 1990. Chilling injury induction is accompanied by galactolipid degradation in tomato pericarp. – Plant Physiol. Biochem. 28: 283–291.

O'Neill, S., Priestley, D. & Chabot, B. 1981. Temperature and aging effects on leaf membranes of a cold hardy perennial *Fragaria virgeiniana*. – Plant Physiol. 68: 1409–1415.

Raison, J., Berry, J., Armond, P. & Pike, C. 1980. Membrane properties in relation to the adaptation of plants to high and low temperature. – *In* Adaptation of Plants to Water and High Temperature Stress (W. C. Truner and P. J. Kramer, eds), pp. 261–273. Wiley-Interscience, New York, NY. ISBN 0 85198 930 6

Renkonen, O., Kosunen, T. & Renkonen, D. 1963. Extractions of serum inositides and other phosphatides. – Ann. Med. Exp. Biol. Fenn. 41: 269–273.

Rogov, V., Ben Yehoshua, S., Albagli, R. & Fang, D. Q. 1995. Reducing chilling injury and decay of stored citrus fruit by hot water dips. – Postharv. Biol. Technol. 5: 119–127.

Sabehat, A., Weiss, D. & Lurie, S. 1996. The correlation between heat shock protein accumulation and persistence and chilling tolerance in tomato fruit. – Plant Physiol. 110: 536–541.

Thompson, J. E., Mayak, S., Shinitzky, M. & Halevy, A. H. 1982. Acceleration of membrane senescence in cut carnation flowers by treatment with ethylene. – Plant Physiol. 69: 859–863.

Whitaker, B. D. 1988. Changes in the steryl lipid content and composition of tomato fruit during ripening. – Phytochemistry 27: 3411–3416.

– 1991. Changes in lipids of tomato fruit stored at chilling and non-chilling temperatures. – Phytochemistry 30: 757–761.

– 1993. Lipid changes in microsomes and crude plastid fractions during storage of tomato fruits at chilling and nonchilling temperatures. – Phytochemistry 32: 265–271.

– 1994. Lipid changes in mature-green tomatoes during ripening, during chilling, and after rewarming subsequent to chilling. – J. Am. Soc. Hortic. Sci. 199: 994–999.

Yoshida, S. 1984. Chemical and biophysical changes in the plasma membrane during cold acclimation of mulbery bark cells. – Plant Physiol. 76: 257–265.

Edited by C. H. Bornman

THE EFFECT OF HIGH TEMPERATURE ON MAINTAINING QUALITY OF HARVESTED TOMATOES

S. Lurie, A. Handros, A. Sabahat, J.D. Klein, E. Fallik and R. Shapiro[1]
Deptartment of Postharvest Science, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel, and (1) Department of Biochemistry, The Faculty of Agriculture, The Hebrew University, Rehovot, 76100, Israel

Additional Index Words: *Botrytis cinerea, Lycopersicum esculentum*, heat stress, ripening, pathogen decay, chilling injury.

Abstract: **Mature green tomatoes, heated for 3 days at 38°C, ripened more slowly than unheated tomatoes once removed to 20°C. Their color development and softening were delayed, although ethylene production was enhanced. Lower activity and protein content of polygalacturonase in heated tomatoes correlated with slower softening. After storage at 2°C for three weeks, heated tomatoes ripened normally while unheated tomatoes developed chilling injury. Normal ripening was accompanied by increased mRNA levels for polygalacturonase and phytoene synthase in heated tomatoes compared to unheated. Chilling injury in unheated tomatoes was accompanied by loss of membrane phospholipid and increase in ion leakage. The unheated tomatoes also developed more rots than heated tomatoes. The germination and growth of *Botrytis cinerea* was found to be inhibited by the heat treatment.**

An ability to modify metabolism and development in response to environmental changes is essential to nonmobile organisms such as plants. Temperature extremes are an example of environmental stress, and plant tissues show altered gene expression in response to either high or low temperature (Guy, 1990; Sachs and Ho, 1986). In some cases this results in a dramatic change in protein synthesis, with stress proteins accumulating and normal proteins not being synthesized (Matters and Scandalios, 1986). Several studies suggest that these stress proteins, such as heat shock proteins, protect cells from deleterious effects of extreme temperatures, by enhancing the range of thermotolerance (Vierling, 1992).

Harvested fruits differ from most tissue used to investigate stress situations in that they are no longer growing, and as such have more limited means at their disposal to respond to stress situations. Nonetheless, fruits are often exposed to stress in the form of low temperature storage. These conditions can lead to tissue damage or chilling injury. If the fruit is climacteric and not stored at a low temperature, ripening will continue during storage. Therefore, a frequent compromise is often to hold fruit at a temperature which slows ripening without causing storage disorders, which in turn limits the possible period of storage. In an attempt to increase the effective storage life of tomatoes, which are subject to chilling injury, we have tried heat stress treatments (Lurie and Klein, 1991). The results show that this stress can a) reversibly inhibit fruit ripening, b) prevent the development of chilling injury, c) reduce pathogen infection.

Materials and Methods

Treatment of Fruit

Mature green tomatoes (*Lycopersicum esculentum* Mill., cv F144) were picked directly from the greenhouse, and divided into three lots. One lot of fruit was placed at 20°C, a second lot was placed immediately at 2°C, while a third lot was heated at 38°C for 3 days and then transferred to 2°C. Heating was in a thermostatically controlled chamber with the fruit in plastic trays inside nonsealed plastic bags to retard water loss. At the end of 3 days of heating, half of the fruit was placed at 20°C, and the other half at 2°C.

A. Ait-Oubahou and M. El-Otmani (eds.)
Postharvest Physiology, Pathology and Technologies for Horticultural Commodities : Recent Advances, 234-240
© 1995 Institut Agronomique & Vétérinaire Hassan II, Agadir, Morocco.

After 3 weeks at 2°C, fruits were removed to 20°C and monitored for color development, softening, chilling injury symptoms and rot development. Color development was monitored objectively as the "a" value on a Minolta Colorometer, as well as subjectively by determining the percentage of red fruit. Firmness was measured with a Durometer on two sides of the fruit. Twenty fruit were measured from each treatment. Chilling injury was monitored visually.

Ethylene was monitored by placing two fruits in 1 liter jars and closing them for 1 h each day. Four replicate pairs of fruit from each treatment were assayed. Gas samples were withdrawn with a syringe through a septum in the jar lid. Ethylene was measured on a gas chromatograph using a flame ionization detector with an alumina column kept at 80°C with N as the carrier gas.

Nucleic acid isolation and dot blots

Total RNA was extracted following the method of DellaPenna et al. (1986). Twenty g of frozen pericarp tissue were ground to a powder in a mortar and pestle and homogenized in 40 ml of lysis buffer (5M guanadine thiocyanate, 10 mM EDTA, 300 mM Tris-HCl, pH 7.6, 8% β-mercaptethanol). Following centrifugation at 3000g for 10 min, the supernatant was filtered through Miracloth and precipitated overnight at 4°C in 7 vol of 4 M LiCl. The sample was centrifuged at 8,000g for 90 min, washed with 3 M LiCl and resuspended.

The sample was extracted twice with phenol:chlorophorm and once with chloroform. Total RNA was precipitated with 2.2 vol ethanol and 0.1 vol of 3 M sodium acetate, pH 5.5 at -20°C. After precipitation and resuspension in DEPC-treated water the RNA was used for dot blot analysis. Ten μg total RNA per dot were blotted onto nylon membranes and probed with 1 x 10^7 cpm of labeled cDNA inserts as described by Sambrook et al. (1989).

Protein preparation and immunoblotting

Two g of frozen pericarp tissue were ground in 10 ml of 0.1 M Tris-HCl, pH 7.2, 5% sucrose, 4% SDS, 5% β-mercaptoethanol and 2 mM PMSF in a mortar and pestle. The slurry was incubated 10 min at 4°C, filtered through cheesecloth and an equal volume of water saturated phenol added. This mixture was shaken, centrifuged and the phenol phase reextracted with fresh extraction buffer. Proteins were precipitated by adding 5 vol of 0.1 M ammonium acetate in methanol and leaving at -20°C overnight. The mixture was then centrifuged, washed 3 times with ammonium acetate in methanol, once with acetone, dried under nitrogen and resuspended in Laemmli's buffer (Laemmli, 1970).

Electrophoresis of proteins was carried out in denaturing gels (12%) as described by Laemmli (1970). Protein samples (20 μg/lane) were loaded onto a Protean II mini-gel (BioRad, Hercules, CA) and electrophoresed 1 h at 150 V constant voltage. Protein was electroblotted onto nitrocellulose membrane for 2 h at 350 V. The nitrocellulose filter was probed with anti-tomato polygalacturonase antiserum (DellaPenna et al. 1986).

Fruit Inoculation

Both mature green and pink tomatoes were harvested and divided into two lots, inoculated and control. Using a 25 gauge needle, fruits were inoculated with 100 μl of a $5x10^4$ spores/ml suspension of *Botrytis cinerea* 3 mm into the pericarp. Control fruits were injected similarly with 100 μl sterile water. Half of the inoculated and control fruit was placed at 20°C and half held at 38°C. Ten fruit from each treatment were removed for the heat chamber every 24 h and placed at 20°C for 12 days in order to allow decay development. At the end of shelf life disease severity was recorded subjectively on a scale of 0-5 where 0=no decay development, 1=decay up to 0.5 cm diam. without sporulation, 2=decay between 0.5-1.0 cm diam. with sporulation, 3=decay between 1.0-2.5 cm diam. with sporulation, 4=decay between 2.5-4.0 cm diam. with sporulation and mycelium, and 5=fruit heavily covered with mycelium

Results and Discussion

Effects of heat treatment on fruit ripening

When mature green tomatoes were heated and then placed at 20°C for ripening, the rate of fruit color development and softening was slower than that of unheated fruit (Figs 1 and 2). The color of both lots of fruit eventually reached full red, but heated fruit took 10 to 14 days to achieve this, while most of the unheated fruit was red after a week at 20°C. In addition, the heat treatment appeared to synchronize color development so that all the fruits were at a similar stage of color development. In contrast, the unheated fruits showed greater variation, as seen by the distribution of color among the unheated fruit after one week compared to heated fruit (Table 1). This feature of the heat treatment is of importance for countries such as the United States where tomatoes are harvested mature green and given an ethylene treatment to promote ripening. Often the fruits which do not respond are given a second treatment with ethylene. A heat treatment may eliminate the need for the ethylene treatment.

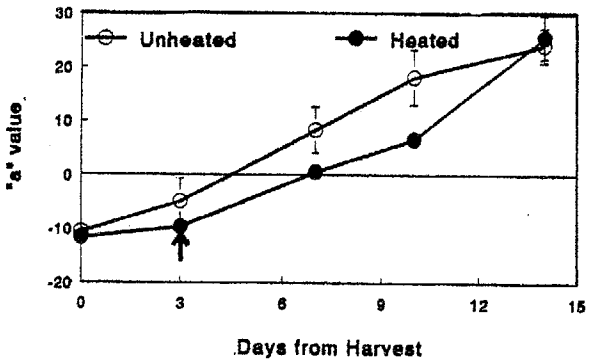


Fig. 1. Change in fruit color of unheated and heated fruits from green to red during holding at 20°C. Arrow indicates when heated fruits were transferred to 20°C. Standard deviation is indicated.

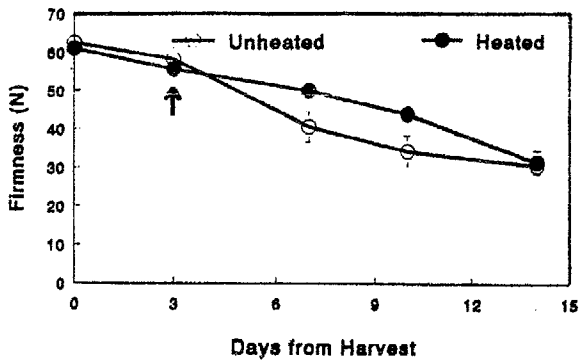


Fig. 2. Change in fruit firmness of unheated and heated fruits during holding at 20°C. Arrow indicates when heated fruits were transferred to 20°C. Standard deviation is indicated.

Table 1. Percentage of green, pink and red fruits in unheated or heated tomatoes after one and two wee of holding at 20°C.

Holding period at 20°C	Treatment	Percentage of Fruits		
		Green	Pink	Red
7 Days	Unheated	20	30	50
	Heated	0	70	30
14 Days	Unheated	5	15	80
	Heated	0	0	100

At the end of the heat treatment the fruits were softer than fruits held at 20°C (Fig. 2). Howe during the ripening period they softened more slowly and were firmer at the end of the ripening per This is reflected in the presence of polygalacturonase, one of the cell wall degrading enzymes, in he and unheated fruit (Fig. 3). At harvest the enzyme was not apparent, but during the ripening peric increased in inverse correlation to the fruit softening. During the heat treatment expression polygalacturonase was inhibited, while holding fruit at 20°C following the heating allowed accumulation of the enzyme, though to a lower extent than in unheated fruit.

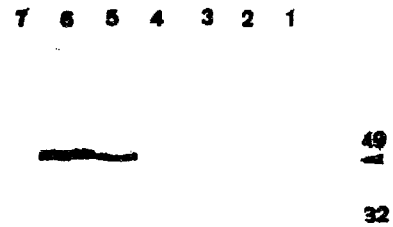


Fig. 3. Western blot of polygalacturonase protein. Protein was not detected at harvest (lane 1) or du 1 day at 20°C (lane 2) or 1 or 3 days at 38°C (lanes 3,4). However, after 7 days at 20°C, both h (lane 5) and unheated (lane 6) fruits had detectable polygalacturonase. The unheated fruits had a hi level of protein than the heated fruits. Molecular weight markers are indicated.

Ethylene production was inhibited in fruit during the heat treatment (Fig. 4). This was shown to b to a decrease in both ACC synthase and ACC oxidase (Biggs et al., 1988; Klein, 1989). It is prob that the decrease in enzyme activity was due to decreased synthesis of the enzymes, since we have f that the heat treatment decreased the level of the ACC oxidase mRNA (Lurie et al, 1993). Once the were removed from heat ethylene production recovered rapidly and the climacteric rise was great heated than in unheated fruit. However, this increased ethylene production did not cause faster ripe in the heated fruit, an indication that after heat treatment fruits were less sensitive to ethylene.

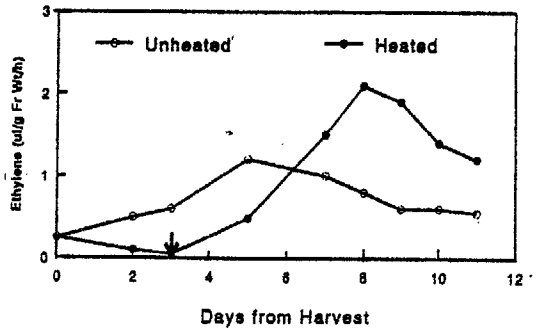


Fig. 4. Ethylene production in unheated and heated tomatoes during ripening at 20°C. Arrow indicates transfer from 38°C to 20°C.

Effect of heat treatment on chilling injury

Prestorage heating of tomatoes prevented the chilling injury which developed on unheated tomatoes stored for 3 weeks at 2°C (Table 2).

Table 2. The development of chilling injury as evidenced by necrotic areas, the absence of color development, and suceptibility to rots on mature green tomatoes stored for 3 weeks at 2°C plus 7 days at 20°C.

Treatment	Necrotic Areas (%)	Red Fruit (%)	Rots (%)
Unheated	60	39	30
Heated	0	90	5

The chilling injury appeared as an uneven development of red color, the development of necrotic areas on the fruit peel, and increased susceptibility to fungal rots. The heated fruits ripened normally, developing normal red color and softening. The inhibition of ripening found after prolonged low temperature storage may be caused by the inability of the unheated fruits to synthesize the enzymes involved in the ripening process. An examination of the mRNA for both polygalacturonase and phytoene synthase (involved in lycopene synthesis) showed that the message was low during storage in fruits from both treatments, and increased more in the heated fruit than in the unheated when they were removed from storage (Fig. 5).

Chilling injury is thought to be a membrane related disorder (Lyons, 1973) and there is a large body of evidence that alteration in the amount and composition of membrane phospholipids and sterols can protect tissues from chilling injury (Guy, 1990). Recently, Murata and coworkers (1992) caused overexpression of a lipid acyl transferase in tobacco and demonstrated that these plants were more resistant to chilling injury. The necrotic areas of the peel which develop in chilled tomatoes were correlated with a decreased phospholipid content from the microsomal membrane fraction with no change in sterol content (Table 3). This decrease in phospholipid was accompanied by increased leakage in fruit discs. A prestorage heat treatment prevented both of these phenomena. Our hypothesis is tha consequence of the heat stress synthesis of enzymes(s) responsible for phospholipid degradatio inhibited.

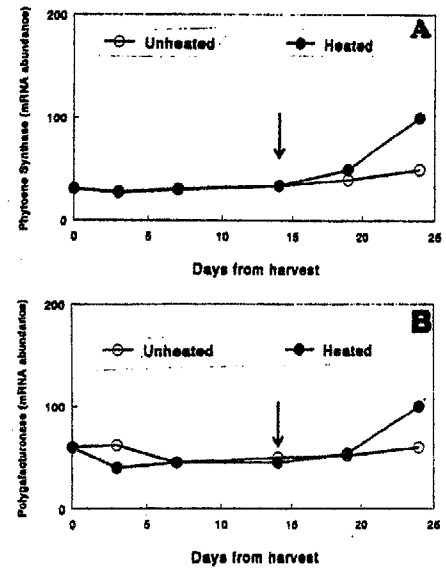


Fig. 5. Relative abundance of phytoene synthase (A) and polygalacturonase (B) mRNA in unheat heated tomato fruit during and after storage at 2°C. Arrow indicates transfer from 2°C to 20°C.

Table 3. Tomato microsomal membrane phospholipid and sterol content and potassium leakage tomato discs from fruit stored for 3 weeks at 2°C.

Treatment	Phospholipid (μg/50 g Fr Wt)	Sterol (μg/50 g Fr Wt)	Potassium Leakage (% of total)
Unheated	804	190	49
Heated	1350	178	34

Effect of heat treatment on pathogen infection

As fruits ripen, they become more prone to pathogen attack. This is due to a number of factors, a them disappearance of antifungal compounds from the tissues and the weakening of the physical b of the cell wall as the fruits soften. By inhibiting the ripening process the heat treatment lowe possibility of fungal invasion. Heat treatment decreased the amount of natural infection occurri ripening tomatoes (Table 2). The heat treatment was found to be effective in abolishing infecti *Botrytis cinerea* on tomatoes (Fallik et al., 1993). The germination of spores was inhibited with first 24 h at 38°C, and after longer times of heat stress the development of germinated spore inhibited. If mature green or pink fruits were inoculated before the heat treatment the developm

the fungus was inhibited within 2 days of heating at 38°C (Fig. 6). Thus, heat stress can be beneficial in reducing pathogen infection by action on either the host or the pathogen.

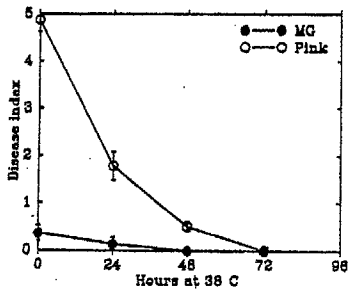


Fig. 6. Disease index of mature green and pink tomatoes inoculated with *Botrytis cinerea*, as affected by length of exposure to 38°C. Fruits were examined after 12 days at 20°C. Bars indicate standard error.

Literature Cited

Biggs, M.S., Woodson, W.R. and Handa, A.K. 1988. Biochemical basis of high temperature inhibition of ethylene biosynthesis in ripening tomato fruit. Physiol. Plant. 72:572-578.

DellaPenna, D., Alexander, D.C. and Bennett, A.B. 1986. Molecular cloning of tomato fruit polygalacturonase: analysis of polygalacturonase mRNA levels during ripening. Proc. Natl. Acad. Sci., USA 83:6420-6424.

Fallik, E., Klein, J.D., Grinberg, S., Leomniec, E., Lurie, S., Lalazar, E. 1993. Postharvest heat treatment of tomatoes: Effect on *Botrytis cinerea* and fruit ripening. Plant Dis. 77:985-988.

Guy, C.L. 1990. Cold acclimation and freezing stress tolerance: role of protein metabilism. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41:187-223.

Klein, J.D. 1989. Ethylene biosynthesis in heat treated apples. In: Biochemical and physiological aspects of ethylene production in lower and higher plants. (eds. H. Clijsters, M. de Profit, R. Marcelle and M. van Pouche), Kluwer Acad. Press, Dordercht, The Netherlands, pp. 181-189.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the heat of the bacteriophage T4. Nature 227:680-685.

Lurie, S. and Klein, J.D. 1991. Acquisition of low temperature tolerance in tomatoes by exposure to high temperature stress. J. Amer. Soc. Hort. Sci. 116:1007-1012.

Lurie, S., Watkins, C. , Ross, G., Klein, J.D. and Ferguson, I.B. 1993. Cell wall changes in nectarines during air and CA storage. In: Physiol. Basis of Postharvest Technol. (M. Saltveit, ed.) Acta Hortic., 343:204-208.

Lyons, J.M. 1973. Chilling injury in plants. Annu. Rev. Plant Physiol. 24:445-466.

Matters, G.L., Scandalios, J.G. 1986. Changes in plant gene expression during stress. Dev. Genetics 7:167-175.

Murata, N., Ishizaki-Nishizawa, O., Higashi, S., Hayashi, H., Tasaka, Y., Nishida, I. 1992. Genetically engineered alteration in the chilling sensitivity of plants. Nature 356:710-713.

Sachs, M.M. and Ho, T. H. 1986. Alteration of gene expression during environmental stress in plants. Annu. Rev. Plant Physiol. 37:363-376.

Sambrook, J. Fritsch, E.F. and Maniatis, T. 1989. Molecular cloning: a laboratory manual 2nd edition. Cold Spring Harbor, N.Y.

Vierling, E. 1991. The role of heat shock proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:579-620.

5. Methods and Results

2. prevent fungal infection of harvested fruit.

A dry heat treatment was developed for apples. This treatment was previously found to maintain fruit firmness and prevent the storage disorder superficial scald. In the present study it prevented the development of *Penicillium expansum* on apples, even if the fruits were stored after the heat treatment. The results are presented in: E. Fallik, S. Grinberg, M. Gambourg, J.D. Klein and S. Lurie. 1995. Prestorage heat treatment reduces pathogenicity of *Penicillium expansum* in apple fruit. Plant Pathology 45:92-97.

Hot water dips were tried to prevent fungal decay in peppers. There is no approved postharvest fungicide for peppers and abiotic methods of decay prevention are very important. The results are presented in: E. Fallik, S. Grinberg, A. Alkalai and S. Lurie. 1996. The effectiveness of postharvest hot water dipping on the control of gray and black mould in sweet red pepper (*Capsicum annuum)*. Plant Pathology 45:644-649.

Plant Pathology (1995) 45, 92–97

Prestorage heat treatment reduces pathogenicity of *Penicillium expansum* in apple fruit

E. FALLIK*†, S. GRINBERG†, M. GAMBOURG†, J. D. KLEIN‡ *and* S. LURIE†
ARO–The Volcani Centre, †Department of Postharvest Science of Fresh Produce, and ‡Department of Agronomy and Natural Resources, Bet-Dagan 50250, Israel

Penicillium expansum is one of the main postharvest pathogens of apples in Israel. Heating apple fruit inoculated with *P. expansum* for 96 h at 38 °C completely inhibited decay development. Fruit held for 24 h at 42°C or 12 h at 46 °C had significantly reduced decay after an additional 14 days incubation at 20 °C, compared with unheated inoculated control fruit. Mycelial growth and percentage spore germination *in vitro* were inversely proportional to length of time of exposure to various temperatures. The ET_{50} for spore germination was 42, 34 and 20 h at 38, 42 and 46 °C, respectively, while the ET_{50} for mycelial growth was 48, 44 and 36 h at those temperatures. When Penicillium spores were incubated on crude extract prepared from the peel of apple fruits held 4 days at 38 °C, germ tube elongation was significantly reduced, while the walls of the tubes were thicker, compared with germ tubes from spores incubated on crude extract prepared from peel of non-heated fruit. The evidence presented here supports the hypothesis that the effect of heating on the decay of apples caused by *P. expansum* is not only the result of direct inhibition of fungal germination and growth by high temperature, but is also partly due to the formation of an inhibitory substance in the heated peel.

INTRODUCTION

Penicillium expansum rot occurs in most areas of the world where apples (*Malus domestica*) are grown 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 is transferred to a warm environment. Although some chemicals are effective in retarding or preventing rots caused by *P. expansum* (Burton & Dewey, 1981), the use of pre- and postharvest chemical treatments is becoming limited due to consumer concerns (How, 1991).

Prestorage heat treatments have been used for many years to control fungal diseases and insect infestation of fruit (Couey, 1989). Porritt & Lidster (1978) showed that a prestorage exposure of apple fruit to 38 °C for four days suppressed both softening and naturally occurring decay, mostly due to *Penicillium* spp., after prolonged storage. Similar beneficial effects on firmness were obtained with apples held at 38, 42, or 46 °C for 96, 24, or 12 h before storage, respectively (Klein & Lurie, 1992). A synergistic effect was observed when heated fruit were dipped in $CaCl_2$ solutions prior to storage (Klein & Lurie, 1994). Sams *et al.* (1993) reported that heating 'Golden Delicious' apples for 4 days at 38 °C and then infiltrating them with calcium reduced decay caused by *P. expansum* by 30% and maintained fruit firmness during 6 months storage at 0 °C. The response of apples to heat treatments may vary according to cultivar, as 'Golden Delicious' and 'Delicious' showed relatively strong tolerance heat (Kim *et al.*, 1993).

The goal of this work was to examine the effect of different heat treatments on reduction of decay development caused by *P. expansum* in harvested apple fruit, and to investigate the mode of action of this treatment.

MATERIALS AND METHODS

The effect of increasing exposure times at 38, 42 and 46°C on spore germination and mycelial growth

Penicillium expansum was isolated from a diseased 'Golden Delicious' apple fruit. To test

* To whom correspondence should be addressed.
Accepted 19 June 1995

the effect of 38, 42 and 46°C *in vitro* on spore germination, spores from a 10-day-old culture were suspended in sterile distilled water and 0·03% Tween-20 and maintained as a single spore culture growth on potato dextrose agar (PDA) at 20°C. Aliquots (100 μl) of the suspension (1×10^5 spore/ml) were streaked aseptically on PDA plates. In a parallel experiment, the effect of temperature on mycelial growth of *P. expansum* was tested by placing a 5-mm diameter disc from the periphery of a 10-day-old culture into the centre of a 9-cm petri-dish with PDA medium. All plates were then incubated at 38, 42 and 46°C in trays covered with plastic bags to prevent desiccation. Every 24 h over a period of 4 days, a set of three plates were transferred from each temperature to 20°C for 8 days. Germinating spores were counted as described previously (Fallik *et al.*, 1993). Mycelial growth was determined as the increase in colony radius following incubation for 7 days at 20°C. The experiment was repeated three times with three replicates in each trial, using fresh spore suspensions each time.

Plant material and decay severity

'Golden Delicious' apples were picked at commercial harvest from an established orchard. Fruit were divided into four equal lots of uniform size and colour of 25 fruit each. Fruit were wounded on two sides to a depth of 1·5 mm by puncturing them with the head of a nail 1·5 mm in diameter. Each wound site was then inoculated with 40 μl of a spore suspension (10^5 spore/ml) of *P. expansum*. The apples were then held at 38, 42 and 46°C for 96, 24 and 12 h, respectively or were placed directly at 20°C. Heating was performed 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 relative humidity at 85%, as measured by a thermohygrograph (Casella, London). Following the heat treatment, fruit were placed at 20°C for an additional 14 days, in order to allow for decay development. To prevent excessive water loss, all fruit held at 20°C were kept in cartons covered with polyethylene bags. After 14 days at 20°C, 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 as a circle.

The results from three experiments were pooled and analysed.

Isolation of *P. expansum* from inoculated heated and non-heated fruit

Samples (about 3 mm wide × 3 mm long × 2 mm deep) from the peel and underlying tissue of each inoculation site were excised and placed on PDA in 9-cm diameter petri dishes. Cultures were incubated at 22 ± 2°C and were examined daily over 8 days for the growth rate and identification of the re-isolated fungus.

Biological activity of extract from heated and non-heated fruit

Fifty grams of apple peel were removed mechanically to a depth of about 1 mm. The tissue was then homogenized with a cold ethanol : methanol mixture (80 : 20) for 3 min in an ice-cooled Sorval Omnimixer operated at full speed. The filtered residue was concentrated *in vacuo* at 45°C to about one-fifth of the original volume. The concentrated extract was partitioned twice with 50 ml dichloromethane. The organic layers were pooled, dried with anhydrous $MgSO_4$, concentrated *in vacuo*, dried under nitrogen and kept at −20°C for bioassay.

A glass slide bioassay was used to evaluate the activity of the crude extract. Forty microlitres of extract dissolved in 95% ethanol were spotted onto a 13-mm diameter Millipore filter (HAWP, 0·45 μm pore size, Millipore Co.). The filter was placed on a glass slide and the ethanol allowed to evaporate. Then, 80 μl of spore suspension of *P. expansum* (10^5 spore/ml) prepared in Lilly medium (Lilly & Barnett, 1951), with 2% glucose and 0·03% Tween 20, were placed on the Millipore filter and incubated in moist petri dishes for 14 and 24 h at 20°C. Germ tube elongation was terminated by fixation of the fungus on the Millipore filter with cotton blue 0·1% in lactophenol. The activity of each extract was determined by comparing the elongation of germ tubes with that of the control using an Olympus BH-2 light microscope at 400 × magnification.

Scanning electron microscopy (SEM) analysis

Pieces of about 3 mm^3 of peel and exocarp were excised from the inoculation sites of two fruits per replicate after 4 days at 20°C and after 4 days at 38°C plus an additional period of 14 days at 20°C. Inoculated non-heated apples served as controls. Samples were prepared for SEM as described by Newbury *et al.* (1986) and were

observed in a JEOL GSM-T300A scanning electron microscope at 10 and 15 kV, 0 and 30 degree tilt and 10 mm working distance.

RESULTS

Effects of exposure times

Percentage spore germination and mycelial growth were inversely proportional to the length of exposure to 38, 42 and 46°C (Fig. 1). Spore germination was more sensitive than mycelial growth at various temperatures. Although control (unheated) spores germinated within 24 h at 20°C, all heated spores exhibited a further 24 h lag in germination after petri dishes were removed from the heat chambers (data not shown). Data in Fig. 1a are shown without the lag in the interest of clarity. The ET_{50} for spore germination was 42, 34 and 20 h, at 38, 42 and 46°C, respectively, whereas for mycelial growth the ET_{50} was 48, 44 and 36 h at those temperatures (Fig. 1). A complete inhibition of spore germination was observed following 72- or 48-h incubation at 42 and 46°C respectively (Fig. 1a). Mycelial growth was inhibited completely following 96 and 72 h incubation at 42 and 46°C, respectively (Fig. 1b).

Decay on fruit

No decay was found on inoculated apple fruit held for 96 h at 38°C and an additional 14-day period at 20°C (Fig. 2). A significant reduction in area of decay was also observed on fruit held for 24 or 12 h at 42 or 46°C, respectively. The decayed area of inoculated unheated fruit was about 19 cm^2, which covered almost 50% of the fruit surface and which was associated with heavy sporulation of the fungus.

Re-isolation of Penicillium

A lag of about 48 h was observed in development of *P. expansum* isolated underneath the tissue of inoculated heated fruit (Fig. 3). Not only was there a lag in growth, but the growth rate of *P. expansum* colonies isolated from heated fruit was 20% slower than that of the colonies isolated from unheated fruit, as shown by the slope calculated 2 days after isolation.

Electron microscope analysis

A scanning electron microscope examination of inoculated heated fruit revealed that after 4 days

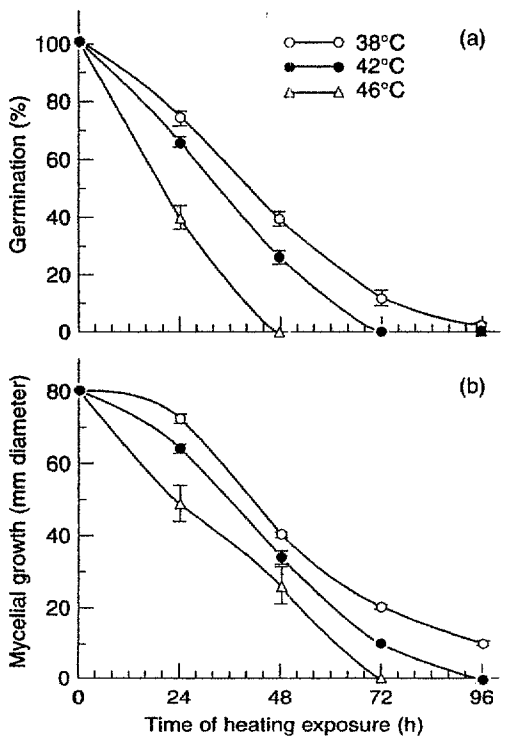


Fig. 1 Inhibition of spore germination (a) and mycelial growth (b) of *Penicillium expansum* by increasing times of exposure to 38, 42 and 46°C. Data in (a) recorded after 24 h incubation at 20°C following treatment; data in (b) recorded after 8 days at 20°C following treatment. SE is indicated for each point.

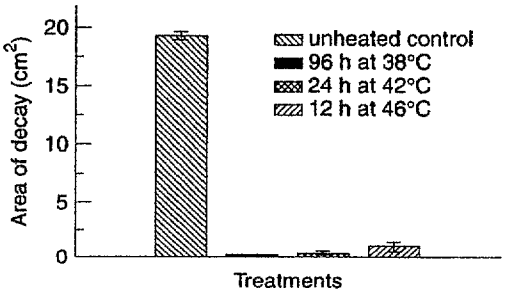


Fig. 2 Effect of heat treatment on decay caused by *P. expansum* on 'Golden Delicious' apple fruit. Fruit were wounded and inoculated and either not heated or heated for 96 h at 38°C, 24 h at 42°C and 12 h at 46°C. After treatments, fruit were held for a 14-day period at 20°C and then inspected for rots. SE is indicated.

of heat treatment and an additional 14-day period at 20°C, very few spores were germinated (Fig. 4a) and the mycelial mat was very thin due to markedly reduced branching (Fig. 4b). In contrast, after only 4 days at 20°C, the mycelial mat on control (unheated) fruit was thick and lush (Fig. 4c). It was impossible to prepare a SEM sample from unheated inoculated fruit (control) after 14 days incubation at 20°C, since there was massive decay and tissue deterioration.

Effect of crude extract from apple peel on *P. expansum*

Germ tube elongation was significantly inhibited by exposure to crude extract prepared from the peel of heated apple fruit, compared to unheated fruit (Table 1). A 77% reduction in tube length was observed after 14 h incubation, whereas the reduction was only 50% when measured after 24 h incubation in crude extract.

DISCUSSION

Holding inoculated apple fruits for 4 days at 38°C provided effective protection against decay caused by *P. expansum*. Similar, although less pronounced, effects were achieved by holding the apples for 24 h at 42°C or for 12 h at 46°C. Holding apples for longer times at the higher temperatures (42 or 46°C) would probably have inhibited *P. expansum* development as well, but at the risk of heat damage to the fruit (Klein & Lurie, 1992). One of our aims in this research is to determine the shortest exposure period at the highest temperature that will inhibit decay-causing organisms without damaging the fruit. We are investigating currently whether another 3 to 6 h at 42 or 46°C, or 24 h less at 38°C would achieve this aim.

The mode of action of the heat treatment appears to be both via direct interaction with the fungus itself and via physiological responses of

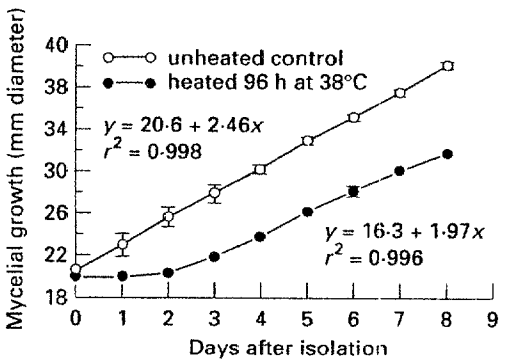


Fig. 3 Effect of heat treatment on growth of *P. expansum* isolated from underneath the peel of inoculated apple fruit. The inoculated tissue was placed on PDA medium and inspected daily for mycelial growth during an 8-day period. SE is indicated.

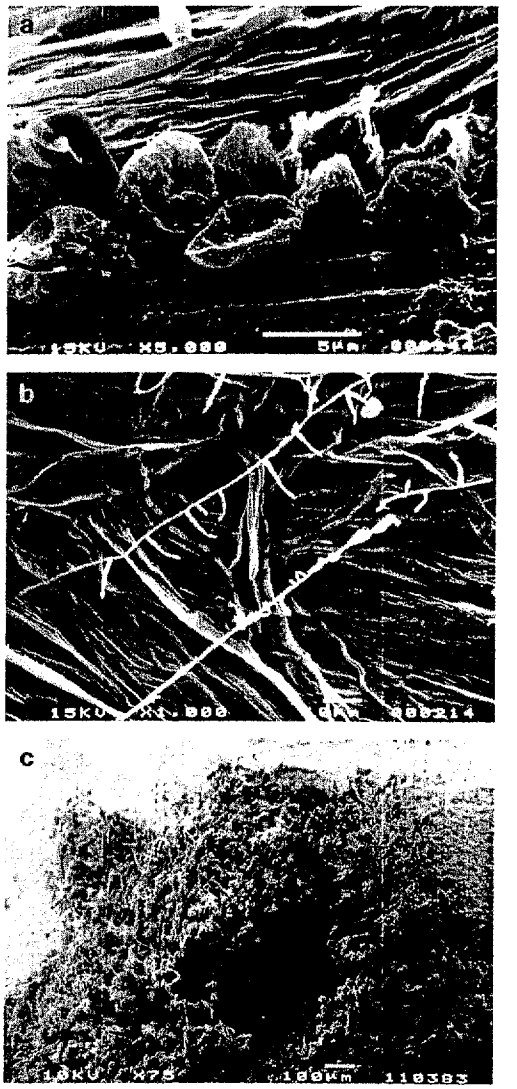


Fig. 4 (a) Spores [× 5000] and (b) mycelium [× 1000] of *P. expansum* growing on inoculated apples after exposure to 38°C for 96 h, plus a further 14-day period at 20°C. (c) *P. expansum* infection site [× 75] on an apple after 4 days at 20°C.

Table 1 Effect of crude extract prepared from heated and unheated apple peel on germ tube elongation of *Penicillium expansum* after 14 and 24 h of incubation at 20°C

	Germ tube length (μm)	
Treatment	14 h	24 h
Unheated	3·11 a	26·06 a
Heated	1·75 b	16·94 b

Means followed by different letters at each observation time are significantly different according to *t*-test at $P = 0{\cdot}05$.
Values are the means of three experiments.

the fruit tissue. Direct effects of heat on the fungus were noted when both germination and growth declined *in vitro* in response to extended periods at higher temperatures (Fig. 1). These results were obtained *in vivo* as well, where the significant reduction in decay development was probably the result of a direct effect of the heat treatment, which, by reducing germination, reduced the effective inoculum size. Preliminary results indicate that decay development in apples inoculated with 10^3 spore/ml is much slower in comparison with fruit inoculated with a higher concentration of *P. expansum* (E. F., unpublished data). Present data with *P. expansum* are similar to those reported by Fallik *et al.* (1993) with *Botrytis cinerea*, in which the prophylactic effect of heating on decay of tomatoes appeared to be by direct interaction with the fungus.

The physiological response of the tissue, and its interaction with the fungus, is seen in the incomplete recovery of germinability of spores after re-isolation of fungal material from inoculated apples and transfer to optimal growth conditions. The partial recovery that was obtained, the lag in initial germination, and the decreased rate of growth (Fig. 3) all indicate that there is a residual 'shock effect' of heating well after application of the treatment. The notion that this holdover effect could have a basis in a physiological phenomenon is strengthened by the finding that adding crude extract from the peel of heated fruit to PDA not only inhibits fungal growth but also results in distorted mycelia (Fig. 4). The effect of crude extract on fungal growth supports the hypothesis that antifungal-like substances are also involved in the resistance of heated apple fruit to *P. expansum*. Heating wounded pear fruits at 37°C reduced Mucor rot and Phialophora side rot even when fruits were inoculated after heating, demonstrating a wound-healing response (Spotts & Chen, 1987). Ben-Yehoshua *et al.* (1988) have reported that a similar heat treatment induced resistance of grapefruits to decay caused by *P. digitatum* by increasing the endogenous concentration of preformed antifungal substances. Short-term exposure to anaerobiotic conditions can also enhance endogenous resistance to plant pathogens (Prusky *et al.*, 1993).

It is possible that heat treatment could be an effective adjunct to other antifungal treatments. Calcium treatments reduce susceptibility of apples to a range of fungal organisms (Conway *et al.*, 1991). In a recent study of 'Golden Delicious' apples inoculated with *B. cinerea*, Conway *et al.* (1994) reported that heat treatment at 38°C for 4 days, pressure infiltration with calcium solutions, or a combination of both significantly reduced decay caused by the fungus while maintaining fruit firmness during prolonged storage.

Chemical means of preserving postharvest fruit quality that are acceptable to consumers are becoming increasingly limited. Heat treatments may be effective either by direct inhibition of fungal germination and growth, or by altering the susceptibility of the host to pathogens. Previous work has shown that a heat treatment improved the keeping and eating quality of apples by reducing the rate of fruit softening and increasing the sugar:acid ratio (Klein & Lurie, 1990). With the trend toward less reliance on chemical control, postharvest use of heat treatment warrants greater study and further development.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Mrs Lilia Arkan of the Department of Virology for her assistance with the SEM work. This research was supported under grant No. C12-081, US–Israel Cooperative Development Research Program, Office of the Science Advisor, US Agency for International Development.

Contribution from the Agricultural Research Organization, The Volcani Centre, Bet Dagan, Israel, No. 1480-E, 1994.

REFERENCES

Ben-Yehoshua S, Shapiro B, Kim JJ, Sharoni J,

Carmeli S, Kashman Y, 1988. Resistance of citrus fruit to pathogen and its enhancement by curing. In: Goren R, Mendel K, eds. *Proceedings of 6th International of Citrus Congress*. Rehovot: Balaban, 1371–9.

Buchanan JR, Sommer NF, Fortlage RJ, Maxie EC, Mitchell FG, Hsieh, DPH, 1974. Patulin from *P. expansum* in stone fruits and pears. *Journal of American Society for Horticultural Science* **99**, 262–5.

Burton CL, Dewey DH, 1981. New fungicide to control benomyl-resistant *Penicillium expansum* in apples. *Plant Disease* **65**, 881–3.

Conway WS, Sams CE, Abott JA, Bruton BD, 1991. Postharvest calcium treatment of apple fruit to provide broad-spectrum protection against postharvest pathogens. *Plant Disease* **75**, 620–2.

Conway WS, Sams CE, Wang CY, Abbott JA, 1994. Additive effects of postharvest calcium and heat treatments on reducing decay and maintaining quality in apples. *Journal of American Society for Horticultural Science* **119**, 49–53.

Couey HM, 1989. Heat treatment for control of postharvest diseases and insect pests of fruits. *HortScience* **24**, 198–202.

Fallik E, Klein J, Grinberg S, Lomaniec E, Lurie S, Lalazar E, 1993. Effect of postharvest heat treatment of tomatoes on fruit ripening and decay caused by *Botrytis cinerea*. *Plant Disease* **77**, 985–8.

How RB, 1991. *Marketing Fresh Fruits and Vegetables*. New York: AVI Book by Van Nostrand Reinhold, 179–203.

Kim DM, Smith NL, Lee CY, 1993. Apple cultivar variations in response to heat treatment and minimal processing. *Journal of Food Science* **58**, 1111–4.

Klein JD, Lurie S, 1990. Prestorage heat treatment as a means of improving poststorage quality of apples. *Journal of American Society for Horticultural Science* **115**, 265–9.

Klein JD, Lurie S, 1992. Prestorage heating of apple fruit for enhanced postharvest quality: Interaction of time and heating temperature. *HortScience* **27**, 326–8.

Klein JD, Lurie S, 1994. Time, temperature, and calcium interact in scald reduction and firmness retention in heated apples. *HortScience* **29**, 194–5.

Lilly VG, Barnett HL, 1951. *Physiology of Fungi*. New York: McGraw-Hill.

Newbury DE, Joy DC, Echlin P, Fiori CE, Goldstein JI, 1986. Advance in specimen preparation for biological SEM. In: *Advances Scanning Electron Microscopy and X-ray Microanalysis*. New York: Plenum Press, 325–65.

Porritt SW, Lidster PD, 1978. The effect of pre-storage heating on ripening and senescence of apples during cold storage. *Journal of American Society for Horticultural Science* **103**, 584–7.

Prusky D, Bazak M, Ben-Arie R, 1985. Development, persistence, survival and strategies for control of thiabendazole-resistant strains of *Penicillium expansum* on pome fruits. *Phytopathology* **75**, 877–82.

Prusky D, Plumbley RA, Kobiler I, Zauberman G, Fuchs Y, 1993. The effect of elevated CO_2 levels on the symptom expression of *Colletotrichum gloeosporioides* on avocado fruits. *Plant Pathology* **42**, 900–4.

Sams CE, Conway WS, Abbott JA, Lewis RJ, Ben-Shalom N, 1993. Firmness and decay of apples following postharvest pressure infiltration of calcium and heat treatment. *Journal of American Society for Horticultural Science* **118**, 623–7.

Spotts RA, Chen PM, 1987. Prestorage heat treatments for control of decay of pear fruit. *Phytopathology* **77**, 1578–82.

BEST AVAILABLE COPY

Plant Pathology (1996) **45**, 000–000 Paper 466

The effectiveness of postharvest hot water dipping on the control of grey and black moulds in sweet red pepper (*Capsicum annuum*)

E. FALLIK*, S. GRINBERG, S. ALKALAI *and* S. LURIE
ARO–The Volcani Centre, Department of Postharvest Science of Fresh Produce, PO Box 6, Bet Dagan 50250, Israel

The effectiveness of hot water dipping on the control of grey mould, caused by *Botrytis cinerea*, and black mould, caused by *Alternaria alternata* on sweet red pepper quality was investigated. Dipping naturally infected or artificially inoculated fruit at 50°C for 3 min completely inhibited, or significantly reduced, decay development caused by *B. cinerea* and *A. alternata*, respectively. Heat damage was observed on fruit dipped for 5 min at 50°C, or at 55°C for 1 min or longer. Damage appeared as cracks and pitting on the fruit surface. Spore germination and germ tube elongation *in vitro* was inversely related to the duration of exposure or to the range of temperature used. The ET_{50} for spore germination for *B. cinerea* was 3·2, 1·5 and 0·8 min, and for *A. alternata* 8·8, 4·2 and 1·4 min, at 45, 50 and 55°C, respectively. The ET_{50} for germ tube elongation for *Botrytis* was 2·6, 0·9 and 0·5 min, and for *Alternaria*, 7·2, 2·5 and 1·6 min, at 45, 50 and 55°C. The mode of action of hot water dips on decay of pepper appears to be by direct interaction with the fungi.

INTRODUCTION

Postharvest decay is the major factor limiting the extension of storage life of sweet red bell pepper (*Capsicum annuum*). The two main fungi responsible for storage decay in Israel are *Botrytis cinerea* and *Alternaria alternata*, the causal organisms of grey and black moulds, respectively (Barkai-Golan, 1981). There is no registered postharvest fungicide for control of decay in bell peppers in Israel, and this has prompted research into alternative, nonchemical treatments for pathogen control.

Postharvest heat treatments of fruit and vegetables for disease control and insect disinfestation have been used for many years (Barkai-Golan & Philips, 1991; Klein & Lurie, 1991). Heat can be applied to fruits and vegetables as hot water dips, vapour heat, or hot dry air. Dipping oranges for 42 min in water heated to 45°C significantly reduced decay caused by *Colletotrichum gloeosporioides*, *Penicillium digitatum* and *P. italicum* (Williams *et al.*, 1994). Vapour heat at temperatures ranging from 47 to 49°C controlled *C. gloeosporioides* in mangoes (Coates *et al.*, 1993), whilst exposing tomato fruits to 38°C for 3 days prior to storage inhibited decay due to *B. cinerea* Pers. (Fallik *et al.*, 1993). Heat is used commercially for disinfestation of mangoes and papaya (Klein & Lurie, 1991). Heat treatments can inhibit the ripening of many fruits and vegetables, and can alleviate storage disorders (Paull, 1990; Klein & Lurie, 1991).

The objective of this work was to identify optimum treatment times and temperatures to control decay in naturally infected and artificially inoculated sweet red bell pepper during storage.

MATERIALS AND METHODS

The effect of exposure times at various temperatures on spore germination and germ tube elongation

Single-spore cultures of *B. cinerea* and *A. alternata*, isolated from red pepper fruit, were grown for 10 days at 20°C and used for preparing spore suspensions in sterile water supplemented with 0·03% Tween-20 to maintain uniform spore distribution. Glass test tubes (16 mm diameter) containing 4 mL of water-Tween were placed in a heating block at 45, 50, or 55°C for 30 min, to equilibrate the mix prior to adding the spore suspensions. The spore suspensions (100 μL;

* To whom correspondence should be sent.

Accepted 29 February 1996.

BEST AVAILABLE COPY

1×10^5 spores mL^{-1} final concentration in 4 mL) were then added to the tubes and heated for an additional 1, 3, 5 or 10 min. Unheated spore suspensions served as controls. Aliquots (100 μL) of the treated suspensions were streaked aseptically onto potato-dextrose agar (PDA-Difco) plates. After 24 h incubation at 20°C, conidial germination was measured as described previously (Fallik *et al.*, 1993). Germ tube elongation was measured using a Zeiss Axiolab light microscope at × 100 magnification. The experiment was conducted three times, with four replicates in each experiment.

Effect of hot water dipping on postharvest decay and fruit quality

Naturally infected fruit. Sweet red bell peppers (cv. Maor) of uniform medium size (about 100 g each) were harvested from a commercial greenhouse. Fruits free of any damage were dipped for 1, 3 or 5 min in a 30 L insulated, thermostatically controlled, circulating water bath (Fried Electric, Haifa, Israel) maintained at a constant temperature of 45, 50 or 55°C. Undipped fruits or fruit dipped in water for 3 min served as controls. Following dipping, the fruits were allowed to air dry on blotting paper at ambient temperature. Fruits were then stored for 14 days at 6°C and RH 90%, as measured by a thermohygrograph (Casella, London, UK), and an additional 3 days at 20°C (simulated sea transport and marketing). After storage and a marketing simulation period, the fruit quality was evaluated by weight loss, firmness and decay incidence as described by Meir *et al.* (1995). Heat damage was expressed as the percentage of fruit having pits of 0·5–1·5 mm diameter.

Five experiments were conducted, with 25 fruit per treatment in each experiment.

Artificial inoculation. Fruit were surface-sterilized with 70% ethyl alcohol and then wounded on two sides to a depth of 1·5 mm by puncturing them with the point of a 1·5 mm diameter nail. Each wound site was inoculated with 40 μL of a spore suspension (10^5 spores mL^{-1}) of *B. cinerea* or *A. alternata*. Fruits were then kept inside a folded-closed polyethylene bag for 6 h to allow drying of the spore suspensions, followed by dipping the fruit for 1, 3 and 5 min at 50°C as described above. Cold-water-dipped (3 min) or undipped-inoculated fruits served as controls. Following the heat treatments, fruits were immediately placed, at 20°C, inside folded-closed polyethylene bags for 10 days in order to permit decay to develop. After 10 days at 20°C, the peppers were scored for decay severity. Severity was expressed as the area of circle whose diameter was the mean of the length and width of the decayed area. The experiment was repeated three times with 10 fruits per treatment. Samples (about 3-mm wide by 3-mm long by 2-mm deep) from the inoculation site were excized and placed on PDA in 9-cm-diameter Petri dishes for the examination of viable fungi.

Statistical analysis

Hot water treatments and the interaction between three temperatures (45, 50 and 55°C) and time of exposure (1, 3 and 5 min) were analysed using analysis of variance and Duncan's multiple range test for a randomized block design. The angular transformation was applied before analysis of decay incidence.

Scanning electron microscopy (SEM) analysis

Ten days after incubation at 20°C pieces of exocarp and mesocarp, about 1–2 mm^2, were excized from undipped fruit, or from fruit dipped for 3 min at 50 or 55°C. Immediately after excising, samples were frozen by plunging into a copper block cooled by liquid nitrogen. The frozen samples were further dried by sublimation in a high-vacuum device as described by Newbury *et al.* (1986) and were examined using a JEOL GSM-T300 A scanning electron microscope at 10 and 15 kV, 0 and 30 degree tilt and 10 mm working distance.

RESULTS

The effect of increasing exposure times at various temperatures on spore germination and germ tube elongation

In general, *B. cinerea* was more sensitive than *A. alternata* to hot water treatment *in vitro*. Percentage score germination (Fig. 1) and germ tube elongation (Fig. 2) of *Botrytis* and *Alternaria* were inversely proportional to the length of exposure to 45, 50 and 55°C. The exposure time (ET_{50}) for spore germination for *Botrytis* was 3·2, 1·5 and 0·8 min at 45, 50 and 55°C (Fig. 1 a), whereas for *Alternaria* the ET_{50} was 8·8, 4·2 and 1·4 min at those temperatures (Fig. 1 b). Spore germination of *Botrytis* was completely inhibited following 5 or 3 min exposure at 50 and

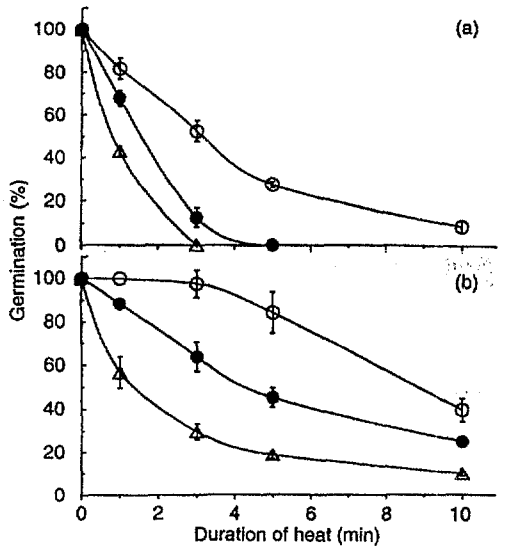


Fig. 1. Inhibition of spore germination of *Botrytis cinerea* (a) and *Alternaria alternata* (b) with increasing times of exposure to 45 (○–○), 50 (●–●) and 55°C (△–△). Data recorded after 24 h incubation at 20°C following treatment. Bars indicated SE for each point.

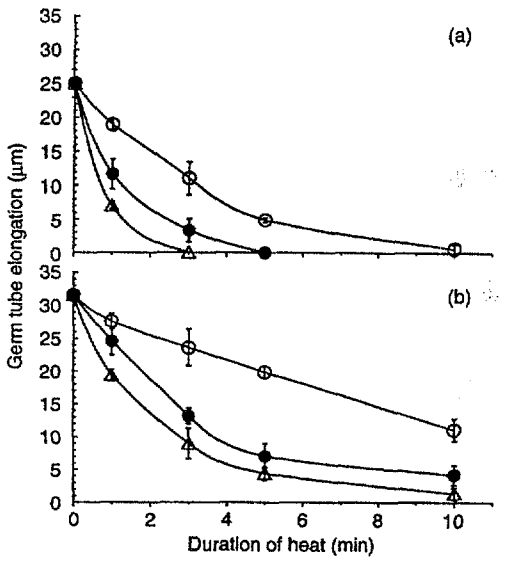


Fig. 2. Inhibition of germ tube elongation of *Botrytis cinerea* (a) and *Alternaria alternata* (b) with increasing times of exposure to 45 (○–○), 50 (●–●) and 55°C (△–△). Data recorded after 24 h incubation at 20°C following treatment. Bars indicated SE for each point.

55°C, respectively (Fig. 1 a); however, complete inhibition of *Alternaria* spore germination was not observed (Fig. 1 b).

The effect of hot water treatment on germ tube elongation was found to be more effective than on spore germination, as measured by the ET_{50}. The ET_{50} for germ elongation for *Botrytis* was 2·6, 0·9 and 0·5 min at 45, 50 and 55°C (Fig. 2 a), and for *Alternaria* the ET_{50} was 7·2, 2·5 and 1·6 min at those temperatures (Fig. 2 b).

The effect of hot water dips on naturally infected fruit quality and decay development

Pepper fruits dipped at 50°C for 5 min, or at 55°C for 1, 3 or 5 min lost significantly more weight during storage and simulated marketing compared with unheated fruit or with other treatments. Temperatures, the time of dipping and the interaction between these factors were found to be significant (Table 1).

Undipped and water-dipped controls, or fruit dipped at 45°C for 1, 3 or 5 min, or at 50°C for 1 or 3 min tended to be firm (2–3 mm deformation), whilst fruit dipped at 50°C for 5 min, or at 55°C were soft (≥3.0 mm deformation). There was no significant interaction between temperature and time of dipping (F-value = 1·7) (Table 1). Heat damage was observed on fruit dipped for 5 min at 50°C, and for all three dipping times at 55°C. Heat damage was proportional to the dipping duration at 50 or 55°C (Table 1).

SEM analysis revealed external and internal heat damage. The exocarp cells of the fruit dipped at 55°C for 3 min were found to be thinner (Fig. 3 e) with large hypoderm cells (Fig. 3 f), and cracks and pitting were observed on the fruit surface (Fig. 3 e,f). The exocarp cells of unheated fruit or fruit dipped at 50°C for 3 min were thicker with small hypoderm cells (Figs. 3 a–d).

Hot water dips at 50°C for 3 min, or at 55°C for 1 min significantly reduced decay incidence compared to other treatments (Table 1). *B. cinerea* and *A. alternata* were the main infective agents that developed on undipped or water-dipped fruits and fruits dipped at 45°C (Table 1), whereas *Alternaria* was the only decay-causing fungus that developed on fruit dipped at 50°C for 3 or 5 min, or at 55°C (Table 1). Significant differences in the incidence of decay were found between temperatures and in the time–temperature interaction, but not between times of dipping (Table 1).

The effect of hot water dips on decay development in artificially inoculated fruit

Dipping artificially inoculated sweet red pepper fruit at 50°C for 3 min completely inhibited decay development caused by *B. cinerea* and significantly reduced decay development caused by *A. alternata* compared with undipped or water-dipped fruit and other hot water treatments (Table 2). An incomplete but significant reduction in area of decay was observed in fruit inoculated with *Botrytis* dipped for 1 and 5 min (Table 2). Similarly, dipping *Alternaria*-inoculated fruit at 50°C for 5 min significantly reduced decay development compared with undipped control or water-dipped control fruit (Table 2). Heat injuries were observed on some fruits dipped for 5 min (data not shown). No viable *Botrytis* spores were isolated from the infection site of fruit dipped for 3 min.

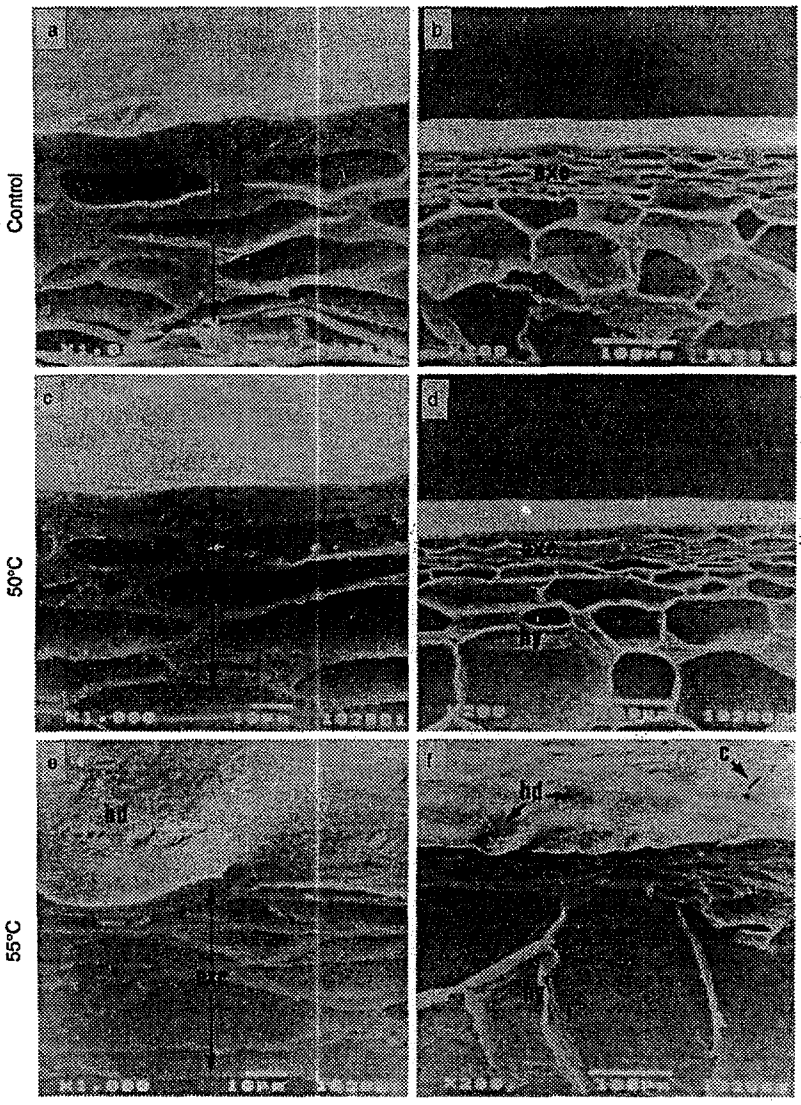


Fig. 3. Scanning electron microscopy on the surface, exocarp (exc) and hypoderm cells (hy) of unheated control sweet red pepper (a,b), fruit dipped at 50°C for 3 min (c, d), and fruit dipped at 55°C for 3 min (e,f). Heat damage (hd) is shown by pitting on the surface of the fruit and by cracks (C).

Table 1 The effect of hot water dips on the quality of naturally infected sweet red pepper after 14 days' storage at 6°C and an additional 3 days at 20°C

Treatment	Weight loss (%)	Firmness (mm)	Heat damage (%)	Decay incidence[b] (%)	Decay causing fungi
Undipped	7·8 e[a]	2·6 de	0·0 d	25·6 a	A + B
Water 3 min	7·8 e	2·6 de	0·0 d	23·0 a	A + B
45°C 1 min	7·4 e	2·3 f	0·0 d	25·4 a	A + B
45°C 3 min	7·4 e	2·4 ef	0·0 d	23·6 a	A + B
45°C 5 min	8·0 de	2·6 de	0·0 d	11·4 bc	A
50°C 1 min	7·2 e	2·3 f	0·0 d	9·4 c	A
50°C 3 min	7·1 e	2·3 f	0·0 d	3·4 d	A
50°C 5 min	8·6 d	2·8 cd	3·6 d	8·6 c	A
55°C 1 min	9·5 c	3·0 c	32·4 c	3·0 d	A
55°C 3 min	11·8 b	3·1 b	85·2 b	18·8 ab	A
55°C 5 min	14·5 a	3·5 a	100·0 a	19·6 ab	A
		Analysis of variance (*F*-value)			
Temperature	436***[a]	110***		24***	
Time	98***	30***		0·8 NS	
T × T	31***	1·7 NS		17***	

[a]Means within columns followed by the same letter do not differ significantly at $P \leq 0{\cdot}05$ according to Duncan's multiple range test.
[b]The angular transformation was applied before analysis of decay incidence. Means presented in the table were transformed back to the original values.
A, *Alternaria alternata*; B, *Botrytis cinerea*.
Firmness: <1·9, very firm; 2–3, firm; >3, soft.
[c]*, **, ***, significant deviation at the 0·05, 0·01, and 0·001 levels, respectively; NS, not significant.

DISCUSSION

Amongst the several technologies available to extend the storability and shelf life of fruits and vegetables, pre-storage hot water dipping appears to be one of the most promising in postharvest control of decay (Couey, 1989). The present investigation demonstrated that a hot water dipping of sweet red pepper at 50°C for 3 min provided effective, nontoxic treatment against the two main storage fungi *B. cinerea* and *A. alternata*. This was expressed by reduced spore germination and germ tube elongation *in vitro*, as well as by inhibiting or reduced rotting of naturally infected and artificially inoculated fruit.

Table 2 The effect of hot water dips on decay development of sweet red pepper artificially inoculated with *B. cinerea* or *A. alternata* after 10 days' storage at 20°C

Treatment	Decayed area (cm^2) *B. cinerea*	*A. alternata*
Control	10·53 a	5·52 b
Water 3 min	10·19 a	5·91 a
50°C 1 min	0·94 b	5·15 c
50°C 3 min	0·00 c	1·47 e
50°C 5 min	0·78 b	1·87 d

Means within columns followed by the same letter do not differ significantly at $P = 0{\cdot}05$ according to Duncans multiple range test.

There is considerable variation in sensitivity to high temperature amongst various fungi (Barkai-Golan & Phillips, 1991). *B. cinerea* was found to be more susceptible to hot water treatment than *A. alternata* both in *in-vitro* and in-vivo experiments. The use of hot water dips at 39–52°C for 2–10 min has been reported to control *in-vitro* and *in-vivo* spore germination of postharvest fungi and decay development in broccoli (Forney, 1995), melon (Teitel *et al.*, 1991), tomato (Barkai-Golan, 1973) and papaya (Couey *et al.*, 1984). the mode of action of hot water dips on decay of pepper appears to be by direct interaction with the fungi, perhaps by

killing ungerminated spores and slowing germ tube elongation, thus slowing the growth rate of the fungus on the infected fruit. It is also possible that hot water treatment inactivated germinated spores as reported by Barkai-Golan (1973). Hot water immersions affect decay development in Valencia oranges by affecting the spore survival of various *Penicillia* (Willliams *et al.*, 1994). Direct effects on the fungi were noted when both germination and germ tube elongation declined *in vitro* in response to extended exposure times and high temperatures. Similar results were reported with *B. cinerea* on decay of tomato (Fallik *et al.*, 1993).

Dipping the fruit at 50°C for 3 min maintained fruit quality when measured by weight loss and firmness. The significant water loss and softness of fruit dipped at 55°C is due to heat damage causing cracks and pitting on the surface of the treated fruit and the expansion and collapse of the hypoderm cells. The relatively greater turgidity of fruit dipped at 50°C for 3 min is perhaps due to recrystallization or 'melting' of the wax layer which sealed invisible cracks. Similar observation was reported by Roy *et al.* (1994) with heated apples. Alternatively, the heat treatment may have stimulated an increase in the synthesis of wax to fill the cracks, as suggested by Baker (1974).

In recent years, increased public awareness has brought about a resurgence of interest in the use of nonchemical treatments for preservation of fresh produce. This study has demonstrated that hot water dipping at 50°C for 3 min reduced postharvest decay development whilst maintaining fruit quality. A further advantage of hot water dipping is its ability to remove most of the dust found on bell pepper fruits grown in Israel under netted shades in the desert valley.

ACKNOWLEDGEMENTS

This research was supported by grant no. 406-0769-94/95 (Israeli Chief Scientist, Ministry of Agriculture) and a contribution from the Agricultural Research Organization (The Volcani Centre, Bet Dagan, no. 1762-E, 1995 Series).

REFERENCES

Baker EA, 1974. The influence of environment on leaf wax development in *Brassica oleracea gemmifera*. *New Phytologist* **73**, 955–66.

Barkai-Golan R, 1973. Postharvest heat treatment to control *Alternaria tenuis* Auct. rot in tomato. *Phytopathologia Mediterranea*, **12**, 108–11.

Barkai-Golan R, 1981. Annotated check-list of fungi causing postharvest diseases of fruits and vegetables in Israel. *Agricultural Research organization, The Volcani Center, Bet Dagan*, Special publication no. 194.

Barkai-Golan R, Phillips DJ, 1991. Postharvest heat treatment of fresh fruits and vegetables for decay control. *Plant Diseases* **75**, 1085–9.

Coates LM, Johnson GI, Cooke AW, 1993. Postharvest disease control in mangoes using high humidity hot air and fungicide treatments. *Annals of Applied Biology* **123**, 441–8.

Couey HM, 1989. Heat treatment for control of postharvest diseases and insect pests of fruits. *HortScience* **24**, 198–202.

Couey HM, Alvarez AM, Nelson MG, 1984. Comparison of hot water spray and immersion treatments for control of postharvest decay of papaya. *Plant Disease* **68**, 436–7.

Fallik E, Klein J, Grinberg S, Lomaniec E, Lurie S, Lalazar E, 1993. Effect of postharvest heat treatment of tomatoes on fruit ripening and decay caused by *Botrytis cinerea*. *Plant Disease* **77**, 985–8.

Forney CF, 1995. Hot-water dips extent the shelf life of fresh broccoli. *Hortscience* **30**, 1054–7.

Klein JD, Lurie S, 1991. Postharvest heat treatment and fruit quality. *Postharvest News and Information*. **2**, 15–19.

Meir S, Rosenberger I, Aharon Z, Grinberg S, Fallik E, 1995. Improvement of the postharvest keeping quality and colour development of bell pepper (cv. 'Maor') by packaging with polyethylene bags at a reduced temperature. *Postharvest Biology and Technology* **5**, 303–9.

Newbury DE, Joy DC, Echlin P, Fiori CE, Goldstein JI, 1986. Advances in specimen preparation for biological SEM. In: *Advances Scanning Electron Microscopy and X-ray Microanalysis*, pp. 325–65. New York: Plenum Press.

Paull RE, 1990. Postharvest heat treatments and fruit ripening. *Postharvest News and Information* **1**, 355–63.

Roy S, Conway WS, Watada AE, Sams CE, Erbe EF, Wergin WP, 1994. Heat treatment affects epicuticular wax structure and postharvest calcium uptake in 'Golden Delicious' apples. *HortScience* **29**, 1056–8.

Teitel DC, Barkai-Golan R, Aharoni Y, Copel A, Davidson H, 1991. Toward a practical postharvest geat treatment for 'Galia' melons. *Scientia Horticulturae* **45**, 339–44.

Williams MH, Brown MA, Vesk M, Brady C, 1994. Effect of postharvest heat treatments on fruit quality, surface structure, and fungal disease in Valencia oranges. *Australia Journal of Experimental Agriculture* **34**, 1183–90.

5. Methods and Results

3. prevent insect infestation of harvested fruit

At the beginning the survival of different developmental stages of *Drosophila melanogaster* was determined in hot water (Tables 1 and 2). It was found that early developmental stages were more sensitive than later stages, but that all stages were sensitive to temperatures between 50 and 60°C. This was used as the basis for developing hot water dips to control decay on pepper as a treatment which will also prevent insect infestation (see section 5.2)

Following studies with the insect model system, we examined pests which can infest fruits, San Jose scale on apples, and Mediterranean fruit fly on tomatoes. Both these pests have quarantine restrictions in various countries, and it is important to prevent their presence on exported fruit. A dry heat treatment was developed for both these pests, which also controlled fungal pathogens and did not detrimentally affect fruit quality. The results are presented in two papers under preparation: S. Lurie, E. Fallik, J.D. Klein, L. Varjas and K.C. Kovacs. Heat treatment of tomato fruit to control Mediterranean fruit fly and maintain fruit quality, and, S. Lurie, E. Fallik, J.D. Klein, F. Kozar and K.C. Kovacs. Postharvest heat treatment of apples to control insects, fungal rots and maintain fruit firmness.

Progress report: THE EFFECT OF HEAT TREATMENT ON *DROSOPHILA* EMBRYOS, LARVAE, AND PUPAE

Task: Effect of heat treatment on different ontogenetic stages of *Drosophila melanogaster*.

Experimental system: Zygotes of well defined developmental stages (determined as the time passed from embryogenesis initiation, i.e., from the moment of egg deposition) were collected. 30-100 zygotes were transferred to plastic baskets equipped with a thin net at the bottom. The basket was subsequently immersed into stirred water bath with precisely controlled temperature. After heat treatment excess water was quickly removed on a block of filter papers. The zygotes were then cultivated on standard *Drosophila* food where the survivors continued development. Zygotes that developed to adulthood were counted.

A control group was established parallel with every experimental groups. Zygotes in the same developmental stage were used, these controls were immersed into a water bath at room temperature, otherwise they were treated the same way as the experimental samples.

Three sets of measurements were conducted at each point where average and standard deviation is indicated. The attached table presents the rate of survival as the fraction of the control and summarises the results collected so far.

The most apparent conclusion is that a very short treatment at elevated temperature has a detrimental effect on zygotes at younger stages. Thus the *Drosophila* system can be a useful model to test physiological and biochemical changes associated with heat treatment.

Table 1.
The survival of *Drosophila melanogaster* zygotes (exposed as fraction of the control ± standard deviation) exposed to different temperatures for different amount of time at different developmental stages.

Stage of exposed zygotes	Time of treatment (sec)	40°C	50°C	60°C	70°C
Blastoderm (3 hours after egg deposition hAEL)	5	1.09±0.36	0 (3)	0 (3)	0 (1)
	10	0.98±0.28	0 (3)	0 (1)	0 (1)
	20	0.73±0.17	0 (3)	0 (1)	0 (1)
	40	0.64±0.13	0 (3)	0 (1)	0 (1)
	80	0.54±0.15	0 (1)	0 (1)	0 (1)
First instar larva (24-28 hAEL)	5	1.15±0.08	0 (3)	0 (2)	0 (1)
	10	1.06±0.20	0 (2)	0 (2)	0 (1)
	20	0.96±0.03	0 (1)	0 (1)	0 (1)
	40	1.02±0.23	0 (1)	0 (1)	0 (1)
	80	0.75±0.11	0 (1)	0 (1)	0 (1)
Second instar larva (48-52 hAEL)	5	0.75±0.15	0 (3)	0 (3)	0 (3)
	10	0.94±0.14	0 (3)	0 (3)	0 (3)
	20	0.84±0.10	0 (3)	0 (2)	ND
	40	0.78±0.17	0 (1)	0 (1)	ND
	80	0.71±0.16	0 (1)	ND	
Third instar larva (72-76 hAEL)	5	0.80±0.14	0 (3)	0 (2)	0 (2)
	10	0.63±0.09	0 (2)	0 (2)	0 (2)
	20	0.71±0.21	0 (1)	0 (2)	0 (2)
	40	0.66±0.09	0 (1)	ND	ND
	80	0.71±0.16	0 (1)	ND	ND
Third instar larva (96-100 hAEL)	5	1.02±0.24	0.44±0.02	0 (3)	0 (2)
	10	1.04±0.05	0 (3)	0 (2)	0 (2)
	20	0.92±0.19	0 (1)	0 (2)	0 (1)
	40	0.72±0.21	0 (2)	0 (1)	0 (2)
	80	0.71±0.26	0 (1)	0 (1)	0 (1)
Young pupa (120 hAEL)	5	1.00±0.14	0.22±0.19	0 (3)	0 (1)
	10	1.02±0.11	0 (3)	0 (3)	0 (2)
	20	0.88±0.09	0 (2)	0 (3)	ND
	40	0.84±0.09	0 (2)	0 (2)	ND
	80	0 (3)	0 (2)	0 (1)	ND
Pupa (168 hAEL)	5	0.92±0.18	0.79±0.13	0 (3)	0 (2)
	10	0.92±0.13	0.75±0.29	0 (3)	ND
	20	0.82±0.31	0.16±0.28	0 (3)	ND
	40	0.91±0.08	0 (3)	0 (2)	ND
	80	1.29±0.21	0 (2)	ND	ND

Notes. - In brackets: the number of experiments.
- ND = not determined.
- Three experiments were conducted where average and standard deviation are given.

REPORT ON THE EFFECT OF HEAT TREATMENT ON DROSOPHILA EMBRYOS, LARVAE AND PUPAE

Aims.

We conducted a study on the effects of heat treatments on different stages of *Drosophila melanogaster* zygotes. Embryos, larvae and pupae of well-defined stages were treated with different temperature water for a different amount of time and the rate of survival to adulthood was measured.

Methods.

Age of the zygotes was determined as the time passed from initiation of embryogenesis, i.e. the moment of egg deposition. At least 100 zygotes were transferred to plastic baskets with a thin net on the bottom. The basket was subsequently immersed into a stirred water bath with precisely controlled temperature. The excess water was subsequently quickly removed on a block of filter papers. The zygotes were than transferred to standard Drosophila food where the survivors continued development. Zygotes that developed to adulthood were counted.

A control group was established along with every of the experimental groups. The control zygotes were immersed into a room temperature water bath and otherwise treated the same way as the experimental samples. Three measurements were conducted in most of the experiments. The average relative rate of survival (as compared to the control) and the standard deviation were calculated.

Results.

The attached table presents the relative rate of survival at different temperatures and for different duration of treatment. For illustration, two dimensional plots of the data are also presented. The main conclusions are as follows.

1. It is apparent that a 40 second treatment at 50°C eliminates even pupae of Drosophila, the otherwise most resistant specimen to heat.

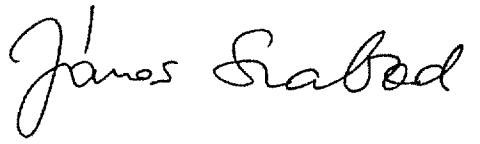
2. At 40°C, about 5-6 minutes are required to induce death of the embryos and larvae. Most of the pupae will survive a 10 min treatment at 40°C.

3. The older the zygotes become the more tolerant they become to heat treatment. However, a 3 min. treatment is apparently sufficient to induce the death of the drosophila zygotes at 46°C.

These are the data. Correlation between the mass of the zygotes and their heat tolerance needs further analysis.

Summary.
Heat treatment for a short period of time (up to half a minute) at moderately hig(46-50°C) provides a way to induce the death of the *Drosophila melnogaster* zygotes. Whether heat tolerance of other pest insect species follows the Drosophila pattern needs further investigations.

Szeged, June 1994



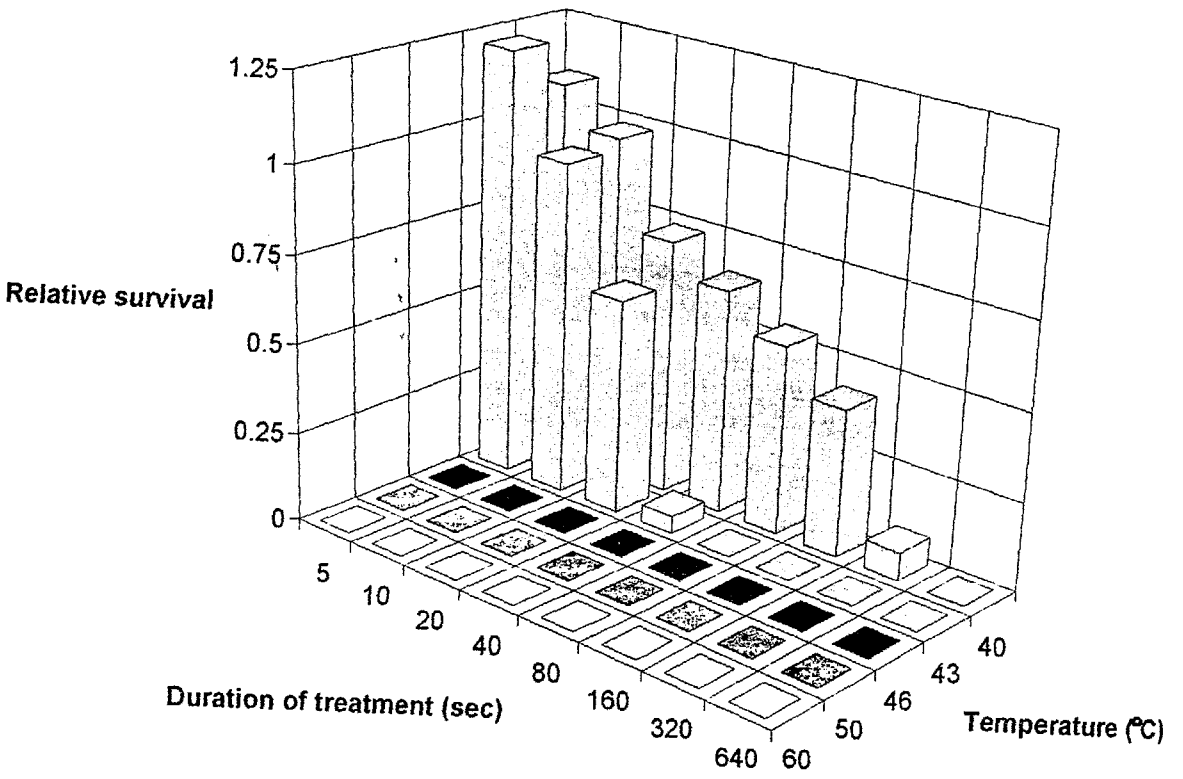
Prof. János Szabad
Department of Biology
Albert Szent-Györgyi Medical Universtiy
H-6720 Szeged, Somogyi u. 4.
Tel./Fax: 36 (62) 312 622

Table 2
The survival of *Drosophila melanogaster* zygotes, presented as the fraction of the control ± standard deviation, exposed to different temperatures for different amount of time at different developmental stages.

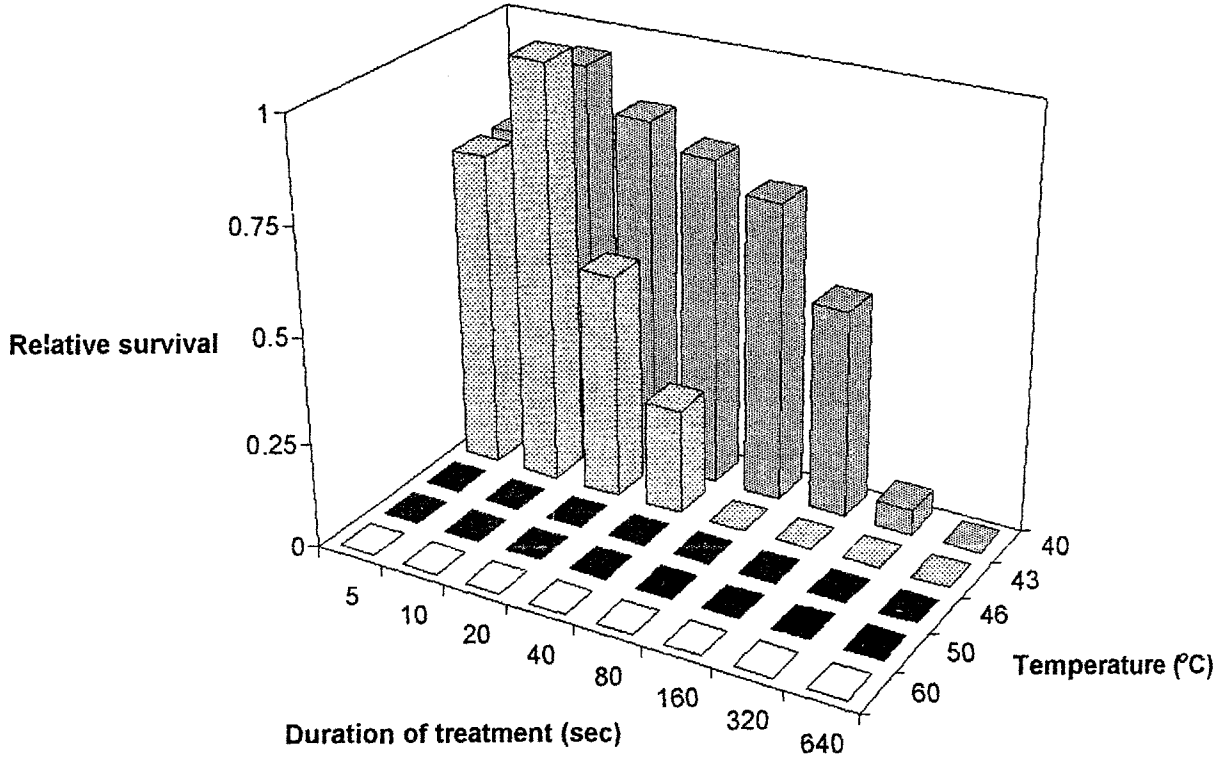
Stage of development	Duration of treatment (sec)	Temperature 40°C	43°C	46°C	50°C	60°C
Blastoderm (3 hours after egg deposition hAEL)	5	1.09±0.36	1.22±0.37	0 (3)	0 (2)	
	10	0.98±0.28	0.95±0.08	0 (2)		
	20	0.73±0.17	0.61±0.31			
	40	0.64±0.13	0.05±0.08			
	80	0.54±0.15	0 (3)			
	160	0.42±0.14	0 (2)			
	320	0.08±0.14	0 (1)			
	640	0 (3)				
First instar larva (24-28 hAEL)	5	1.15±0.08	0.83±0.21	0 (3)	0 (2)	
	10	1.06±0.20	0.88±0.08	0 (2)		
	20	0.96±0.03	1.01±0.14			
	40	1.02±0.23	0.66±0.13			
	80	0.96±0.05	0.21±0.15			
	160	0.75±0.11	0 (3)			
	320	0.42±0.16	0 (1)			
	640	0 (3)				
Second instar larva (48-52 hAEL)	5	0.75±0.15	0.75±0.11	0 (3)	0 (2)	
	10	0.94±0.14	1.03±0.15	0 (2)		
	20	0.84±0.10	0.53±0.13			
	40	0.78±0.17	0.25±0.09			
	80	0.71±0.16	0 (3)			
	160	0.49±0.09	0 (2)			
	320	0.06±0.10				
	640	0 (2)				
Early third instar larva (72-76 hAEL)	5	0.80±0.14	0.87±0.08	0 (3)	0 (2)	
	10	0.63±0.09	0.71±0.05	0 (2)		
	20	0.71±0.21	0.70±0.21			
	40	0.66±0.09	0.40±0.11			
	80	0.66±0.16	0 (3)			
	160	0.42±0.17	0 (2)			
	320	0 (2)				
Mid third instar larva (96-100 hAEL)	5	1.02±0.24	0.88±0.06	0.34±0.02	0 (3)	
	10	1.04±0.05	0.75±0.12	0 (3)		
	20	0.92±0.19	0.83±0.06	0 (2)		
	40	0.72±0.21	0.58±0.13			
	80	0.71±0.26	0 (3)			
	160	0.08±0.13	0 (2)			
	360	0 (2)				
Young pupa (120 hAEL)	5	1.00±0.14	0.90±0.16	0.44±0.29	0.22±0.19	0 (3)
	10	1.02±0.11	0.86±0.08	0 (3)	0 (3)	
	20	0.88±0.09	0.61±0.31	0 (2)	0 (2)	
	40	0.84±0.09	0.50±0.21			
	80	0.62±0.19	0 (3)			
	160	0.09±0.16	0 (2)			
	320	0 (2)				
Pupa (168 hAEL)	5	0.92±0.18	0.95±0.02	0.72±0.37	0.79±0.13	0 (3)
	10	0.92±0.13	0.91±0.05	0.87±0.04	0.75±0.29	
	20	0.82±0.31	1.00±0.02	0.88±0.09	0.16±0.28	
	40	0.91±0.08	0.95±0.13	0.71±0.08	0 (3)	
	80	1.29±0.21	0.94±0.09	0.69±0.17	0 (2)	
	160	0.96±0.06	0.76±0.24	0.07±0.13		
	320	0.94±0.05	0.74±0.26	0 (3)		
	640	0.84±0.24	0 (3)	0 (2)		

Notes. - Three experiments were conducted where average and standard deviation are given.
In br ck t h numb

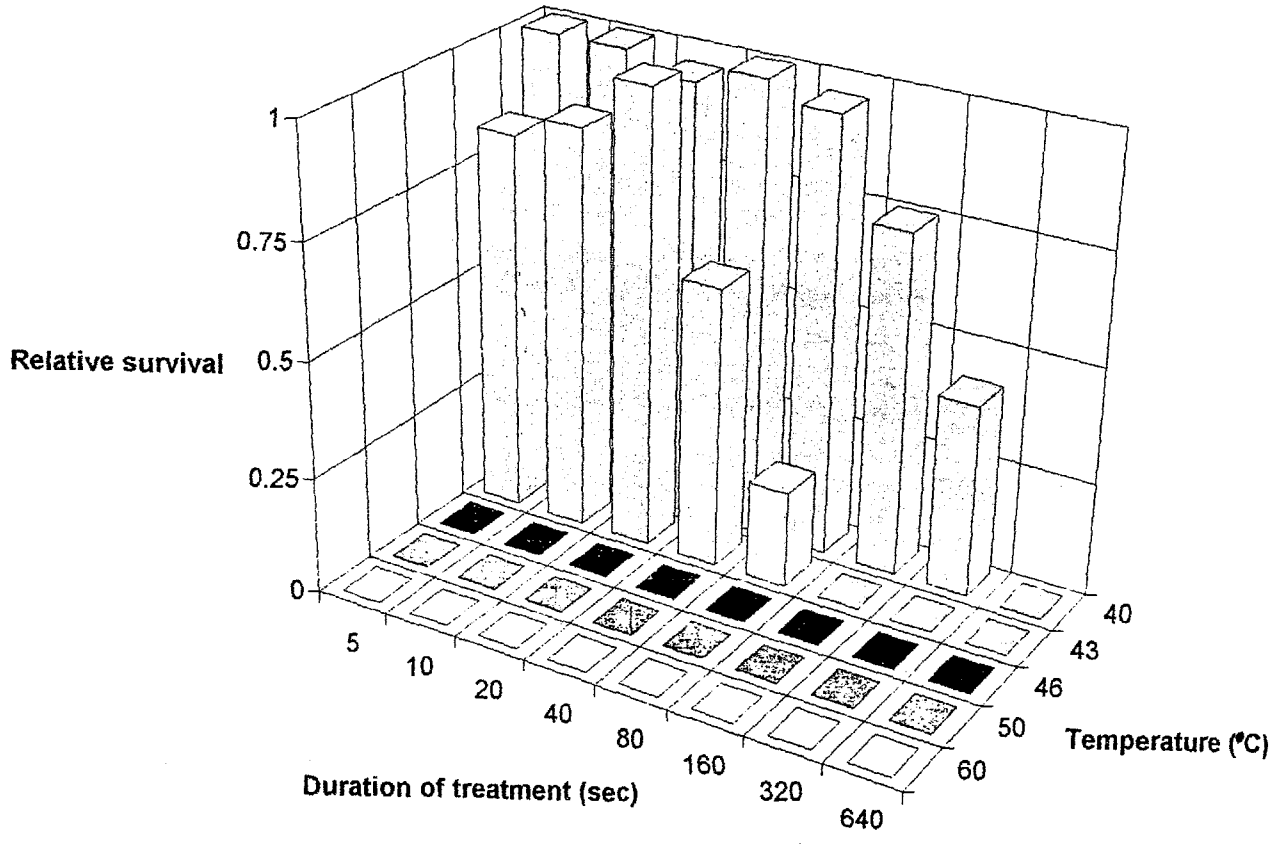
Blastoderm (3 hours after egg position; hAEL)



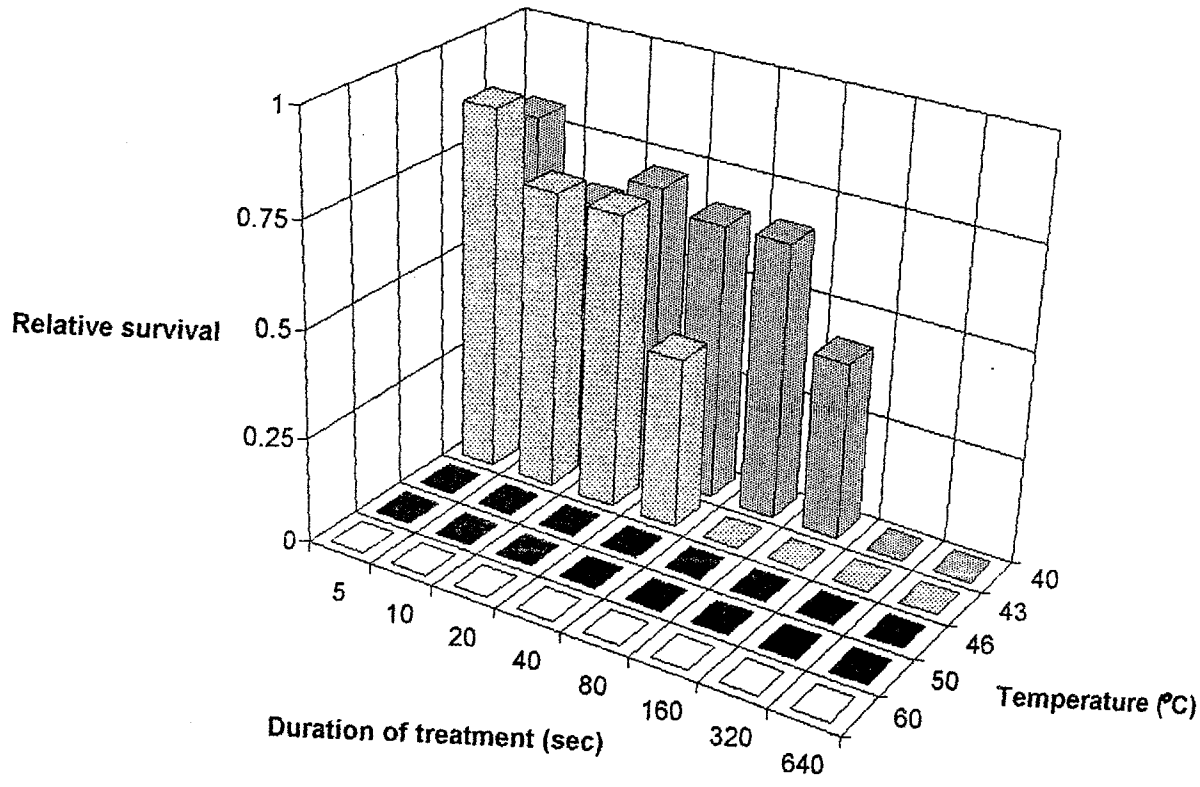
Second instar larva (48 - 52; hAEL)



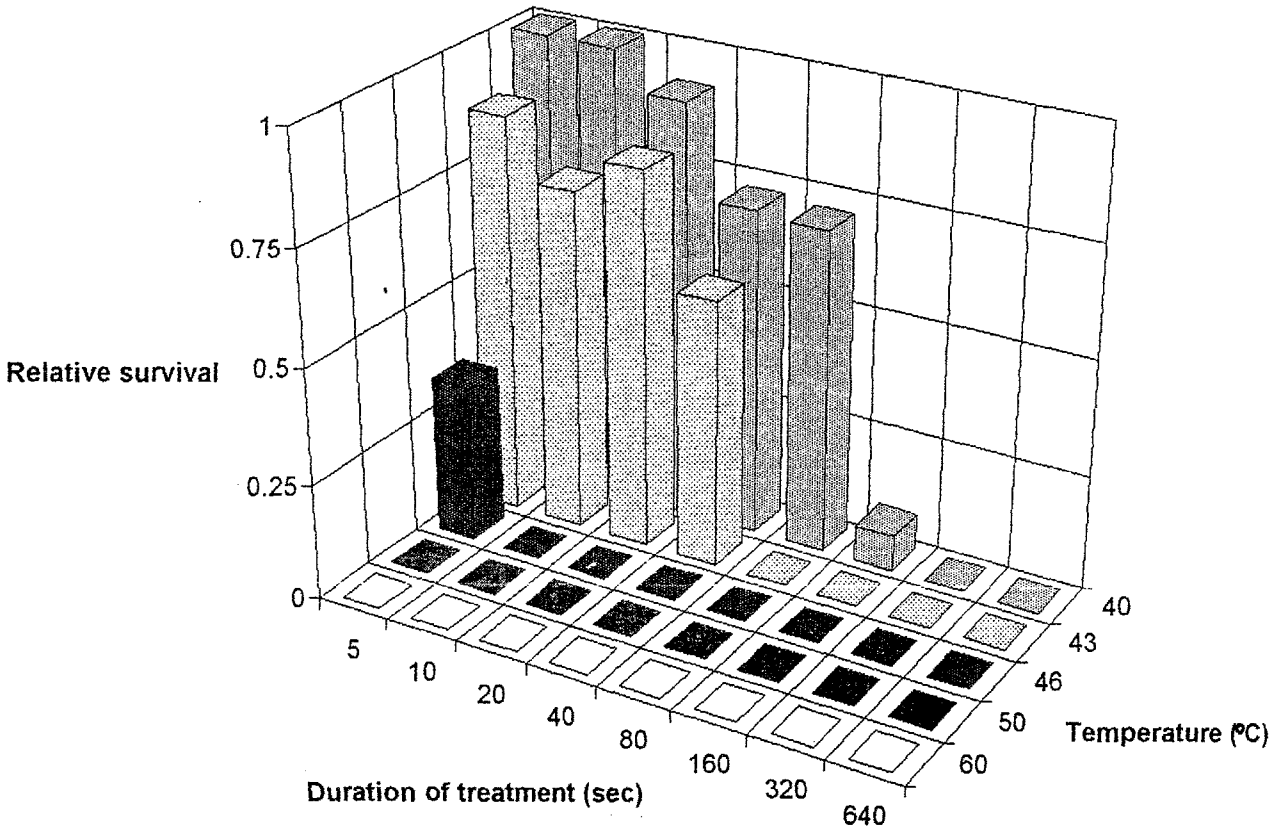
First instar larva (24 - 28; hAEL)



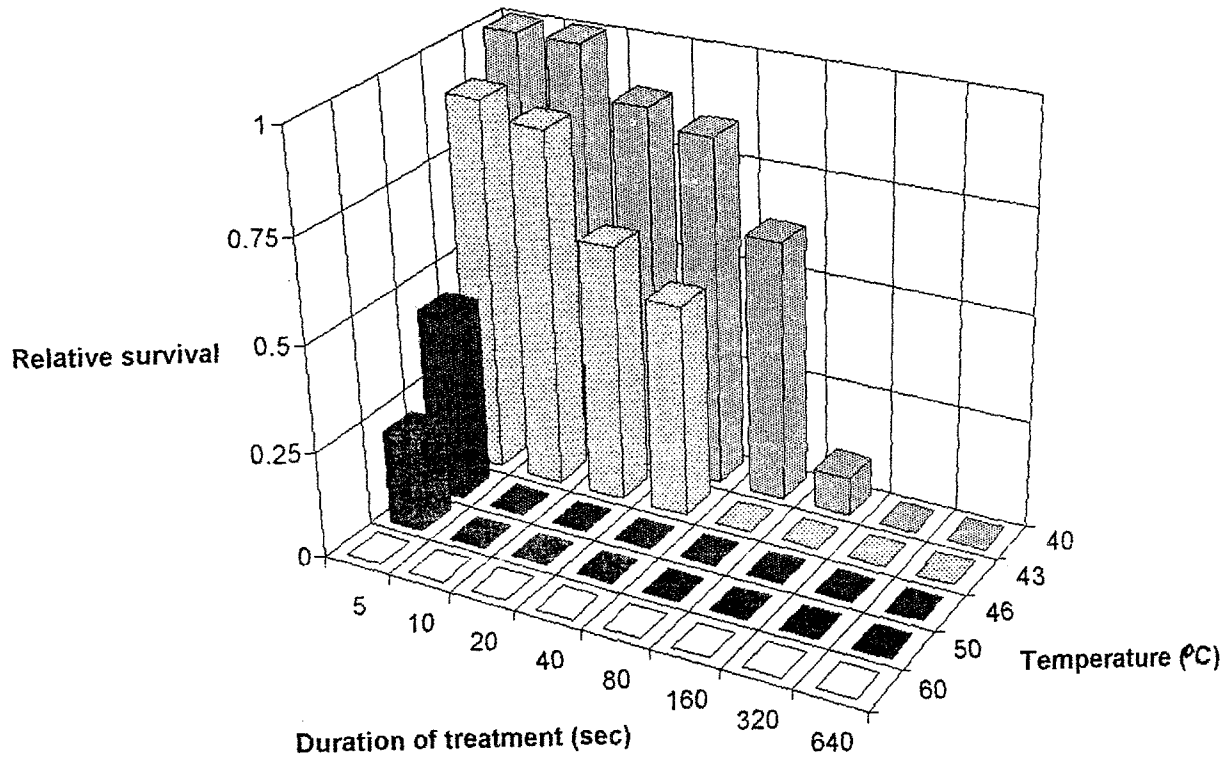
Early third instar larva (72 - 76; hAEL)



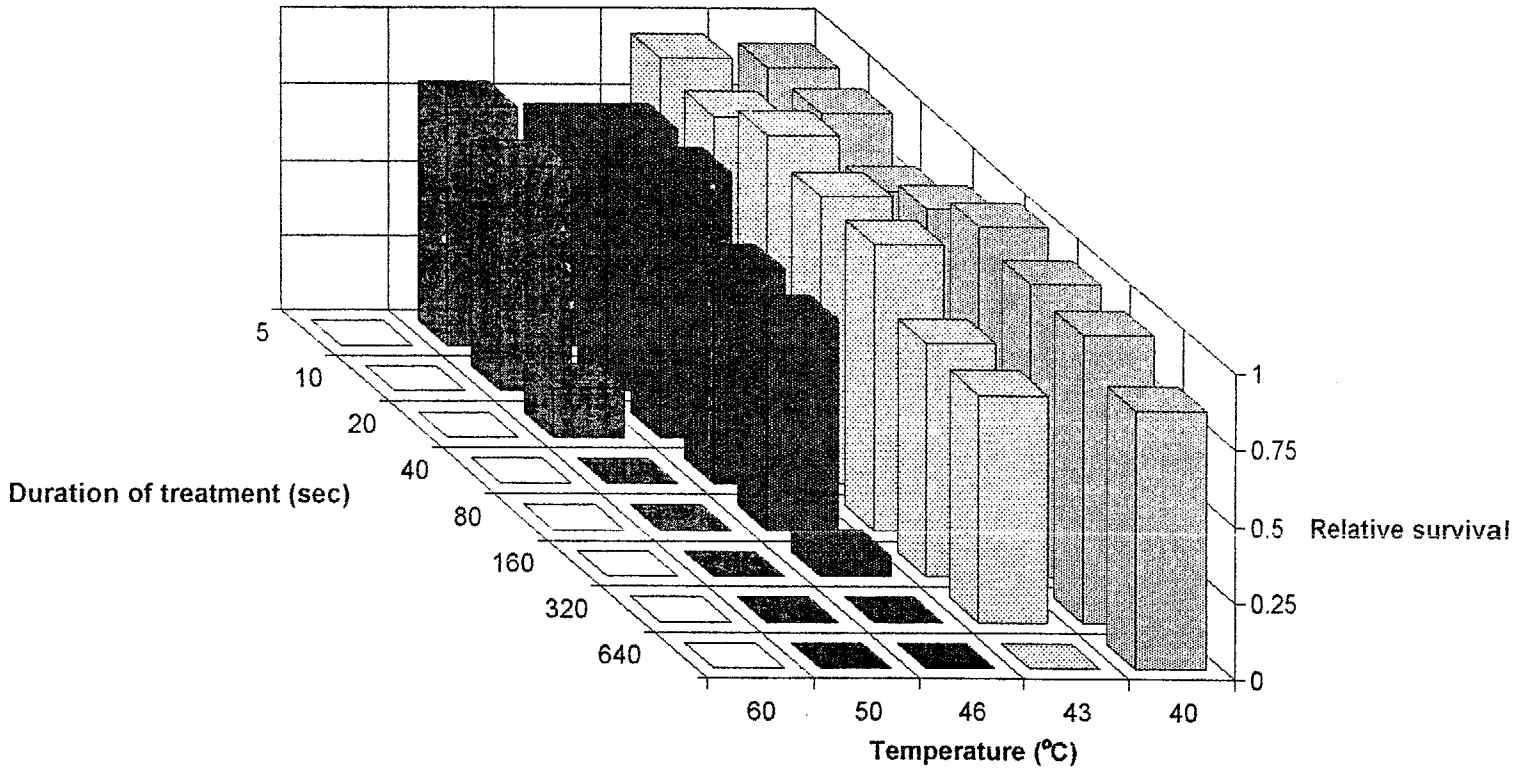
Mid third instar larva (96 - 100; hAEL)



Young pupa (120; hAEL)



Pupa (168; hAEL)



Postharvest Heat Treatment of Apples to Control Apple Scale (*Quadraspidiotus perniciosus*), Green Mold (*Penicillium expansum*), and Maintain Fruit Firmness

Susan Lurie[1], Elazar Fallik[1], Joshua D. Klein[2], Frerenc Kozar[3], Kornel Kovacs[4]

[1]Department of Postharvest Science, ARO, The Volcani Center, Bet Dagan 50250, Israel

[2]Department of Field Crops, ARO, The Volcani Center, Bet Dagan 50250, Israel

[3]Institute of Plant Protection, Hungarian Academy of Sciences, Budapest, Hungary

[4]Biological Research Centre, hungarian Academy of Sciences, H-6701 Szeged, Hungary

Abstract

Postharvest heat treatments were applied to three apple cultivars, 'Anna', 'Golden Delicious' and 'Jonathon'. The temperatures ranged from 50°C to 38°C and from 5 to 96 h. The temperatures of 50°C for 5 or 10 h and 46°C for 10 h controlled all developmental stages of San Jose apple scale (*Quadraspidiotus perniciosus* Comstock) on both 'Golden Delicious' and 'Jonathon' fruit. Forty six, 42° and 38°C were able to prevent germination of *Penicillium expansum* after 28, 34 and 42 h, respectively. The time needed to control the fungus was longer than that required to kill the insect. Fifty degrees caused damage to the apple fruit, while the apples could withstand over 12 h at 46°C and over 24 h at 42°C. At 38°C no damage was found on preclimacteric apples even after 96 h, but if postclimacteric fruit were heated at 38°C, heat damage occurred. The treatments

which 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.

Introduction

Apples are an important world wide 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 growing problems to using chemicals for postharvest, in that both pathogens and insects are developing resistance to the compounds. In addition, consumers are increasingly requesting chemical-free produce.

Penicillium expansum rot occurs in most areas of the world on apples (*Malus domestica)* 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 is transferred to a warm environment. Although some chemicals are effective in retarding or preventing rots caused by *P. expansum* (Burton and Dewey, 1981), 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 (*Quadraspidiotus perniciosus* Comstock), are among the major insect pests of apples. Some countries are free of this insect and have restrictions against importing apples which may be contaminated. Fumigation by methyl bromide or hydrogen cyanide against scale

insects has been used for a long time on both nursery material and on fruits (Riehl, 1990; Mitsume et al., 1994). However, the use of these chemicals poses hazards, and is restricted in different 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 apple fruit to 38°C for four days suppressed both softening and naturally occurring decay, mostly due to *Penicillium* spp., after prolonged storage. Similar benefits on firmness were 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 days at 38°C reduced decay caused by *P. expansum* 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 showed relatively strong tolerance to heat (Kim et al., 1993).

The goal of this work was to determine a postharvest heat treatment which would control both fungal and insect pests on stored apples without decreasing the fruit quality.

Materials and Methods

Heat Treatment of Fruit

Fruit were obtained from different orchards at commercial harvest. Three varieties were used in different experiments; 'Anna', 'Jonathon' and 'Golden Delicious'. The fruits were heated in a temperature-controlled chamber with the

fruit in plastic trays inside nonsealed palstic bags to retard water loss. A water bath was placed inside the chamber to maintain relative humidity at 85%, as measured by a thermohydgrograph. Depending on the experiment, the fruit were either inoculated with fungus, infected with insects, or not treated before the heat treatment. Following the heat treatment the fruits 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*) at 25°C and LD 18:6 photoperiod. Transfer of insects to apples was accomplished by placing them in contact with the watermelon for a 3 day period. The heat treatments were conducted 14 or 21 days after the last day of exposure to scale for 'Jonathon' or 'Golden Delicious', respectively. At this point, as a check of the apples revealed, the *Q. perniciousus* population consisted of the 1st and 2nd instar larva of both sexes, and pronymphal and nymphal males. Experiments on female insects were carried out on 40 to 42 day old insects (about 14 day old females).

Testing of 1st instar diapausing larvae (black scale stage) was conducted on naturally infested fruit collected from a Jonathan orchard. The insects were in diapause for three months.

The fruits were held in the heat chamber for 2 exposure times, 5 and 10 h, and 3 temperatures, 50, 46 and 42°C, as well as a control temperature of 20°C. Following treatment the fruits were placed at 20°C for 5 days 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. The number of insects was 148 to 1380 specimens for each treatment.

Fungal culture and inoculation of fruit

Penicillium expansum was isolated from a diseased 'Golden Delicious' apple and maintained as a single spore culture on PDA at 20°C. For fruit inoculation or *in vitro* tests spores from a 10 day old culture were suspended in sterile distilled water and 0.03% Tween-20. Aliquots (100 μl) were streaked aseptically of PDA plates and exposed to various temperatures. In addition a 5 mm diameter disc from the periphery of a 10 day old culture was placed in a fresh petri dish with PDA and exposed to various temperatures. Germination was determined according to Fallik et al. (1993) and mycelial growth was determined as the increase in colony radius. Both measurements were made 7 days after the plates were transferred from heat to 20°C.

For fruit inoculation, 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 40μl of spore suspension (10^5 spore ml^{-1}) of *P. expansum*. 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.

The apples were then held at 3 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 one month and 'Golden Delicious' apples for four months. Control apples were inoculated and held at either 0° or 20°C without heating. After 14 days at 20°C, with or without a period of storage, 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

Fruits, 'Anna' and 'Golden Delicious', were heated at three temperatures, 46, 42 and 38, for various times before being placed at 0°C storage for one month ('Anna') or four months ('Golden Delicious'). At harvest, at the end of storage and during 7 days 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. In one experiment, 'Anna' apples were held at 20°C and their respiration and ethylene measured daily until the climacteric was reached, at which time they were heat treated.

Results

Heat treatment on insect mortality

Q. perniciousus females were more sensitive to high temperature than a mixed population (Tables 1 and 2). 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 'Jonathon' 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 'Jonathon' and 45% 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). The insects in one stage of development were not more sensitive than those of another. There was a significant interaction between time and temperature on both the female population and the mixed population. Apple cultivar did not affect the mortality of the female insects, but in the mixed population there was greater mortality at lower temperatures on 'Jonathon' apples than on 'Golden Delicious' and therefore a significant interaction of insect with cultivar.

The effect of heat on diapausing first instar of *Q. perniciosus* was tested on naturally infested 'Jonathon' fruit (Table 3). In this developmental stage, both temperature and time of exposure showed a significance of $p = 0.001$. When the naturally infested fruits were heat treated, only 50°C for 5 or 10 h and 10 h at 46°C controlled scale. Five h at 46°C or exposure to 42°C allowed unacceptably high rates of insect survival. The mortality of scale insects in the control group was 34%.

Effect of heat treatment on P. expansum development

Both spore germination and myceliar growth *P. expansum* was found to be sensitive to elevated temperature (Fig. 1). Percentage spore germination and myceliar growth were inversely proportional to length of exposure to 38, 42 and 46°C. Spore germination was more sensitive than myceliar growth at various temperatures. The ET_{50} for spore germination was 42, 34 and 28 h at 38, 42 and 46°C, respectively, whereas for myceliar growth the ET_{50} was 48, 44 and 36 h at those temperatures.

When apples were inoculated with spores which had previously been heated, the severity of the infection was much less than for unheated spores

(Table 4). The difference between decay development of heated and unheated spores increased as the concentration of the spores used for inoculation decreased. The normal inoculation concentration of 10^5 spores ml^{-1} showed a 12 fold difference in the decayed area on the fruit if the spores were previously heated for 4 days at 38°C. 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 apply cultivars inoculated after heating at different temperatures was measured with immediately after treatment or after storing the apples (Table 5). In both varieties tested, storage did not reduce the efficiency of the heat treatment. Fifty degrees heating 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.

P. expansum 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 6). 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 96 h at 38°C prevented decay development in both types of wounds (Table 6). If the apples were first heated and then inoculated decay development was significantly 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. Fifty degrees was found to cause injury 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 7.). At 38°C apples were heated for up to 96 h with no injury.

Apples are generally stored when they are preclimateric, and this ripeness stage may be more or less sensitive to high temperatures than apples more advanced in ripening. To check this 'Anna' apples were heat treated at defined maturity stages (Fig. 2). 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.

Heated apples maintained firmness during storage and shelf life better than unheated fruit (Table 7). 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, both at removal and after shelf life.

Discussion

From this study it appears that there is a wide temperature range of heat treatment which can control insects and fungi and not damage apple fruit. Insects

were more sensitive to high temperature than fungi, perhaps because they are more complicated organisms (Armstrong, 1994). Ten h at 46°C controlled *Q. perniciosus* while 28 or 36 h at this temperature was necessary for prevention of *P. expansum* spore germination or myceliar growth. The time needed at 46°C to control *Q. perniciosus* did not cause damage to preclimacteric apples, but the longer times needed at this temperature for fungal control did cause heat damage.

At the lower temperatures, 42 and 38°C there was less danger of fruit damage. These temperatures also controlled *P. expansum* 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 (Fig. 1 compared to Table 4). 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 is heated before inoculation, the decay develops more slowly than on unheated fruit (Table 6). We observed such a compound 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 both insect and fungal pests and thereby reducing postharvest use of chemicals. Unfortunately, unlike chemicals, the treatment must be individually tailored to each commodity. First, the limits of tolerance at a range of temperatures must be determined for the commodity for the ripeness stage at which it is harvested for marketing or storage. Then the particular pest to be controlled should be tested within the time-temperature constraints which do not damage the product. Different fungi

and insects have different tolerances to temperature. For example, the postharvest pathogens *Penicillium expansum,* and *Botrytis cinerea* are easily controlled by high temperatures, while *Alternaria alternaria* is much more refractive (Fallik et al., 1993, 1995; Barkai Golan, et al.,1993). For insects different tolerances are also found. A study of heat treatment for persimmon disinfestation found that mealy bugs (*Pseudococcus longispinus* Targioni-Tozetti) were more tolerant than apple moth (*Epiphas postvittana* Walter) to both hot water and hot air treatments (Lester et al., 1995, Dentener et al., 1996).

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 which don't 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.

Acknowledgments: This research was partially funded under Grant No. C12-081, US Cooperative Development Research Program and the Ministry of Economic Cooperation of Germany. This is a contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel No. E, 1997.

References

Armstrong, J.W. ,1994. Heat and cold treatments. In: Insect Pests and Fresh Horticultural Products (R. Paull and J. Armstrong, eds.) CAB International, UK pp. 103-120.

Barkai-Golan, R., Padova, R., Ross, I., Lapidot, M., Davidson, H. and Copel. A.. 1993. Combined hot water and radiation treatments to control decay of tomato fruits. Scient. Hort. 56:101-105.

Burton, C.L. and Dewey, D.H. 1981. New fungicide to control benomyl-resistant *Penicillium expansum* in apples. Plant Dis. 65:881-883.

Dentener, P.R., Alexander, S.M., Lester, P.J., Petry, R.J., Maindonald, J.H. and McDonald, R.M., 1996. Hot air treatment for disinfestation of lightbrown apple moth and longtailed mealy bug on persimmons. Postharv. Biol. and Technol. 8:143-152.

Fallik, E., Klein, J., Grinberg, S., Lomaniec, E. and Lurie, S., 1993. Effect of postharvest heat treatment of tomatoes on fruit ripening and decay caused by *Botrytis cinerea*. Plant Dis. 77:985-988.

Fallik, E., Grinberg, S., Gambourg, M., Klein, J.D. and Lurie, S., 1995. Prestorage heat treatment reduces pathogenicity of *Penicillium expansum* in apple fruit. Plant Path. 45:92-97.

Hara, A. H., Hata, T.Y., Hu, B.K.S., Kaneko, R.T. and Tenbrink, U.L., 1994. Hot water immersion of cape jasmine cuttings for disinfestation of green scale (Homoptera: Coccidae). J. Econ. Entom. 87:1569-1573.

Howe, R.B. 1991. Mareting Fresh Fruits and Vegetables. AVI, Van Nostrand, N.Y. pp. 179-203.

Klein, J.D. and Lurie, S., 1992. Heat treatments for improving postharvest quality of horticultural crops. HortTechnology 2:316-320.

Lester, P.J., Dentener, P.R., Petry, R.J. and Alexander, S.M., 1995. Hot water immersion for disinfestation of lightbrown apple moth (*Epiphyas postvittana*) and

longtailed mealy bug (*Pseudococcus longispinus*) on persimmons. Postharv. Biol. and Technol. 6:349-356.

Misumi, T., Kawakami, F., Mizobuchi, M., Tao, M., Machida, M. and Inoue, T., 1994. Methyl bromide fumigation for quarrantine control of Japanese mealybug and citrus mealybug on Satsuma mandarin. Res. Bull. Plant Protect. Japan 30:57-68.

Prusky, D., Bazak, M. and Ben Arie, R., 1985. Development, persistence, survival and strategies for control of thiobenzadole-resistant strains of *Penicillium expansum* on pome fruits. Phytopath. 75:877-882.

Riehl, L.A., 1990. Chemical control. In: Armored Scale Insects, Their Biology, Natural Enemies and Control (D. Rosen, ed.) Elsevier Science Publ., Amersterdam pp. 365-392.

Sams, C.E., Conway, W.S., Abbott, J.A., Lewis, R.J., Ben Shalom, N., 1993. Firmness and decay of apples following postharvest pressure infiltration of calcium and heat treatment. J. Amer. Soc. Hort. Sci. 118:623-627.

Table 1. Effect of heat treatment on the survival of *Q. perniciousus* females on 'Jonathon' and 'Golden Delicious' apple. fruit. Mortality was determined 5 days after the heat treatment while the fruits were held at 20°C. Standard deviation is indicated.

Treatment Temperature	Time of Exposure (h)	Apple Cultivar	Insect Mortality (%)
50°C	5	Jonathon	100
		Golden Delicious	100
	10	Jonathon	100
		Golden Delicious	100
46°C	5	Jonathon	100
		Golden Delicious	98.7±1.02
	10	Jonathon	100
		Golden Delicious	100
42°C	5	Jonathon	12.3±5.12
		Golden Delicious	18.6±14.64
	10	Jonathon	17.5±7.05
		Golden Delicious	17.6±5.94
Control		Jonathon	15.2±4.59
		Golden Delicious	11.8±9.45

Analysis of Variance

Temperature	***
Time	NS
Time x Temperature	**
Cultivar	NS

Table 2. Effect of heat treatment on the survival of a mixed population of *Q. perniciosus* consisting of first and second instar larva, pronymphal and nymphal males on 'Jonathon' and 'Golden Delicious' apples. Mortalily was determined 5 days following the heat treatment holding the fruit at 20°C. Standard deviation is indicated.

Treatment Temperature	Time of Exposure (h)	Apple Cultivar	Insect Mortality (%)
50°C	5	Jonathon	100
		Golden Delicious	100
	10	Jonathon	100
		Golden Delicious	100
46°C	5	Jonathon	88.0±8.12
		Golden Delicious	57.5±11.70
	10	Jonathon	99.5±1.07
		Golden Delicious	100
42°C	5	Jonathon	59.7±15.61
		Golden Delicious	45.6±5.17
	10	Jonathon	87.9±7.58
		Golden Delicious	62.6±2.53
Control		Jonathon	42.2±4.18
		Golden Delicious	45.6±3.15

Analysis of Variance

Temperature	***
Time	***
Time x Temperature	***
Cultivar	***

Table 3. Effect of heat treatment on the survival of *Q. perniciosus* diapausing first instar on 'Jonathon' apples. Mortality was determined 5 days after the heat treatment. Standard deviation is indicated.

Treatment Temperature	Time (h)	Insect Mortality (%)
50°C	5	100
	10	100
46°C	5	62.8±15.08
	10	100
42°C	5	44.3±2.47
	10	42.9±7.88
Control		34.0±6.94

	Analysis of Variance
Temperature	***
Time	**
Time x Temperature	**

Table 4. The effect of heat treatment on decay development in 'Golden Delicious' apple fruit inoculated with different concentrations of *P. expansum* spores which had either been heated for 4 days at 38°C, or not heated. Decay was determined after 14 d at 20°C. Standard deviation is indicated.

Treatment	Inoculum	Decayed Area (cm^2)	
	(spores ml^{-1}	Unheated spores	Heated spores
Nonheated	10^3	0.75±0.01	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	***

Table 5. The effect of heat treatment and storage on decay development in 'Anna' and 'Golden Delicious' apple fruit inoculated with *P. expansum* after 14 d at 20°C, or one month ('Anna') or four months at 0°C ('Golden Delicious'), and an additional 14 d at 20°C. Standard deviation is indicated.

Treatment Temperature	Time	Cultivar	Decayed Area (cm^2) Posttreatment	Poststorage
46°C	12	Anna	1.7±0.02	1.4±0.01
		Golden Delicious	3.1±0.02	1.3±0.01
42°C	24	Anna	0.3±0.01	0.1±0.005
		Golden Delicious	--	--
38°C	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

Table 6. The effect of the wound size on decay development in 'Golden Delicious' apples infected with *P. expansum*. 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. Standard deviation is indicated.

Treatment	Decayed Area (cm^2)	
	Pin wound	Peeling wound
Inoculated-->20°C	28.1±0.8	35.2±1.1
Inoculated-->38°C 4 d	0	15.8±0.9
Wounded-->38°C 4 d then inoculated	0	0.1±0.01
38°C 4 d then inoculated	25.5±0.7	28.1±0.1

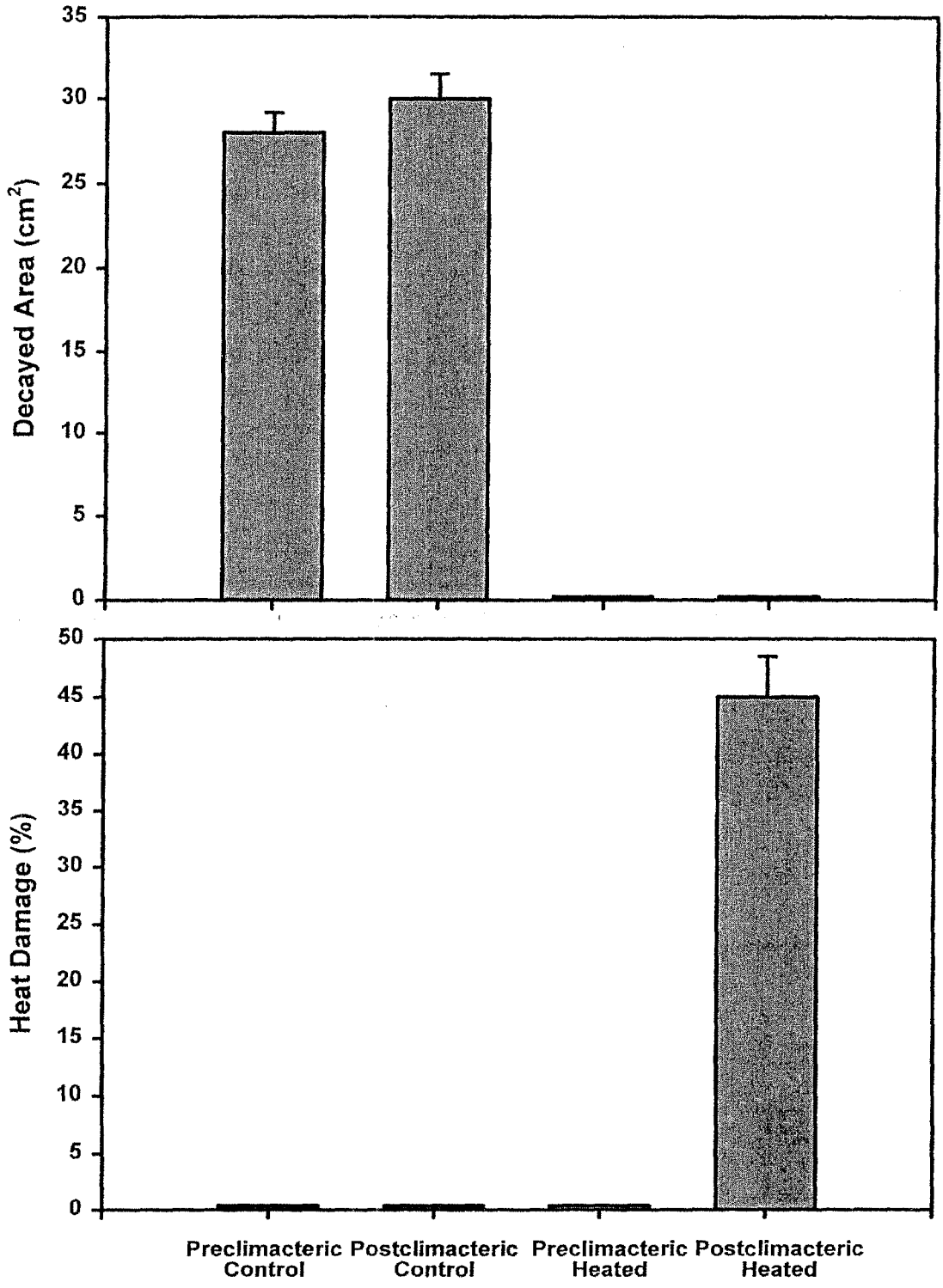
Table 7. Firmness and heat damage of 'Anna' apples after one month 0°C storage. Firmness was measured at removal from storage and after 7 days at 20°C, while heat damage was measured after 7 days at 20°C.

Treatment Temperature	Time	Firmness (N) Removal	Shelf Life	Heat Damage (%)
46°C	12	78	65	0
	24	76	65	100
42°C	24	68	56	0
	48	70	57	83
38°C	72	71	58	0
	96	71	59	0
Control		65	48	0
$LSD_{0.05}$		5.3	5.0	

Figure Legends

Figure 1. Inhibition of spore germination (a) and mycelial growth (b) of *Penicillium expansum* by increasing times of exposure to 38, 42 and 46°C. Data in (a) recorded after 24 h incubation at 20°C following treatment. Data in (b) recorded after 8 days at 20°C following treatment. Standard deviation is indicated.

Figure 2. The effect of heat treatment on decay development and percentage of heat damage in apple fruit cv. 'Anna' inoculated with *Penicillium expansum* as affected by its stage of maturity (pre- or postclimacteric), after 14 days at 20°C.


Decayed Area (cm²)
35
30
25
20
15
10
5
0
Heat Damage (%)
50
45
40
35
30
25
20
15
10
5
0
Preclimacteric Control
Postclimacteric Control
Preclimacteric Heated
Postclimacteric Heated

Heat Treatment of Tomato Fruit to Control Mediterranean Fruit Fly (*Ceratitis capitata* Weidmann) and Maintain Fruit Quality

Susan Lurie[1], Elazar Fallik[1], Joshua D. Klein[2], Laszlo Varjas[3] and Kornel C. Kovacs[4]

[1]Department of Postharvest Science, and [2]Department of Field Crops, ARO, The Volcani Center, Bet Dagan 50250, Israel

[3]Institute of Plant Protection, Hungarian Academy of Sciences, Budapest, Hungary

[4]Biological Research Centre, Hungarian Academy of Sciences, H-6701 Szeged, Hungary

Abstract

Short term (3 or 6 h) and long term (12, 24 and 48 h) heat treatments at 38, 42 or 46°C of pink tomatoes infested with *Ceratitis capitata* Weidmann (Medfly) induced various rates of mortality of the insect pest, depending on the time elapsed after infestation, on the ontogenic phase of the insect, and on the conditions and duration of exposure to heat. The thermotherapy also affected the fruit quality. Short term heat treatments were only effective at 46°C, while 48 h at 38°C was also effective. Times longer than 6 h at 46 or 42°C caused heat damage to the fruit, while 48 or 72 h at 38°C did not cause damage. Organoleptic studies of the 38°C heated fruit showed no decrease in quality compared to unheated fruit. This long term heat treatment shows promise in

being able to control insect infestations. It has previously been shown to decrease fungal infections (Fallik et al., 1993).

Introduction

Heat treatments are increasingly being used to provide quarantine security against pests of fresh agricultural commodities. Two heat treatments have been developed for the Mediterranean fruit fly (*Ceratitis capitata* Wiedmann). One quarantine treatment is a two stage hot water immersion that provides quarantine security against fruit fly eggs (Couey and Hayes, 1986). The second treatment is a vapor heat treatment which involves heating the fruit pulp to 47.2°C in air saturated with water vapor (Armstrong et al., 1989). This treatment is effective against both egg and larval stages of *C. capitata*. Unfortunately, both heat regimes can damage fresh produce, even when used precisely.

High temperatures (37 to 50°) can also inhibit the ripening of many fruits and vegetables (Anthony et al., 1989; Lurie and Klein, 1991; Mitchell, 1986). Furthermore, fruits that are heat treated before storage have been found to be more resistant to low temperature storage than nonheated fruit (Lurie and Klein, 1991; Rodov et al., 1995) Storage of fruits at elevated temperatures is not feasible, as there is excessive weight loss and the ability to ripen normally is lost after extended periods at high temperatures (Eaks, 1978; Tsuji et al., 1984). However, a prestorage heat treatment that would effectively disinfect fruit and slow ripening would be beneficial.

Previous studies on apples showed that no heat injury occurred if they were heated for up to 12 h at 46°C, 24 h at 42°C or 96 h at 38°C (Klein and Lurie, 1992). Tomatoes held for 72 h at 38°C had decreased sensitivity to low temperature and slower and more uniform ripening (Lurie and Klein, 1991; 1992). In addition, this treatment completely inhibited decay caused by *Botrytis cinerea* (Fallik et al., 1993).

In this study, the effect of high temperature on controlling medfly development in tomatoes and the effect of this heat on fruit ripening and quality was also examined.

Materials and Methods

A laboratory culture of the 'Benakio' strain of medfly (*Ceratitis capitata* Weidmann) was established at 25°C and a 12:12 photoperiod. Adult flies were placed into cages of 40x40x60 cm and fed with powdered yeast and sugar plus drinking water. Eggs were collected through a special window of the cage made from a fine net, having holes which could easily be punctured by the egg-laying females. This way eggs were dropped into tap water and collected during a 24 h period. Larvae were reared in small boxes on a special semi-synthetic artificial medium containing dried yeast, sugar, tissue paper and cholesterol.

Tomato fruits were exposed to egg-laying females in the adult cage 4 to 12 days after adult emergence, which period was considered optimal for reproduction. Fruits were kept in the cage for about 6 h. The tomatoes were

then observed under a dissection microscope to localize (and mark with a fine pen) egg-laying punctures on the surface.

Heat treatments were carried out in a thermostated heating chamber equipped with a cooling system and constant air circulation. Heat treatments were set up at three temperatures (38, 42 and 46°C) for varying exposure times (3, 6, 12, 24, 48 h). Treatments were performed 1 or 4 days after infestation which corresponded to egg or L_2-L_3 instar larva, respectively. Heat treated tomatoes and untreated control fruits were held at 25°C.

For evaluation of the efficacy of short-term or long-term thermotherapy, 6 days subsequent to infestation and heat treatment tomato fruits were dissected and the egg chambers (below egg-laying punctures) and the surviving medfly larvae moving in the flesh of fruit were observed. In another experiment, the numbers of *C. capitata* puparia were determined.

For fruit quality experiments, pink fruits were divided into replicates of five fruit, and five replicates (25 fruit) were used for each treatment. The fruits were heated for various times, held for 21 days at 2°C, or for 10 days at 12°C, and then transferred to 20°C for 6 days. At the end of shelf life the fruits were evaluated for ripeness and quality. Firmness was measured nondestructively with a Durometer (Shore Manufacturing, NY) on two sides of each fruit using a scale of 1 to 100. Values above 60 are very firm fruit, and below 30 very soft fruit. Peel color was measured using a Chromameter (Minolta, Japan) using the hue angle scale where 110 was mature green, 80 was pink, and 50 was full red. Soluble solids (SSC) and titratable acidity (TA) were determined on 50 g of

ground tissue from each replicate. SSC was measured with a hand held refractometer (Atago, Japan), and TA by titrating 2 ml of juice to pH 8.2 with 0.1 N NaOH and expressing the result as citric acid. Taste tests were conducted with 40 tasters using the 'smile' system and a scale of 15, from A as best to O as worst (Basker, 1989). The infestation experiments were carried out on commercially purchased Yeoward tomatoes, while the ripening and taste tests were performed on cvs. Daniella (144) and 121.

Results

Short term (3 to 6 h) heat treatment was examined at three temperatures, 46, 42 and 38°C (Table 1). Only 6 h at 46°C prevented totally the development of the insect. Three h at 46°C and 6 h at 42°C decreased insect survival, but did not prevent infestation. Six h at 38°C decreased the number of egg-laying punctures and recovered puparia 30% compared to control fruits.

Increasing time of exposure to lower temperatures was more effective in preventing infestation than the shorter temperatures (Table 2). All times at 46°C prevented hatching of medfly eggs. This was achieved also after 24 h at 42°C and 48 h at 38°C. Twenty-four hours at 38°C also gave good thermotherapeutic action and decreased the number of L_2 and L_3 larvae. Natural mortality as seen in control fruits was not negligible, since 40% of the egg chambers did not contain viable eggs. Forty five percent of the control tomatoes, although all fruits had egg-laying punctures, remained free of medly.

Fungal decay developed on the fruits together with insect infestation. At 46°C, even though infestation was prevented, rots were not, which may reflect heat damage to the tissue. At 42 and 38°C, the times which prevented insect infestation, also prevented fungal infection. The highest level of decay was found in unheated control tomatoes.

If the heat treatment was delayed for 4 days after infestation, the majority of the medly larvae were at the second instar stage. This stage of insect development was more sensitive to heat than late embryonic insects, since 12 h at 42°C was effective here (Table 3) while it was not if the fruits were heated 1 day after infestation (Table 2). However, if the treatment was made 4 days after infestation, the level of fungal rots was extremely high.

Tomatoes which had been heated for 6 h at 46 or 42°C or for longer times at 38°C, reached full ripeness after storage (Table 4). Heating the fruit for 12 or 24 h at 46 or 42°C caused heat damage and high levels of decay. Because of the heat damage no chilling injury could be determined on fruits from these treatments. At 46°C ripening processes were impaired (except for 6 h of heating) and the fruits did not soften or become as red as fruit from other treatments. The unheated control fruits also did not develop full red color after storage, although they softened, and in addition developed a high incidence of chilling injury.

Since 46 and 42°C heating had the potential of causing heat damage, longer times at 38°C, which gave control of medfly with no heat damage, were tested for organoleptic qualities (Table 5). The heated fruit were similar to the

control fruit in their ripeness measurements of peel color, firmness, SSC and TA. A panel of tasters rated the heat treated fruit better tasting, but not significantly so. However, the heated fruit had significantly fewer rots than unheated fruit.

Acknowledgments: This research was partially funded under Grant No. C12-081, US Cooperative Development Research Program and the Ministry of Economic Cooperation of Germany. This is a contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel No. E, 1997.

Table 1. Effect of short term heat treatment on the survival of *Ceratitus capitata* larvae in tomatoes heated 1 day after infestation, when the medfly was in a late embryonic stage of development. Evaluation was based on numbers of recovered puparia 6 days after infestation. The number of fruits used in each treatment was 5 to 13.

Treatment		Egg-laying	Puparia per fruit
Temperature (°C)	Time (h)	punctures per fruit	
46	6	2	0
	3	1.2	2.6
42	6	3.1	4.7
38	6	2	12.7
Control		3.8	17.3

Table 2. Effect of heat treatment on the survival of *Ceratitis capitata* larvae in tomatoes treated 1 day after infestation, when the medfly was in a late stage of embryonic development. Evaluation was based on investigation of dissected fruits 6 days after infestation. The number of fruits used in each treatment was 8 to 22.

Treatment		No. of egg	Percentage of Fruits		
Temperature (°C)	Time (h)	chambers	L_1 larvae	L_2-L_3 larvae	Decay
46	24	37	0	0	29
	12	41	0	0	17
	6	46	0	0	15
42	24	32	0	0	0
	12	37	57	59	6
	6	41	73	85	15
38	48	16	0	0	0
	24	10	10	13	0
Control		35	57	55	41

Table 3. Effect of heat treatment on the survival of *Ceratitis capitata* larvae in tomatoes heated 4 days after infestation, when the medfly was in a late stage of second instar. Evaluation was based on investigation of dissected fruits 6 days after infestation. The number of fruits used in each treatment was 6 to 13.

Treatment		No of egg	Percentage of fruit with	
Temperature (°C)	Time (h)	chambers	L_2-L_3 lavae	Decay
46	24	21	0	67
	12	18	0	83
42	24	20	0	100
	12	22	0	43
Control		34	31	54

Table 4. The effect of heating pink tomato fruits for different times and temperatures on peel color, fruit firmness and fruit damage after 3 weeks storage at 2°C and 6 days at 20°C.

Treatment		Peel	Firmness	Percentage of fruits with		
Temperature (°C)	Time (h)	color (H)	(Durometer units)	Chilling Injury Damage	Rots	Heat
46	24	70.1±4.7	46.2±6.0	-	100	100
	12	65.5±5.2	45.1±5.5	-	68	100
	6	51.6±9.2	35.8±5.4	20	27	0
42	24	58.7±7.6	41.0±5.5	-	50	100
	12	60.1±4.3	40.3±3.1	10	20	50
	6	55.4±6.9	36.9±6.0	14	0	0
38	72	64.1±4.5	30.5±6.2	0	0	0
	48	57.2±3.6	38.1±4.2	4	0	0
Control		75.1±7.2	36.4±4.5	77	33	-

Table 5. The effect of heating tomatoes for 72 h at 38°C on quality parameters and decay after 10 days at 12°C and 6 days at 20°C.

Treatment	Peel color (H)	Firmness (Durometer units)	SSC (%)	TA (%)	Taste*	Decay (%)
Heated	51.8	36.6	4.3	0.41	H	1b
Control	49.2	36.6	4.3	0.43	K	5a
	N.S.	N. S..	N.S.	N.S.	N.S.	

* A= best rating, H=average, O= poorest rating

Letters indicate significant difference at the 0.05 level according to Duncan's multiple range test. N.S. = not significant.

6. Impact, Relevance, and Technology Transfer.

In a time of increased awareness among consumers that many of the chemical treatments of fruits and vegetables to control insects, diseases and physiological disorders are potentially harmful to humans, there is a need to develop effective, non-damaging physical treatments for insect disinfestation and disease control in fresh horticultural products. Heat treatment is one of these possibilities. This project dealt with developing an integrated treatment for controlling insects and pathogens while maintaining commodity quality. The methods were transferred to Hungary and were used both for a model insect system and then on 'real' pests which infest apples and tomatoes. A treatment was developed for both of these commodities which fulfill the aims of the project. In addition, research was conducted on the effect of the treatment in inhibiting ripening which has relevance to the effect of heat on other commodities.

7. Project Activities

The PI attended a meeting on postharvest research in Agadir, Morocco in 1994 and in Taupo, New Zealand in 1996 and presented some results from this project. In addition, a visit was paid to Hungary in 1996 where the scientists met and went over the results which are incorporated in the papers in section 5.3. The Hungarian scientist did not pay a visit to Israel, but a visit was made to the Mediterranean fruit fly research center on Crete to acquire a defined strain of fruit fly and to learn how to propagate and control them.

8. The project accomplished all of the proposed goals.

9. Future Work. The direction of future work is towards shorter periods of heat treatment to make the procedure more commercially attractive. Particularly since we found that a hot water dip could affect the tomato's response to chilling injury similarly to a long hot air treatment, and also hot water dips could control pepper rots, we are trying to examine these treatments more closely. In addition, a machine has been developed to spray hot water on the produce, which appears also to beneficially affect the postharvest life.