

Effect of Postharvest Heat Treatment of Tomatoes on Fruit Ripening and Decay Caused by *Botrytis cinerea*

ELAZAR FALLIK, Department of Fruit and Vegetable Storage, JOSHUA KLEIN, Department of Agronomy and Natural Resources, and SHOSHANA GRINBERG, ELLA LOMANIEC, SUSAN LURIE, and AVRAHAM LALAZAR, Department of Fruit and Vegetable Storage, ARO, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel

ABSTRACT

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

Holding inoculated mature green and pink tomato fruits for 3 days at 38 C completely inhibited decay caused by *Botrytis cinerea*, one of the main postharvest pathogens of tomatoes in Israel. Conidial germination was more sensitive than mycelial growth to 38 C, but inhibition of both processes increased with duration of treatment. The prophylactic effect of heating on decay of tomatoes caused by *B. cinerea* appeared to be by direct interaction with the fungus rather than by inhibitory effects on fruit ripening. Carbon dioxide production by tomatoes increased and ethylene production decreased during heating, but when fruit were moved to 20 C, gas evolution rates returned to those of nonheated fruit. Heating did not affect tomato firmness, color, soluble solids content, or acidity by the end of 7 days at 20 C. Prestorage heating may be a useful nonchemical method of controlling postharvest pathogens.

Additional keywords: disease index, *Lycopersicon esculentum*

Botrytis cinerea Pers.:Fr. is one of the major postharvest pathogens of tomato (*Lycopersicon esculentum* Mill.) fruit, which is highly susceptible to fungal rot during storage (3). This fungus can also initiate latent infections of young fruit through attached withered floral parts in the field and then cause rot during storage (11). There is no registered postharvest fungicide for control of decay in tomatoes caused by *B. cinerea*, which has prompted research into alternative, nonchemical methods of pathogen control.

Heat treatments have been reported to inhibit postharvest fungal germination and growth (8,20). Heat can be applied to fruits and vegetables as hot water dips, vapor heat, or hot dry air. Dipping for 1-2 min in water heated to 55 C was the optimal antifungal treatment for control of *Alternaria*, *Fusarium*, *Rhizopus*, and *Mucor* species on melon fruits (23). Vapor heat (60 min at 44 C) has been used against *B. cinerea* in strawberries (10), while exposing pear fruits to 37 C for 2 days prior to storage inhibited decay due to *Mucor piriformis* E. Fisch. and *Phialophora malorum* (M.N. Kidd & A. Beaumont) McColloch (21).

High temperatures (37-50 C) also inhibit the ripening of many fruits and vegetables (1,15,17). Furthermore, mature green tomatoes that were held for

3 days at 38 C before being stored for 3 wk at 2 C did not develop chilling injury, whereas nontreated tomatoes did (15).

Most research on the effect of hot-air treatments (36-40 C) on tomatoes has focused on the physiological rather than the phytopathological effects and has involved mature green rather than pink or red fruit (15,16,18). However, consumer preference for tomatoes that are as close as possible to "vine ripe" has led the Israeli tomato industry to harvest fruit for export at the pink rather than the mature green stage. The purpose of these experiments was to examine the effect of exposure to 38 C on decay caused by *B. cinerea* in mature green and pink tomatoes and on ripening indices of heated fruit.

MATERIALS AND METHODS

Effects of exposure time. *B. cinerea* was isolated from a diseased tomato fruit and cultured on potato-dextrose agar (PDA). A conidial suspension was prepared in sterile distilled water from a 10-day-old single-conidium culture grown on PDA at 20 C. The suspension was agitated before and during inoculation in order to maintain uniform conidial distribution.

Sample (100- μ l) aliquots of the suspension (5×10^4 ml⁻¹ spores) were streaked aseptically onto PDA plates or on a tomato homogenate (TH) medium. The latter was prepared from mature green or pink tomato and solidified with 1.5% agar (Difco), as described previously (5). A 5-mm-diameter disk from

the periphery of a 10-day-old culture was placed in the center of a 9-cm petri dish containing PDA or TH medium. The plates were then incubated at 38 C in trays covered with plastic bags to prevent desiccation. The temperature of the medium reached 38 C within 30 min (E. Fallik, unpublished). Every 12 hr over a period of 3 days, four plates were transferred from the 38 C incubator to one set at 20 C. After 7 days of incubation at 20 C, conidial germination was measured as described previously (4), while mycelial growth was determined as the average colony radius. The experiment was conducted twice, with four replicates in each trial.

Plant material. Mature green and pink tomatoes (cv. F-144) of uniform size and color were harvested three separate times from a commercial greenhouse. At each harvest, fruit were wiped with cotton pads that had been soaked in sterile distilled water. The fruit were then divided into two lots. One set of fruits was injected with 100 μ l of a 5×10^4 ml⁻¹ conidial suspension 3 mm deep into the pericarp. This inoculum concentration is similar to the natural concentration of *B. cinerea* found in Israeli greenhouses during moderate to heavy infestation (R. Barkai-Golan, personal communication). The second set of fruits was injected similarly with 100 μ l of sterile water. The control and inoculated fruit lots were then each further subdivided into two groups, one of which was placed at 20 C while the other was held at 38 C for 4 days and then transferred to the 20 C chamber. Fruit were placed in plastic trays inside unsealed polyethylene bags to retard water loss and were then heated in a temperature-controlled chamber. A water bath was placed inside the chamber to maintain relative humidity of $85 \pm 3\%$, as measured by a thermohygrograph. Pulp temperature reached 38 C within 4 hr (S. Lurie and J. Klein, unpublished). All fruit held at 20 C were kept in cartons covered with perforated polyethylene bags in order to prevent excessive water loss.

Ten fruit from each treatment were removed from the heat chamber every 24 hr during a period of 96 hr and placed at 20 C for 12 days. Decay severity was measured at the end of shelf life. Ten inoculated fruit at each maturity stage (mature green and pink) were kept at

Contribution No. 3564-E, 1992 series, from the ARO, Volcani Center.

Accepted for publication 14 May 1993.

20 C throughout the experiment to serve as nontreated controls. Disease severity was recorded subjectively on a scale of 0-5, where 0 = no decay development, 1 = decay up to 0.5 cm in diameter without sporulation, 2 = decay between 0.5 and 1.0 cm in diameter with sporulation beginning, 3 = decay between 1.0 and 2.5 cm in diameter with sporulation, 4 = decay between 2.5 and 4.0 cm in diameter with sporulation and mycelium, and 5 = fruit completely rotten and heavily covered with mycelium. The experiment was performed three times.

Ethylene and CO₂ evolution, color measurement, and firmness. The maturity indices and production rates of ethylene and CO₂ were compared with heat-treated and nontreated fruit from each

of the three harvests. One set of fruits was held at 38 C for 3 days and then transferred to 20 C for 7 days, whereas the control fruit were held at 20 C for 10 days. Ethylene and CO₂ evolution was measured by placing one fruit in each of 10 replicate 0.5-L jars for each treatment and closing the jars for 2 hr each day. Headspace gases were sampled by syringe through a septum in the jar lid. The jars were left open and ventilated for 22 hr between measurements. The experiment was conducted three times.

Ethylene was measured with a gas chromatograph equipped with an FID detector and an alumina column held at 80 C, with nitrogen as the carrier gas. Carbon dioxide was measured with a TCD detector with a Poropak N column

held at 25 C, with helium as the carrier gas.

Another set of 10 fruits was measured for weight loss, color development, and softening at 0, 3, 7, and 10 days after harvest. Color was measured on a Minolta Chroma-Meter that was calibrated with a white standard tile. Three sides of each tomato were measured and the results were expressed as hue angle ($\tan^{-1}(b/a)$) (7). Fruit firmness was measured with a Durometer (Shore Instrument and Manufacturing, Jamaica, NY) on three sides of each fruit.

Analysis of total soluble solids (TSS) and titratable acidity. Two replicates, each consisting of 50 g of pericarp tissue from 10 fruits in each treatment, were frozen for 48 hr and then thawed. The juice was expressed manually through cheesecloth for determination of TSS and titratable acidity. TSS was measured by a manual refractometer, and the results were expressed as percentage of TSS. Aliquots (5 ml) of juice were titrated to pH 8.1 with 0.1 N NaOH, and the results were expressed as percentage of citric acid (22). Fruit sampling times were as for the color, firmness, and weight loss measurements.

RESULTS

Effects of exposure times. Mycelial growth and percent conidial germination were inversely proportional to length of time of exposure to 38 C (Fig. 1). The ED₅₀ for conidial germination was approximately 14 hr at 38 C. Tube length of surviving spores was markedly reduced compared with that in controls (*data not shown*). Results from PDA and TH medium were similar.

After 12 days at 20 C, all pink fruits were covered heavily with mycelia and most had collapsed (Fig. 2). In contrast, holding fruit at 38 C for 24 or 48 hr reduced the disease index proportionately, whereas no decay developed in inoculated pink fruit kept at 38 C for 72 hr. Although nonheated mature green fruit were much more resistant to disease than pink fruit, they still benefited from the heat treatment.

Ethylene and CO₂ evolution. By the end of the heating regime, ethylene production by both mature green and pink tomatoes was inhibited (Fig. 3). Peak ethylene production by nonheated mature green fruit occurred on day 7 after harvest, while that by heated mature green fruit occurred on day 8, after 5 days at 20 C. Pink tomatoes that were held at a constant 20 C after harvest never attained a peak of ethylene production.

In contrast to the inhibition of ethylene production, CO₂ production by both mature green and pink tomatoes was enhanced dramatically during the first day at 38 C but subsequently fell. Within 24 hr after removal to 20 C, the CO₂ production rate of heated fruit was the same as that of fruit held continuously at 20 C

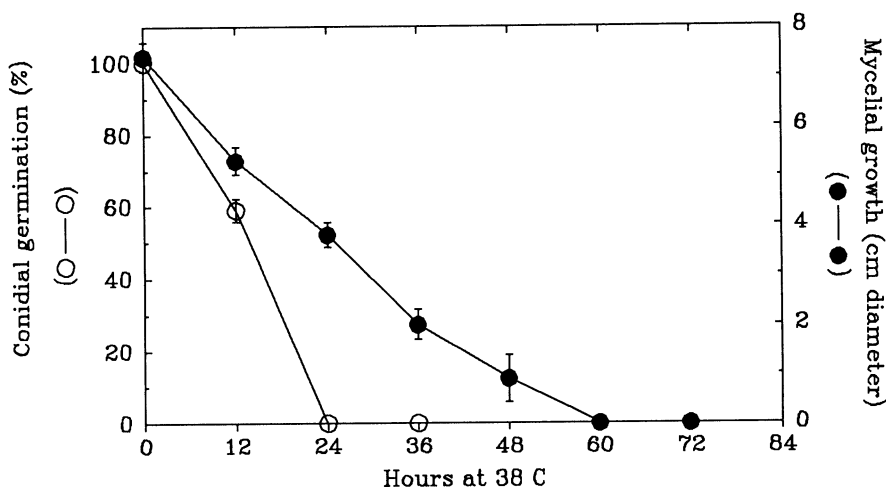


Fig. 1. Germination of conidia and growth of *Botrytis cinerea* mycelium at increasing times of exposure to 38 C. Bars indicate SE.

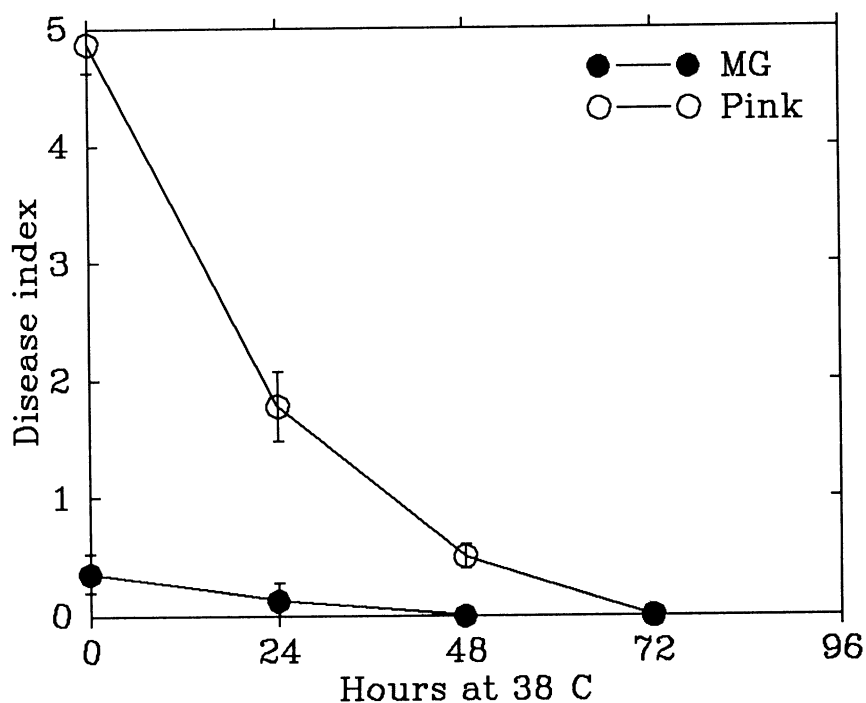


Fig. 2. Disease index of mature green (MG) and pink tomato fruit inoculated with *Botrytis cinerea* as affected by length of exposure to 38 C. Fruits were examined after 12 days at 20 C. Bars indicate SE.

(Fig. 4). The CO₂ production rate of mature green fruit held at 20 C peaked on day 7, whereas the peak CO₂ production of heated mature green fruit was on day 9 after harvest. No CO₂ peak was noted in pink tomatoes at 20 C, whether or not they were heated.

Weight loss. Weight loss by mature green and pink fruit was similar, averaging 2.7% by the end of shelf life, regardless of treatment. In no case did weight loss result in shriveling or cracking.

Ripeness indices. Firmness. Although heated mature green fruit were consistently 3 to 7 N softer during shelf life than nonheated mature green fruit, no such differences were observed between heated and nonheated pink fruit (Fig. 5A). At the end of shelf life (day 10 after harvest), mature green fruit registered a firmness of 55 N (nonheated) or 52 N (heated), as opposed to 40 N for both heated and nonheated pink fruit.

Color. Color development in both mature green and pink fruit stopped during heating. However, by day 10 after harvest, all fruit had attained a uniform red color, regardless of heating treatment or initial stage of maturity (Fig. 5B).

Total soluble solids and citric acid. Heating delayed the rise in TSS percentage in both mature green and pink fruit, but at the end of shelf life, mature green fruit had reached 5.4% TSS, whereas pink fruit had attained 6% TSS, regardless of heating (Fig. 5C). The decrease in citric acid during shelf life was delayed by heating mature green fruit, but not pink fruit. By the end of shelf life, citric acid concentration in mature green fruit was 0.45%, whereas pink fruit had only 0.35% citric acid (Fig. 5D).

DISCUSSION

Holding inoculated mature green and pink tomato fruits for 3 days at 38 C provided effective, nontoxic protection against decay caused by *B. cinerea*, one of the main pathogens of tomatoes in Israel. Heat affected the etiology of the disease itself, as shown by the in vitro inhibition of both spore germination and mycelial growth (Fig. 1), although the former was more susceptible to increasing lengths of exposure to 38 C. We had hypothesized that an additional protective mechanism against fungal attack in heated tomatoes could be the action of heat in delaying fruit ripening (13). Heated tomatoes would therefore be less susceptible to *B. cinerea*, which usually attacks ripe fruit (14).

Although heat treatment inhibited ripening processes during 3 days at 38 C, neither heating nor inoculation substantially affected fruit firmness, color, soluble solids content, or acidity by the end of 7 days shelf life at 20 C (Fig. 5). The 1-day delay in the ethylene climacteric noted in mature green fruit (Fig. 3) was not reflected in the ripeness indices

of those same fruit. Ripening indices of melons were also unaffected by heating (23). No viable conidia were isolated from the inoculation site of pink fruit heated for 72 hr (12). The prophylactic effect of heating on decay of tomatoes caused by *B. cinerea* appears to be by direct interaction with the fungus rather than by inhibition of fruit ripening.

Carbon dioxide production was enhanced and ethylene production decreased during heating, but gas evolution rates returned to those of nonheated fruit upon removal from 38 C. Similar results were found by Lurie and Klein (15,16) with mature green tomatoes, even after storage at 12 C for 2 wk. Barkai-Golan et al (6) found that ethylene production was directly proportional to wound dimension in mature green tomatoes, but the 25-gauge needle we used did not in-

duce ethylene production in water-injected control fruit.

The general appearance of heated fruit did not differ from that of nonheated fruit. Heated fruit did not shrivel or show other signs of thermal distress. The 2-3% weight loss that resulted from an experimental heating regime was no greater than that experienced under commercial conditions, in which tomatoes are shipped in nonwrapped cartons for up to 12 days at 12 C (Y. Fuchs, *personal communication*).

Although mature green fruit remained firmer than pink fruit by the end of shelf life, they still achieved similar red color (Fig. 5A and B). However, the ratio of soluble solids to citric acid was only 12 in mature green fruit, whereas that for pink fruit reached 17. This relative increase in sugars and other soluble solids

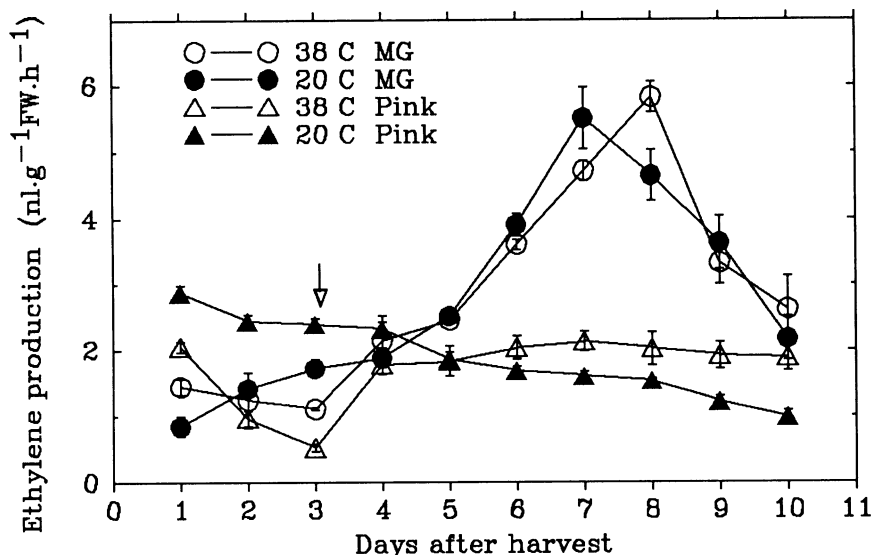


Fig. 3. Ethylene production rate of mature green (MG) and pink tomato fruit as affected by exposure to 38 C for 3 days. Arrow indicates removal from 38 C to 20 C. Bars indicate SE.

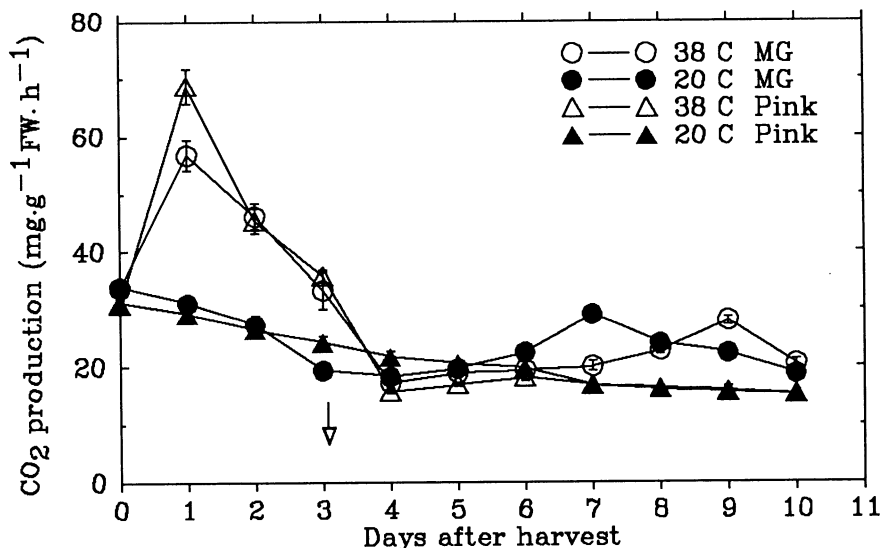


Fig. 4. CO₂ production rate of mature green (MG) and pink tomato fruit as affected by exposure to 38 C for 3 days. Arrow indicates removal from 38 C to 20 C. Bars indicate SE.

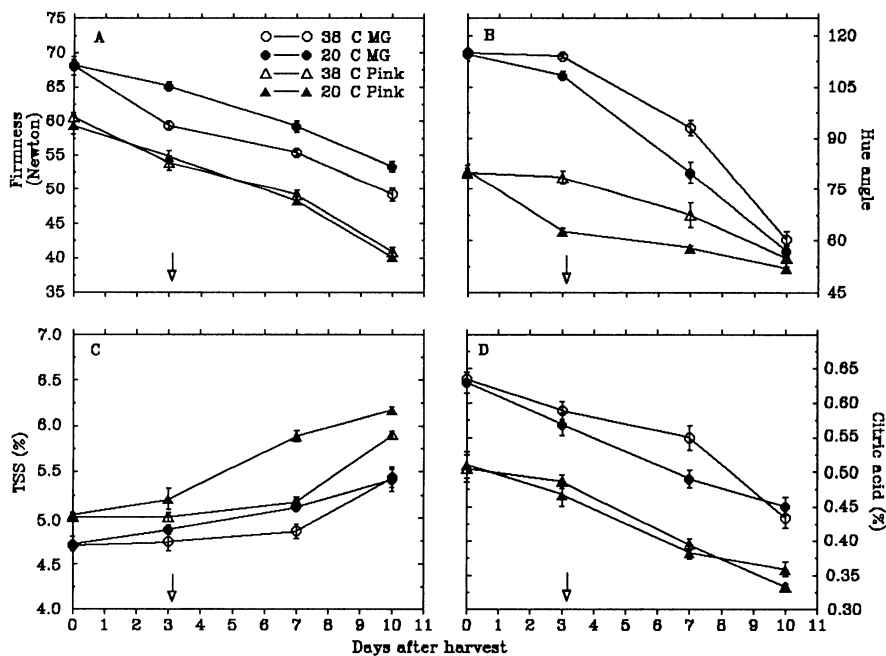


Fig. 5. (A) Firmness, (B) hue angle, (C) percent total soluble solids (TSS), and (D) percent citric acid of mature green (MG) and pink tomato fruit as affected by exposure to 38 C for 3 days. Arrows indicate removal from 38 C to 20 C. Bars indicate SE.

may partially explain why riper fruits are preferred substrates for fungi. Fruit harvested at the pink rather than the mature green stage are also likely to be more acceptable to consumers, regardless of heat treatment. Although heating did not affect objective flavor measurements such as soluble solids and acidity, controlled taste tests are still needed to determine subjective consumer reactions to heat-treated tomatoes. In preliminary experiments, panelists did not discern any off-flavors in heated mature green or pink tomatoes after 6 days at 20 C (12).

Lurie and Klein (15) found that pre-storage heating conferred resistance to chilling injury on mature green tomatoes held for 3 wk at 2 C. Such fruit ripened normally upon removal from storage and did not have the sunken brown spots and rots characteristic of chilling injury. Storage of tomatoes at lower temperatures than those currently practiced could aid in suppressing development of postharvest diseases such as those caused by *Alternaria* sp. In contrast to *B. cinerea*, heating for 3 days at 38 C only reduced in vitro spore germination and mycelial growth of *Alternaria* by approximately 40% (E. Fallik, unpublished). Combining a prestorage high temperature exposure with a low temperature storage regime is currently being investigated for control of postharvest pathogens of tomatoes and cucumbers.

Chemical means of preserving postharvest fruit quality that are acceptable

to consumers are becoming increasingly limited. Although there is considerable variation in sensitivity to high temperature among various fungi (19), postharvest heat treatments of fresh fruits and vegetables have potential as non-chemical control measures against decay-inducing organisms (8,13). The use of water dips or vapor heat at 39–52 C for 2–10 min has been reported to control in vitro and in vivo spore germination and decay development of postharvest fungi in tomato (2), papaya (9), and melons (23). However, only mangoes currently undergo a commercial postharvest antifungal heat treatment (20). Exceeding the recommended exposure times even briefly can cause significant injury with hot-water or vapor treatments (8). In the air-heating method described here, however, the “safety range” of exposure time to heat can be measured in hours rather than minutes, as is the case with hot water treatments. Prestorage heating in air therefore may be of significant benefit as a nonchemical fungicidal treatment.

LITERATURE CITED

1. Anthony, B. R., Phillips, D. J., Badr, S., and Aharoni, Y. 1989. Decay control and quality maintenance after moist air heat treatment of plastic wrapped nectarines. *J. Am. Soc. Hortic. Sci.* 114:946-949.
2. Barkai-Golan, R. 1973. Postharvest heat treatment to control *Alternaria tenuis* Auct. rot in tomato. *Phytopathol. Mediterr.* 12:108-111.
3. Barkai-Golan, R. 1981. Annotated checked list of fungi causing postharvest diseases of fruits

and vegetables in Israel. *Agric. Res. Organ. Volcani Center Spec. Publ.* 194.

4. Barkai-Golan, R., Kahan, R. S., and Padova, R. 1969. Synergistic effects of gamma radiation and heat on the development of *Penicillium digitatum* in vitro and in stored citrus fruits. *Phytopathology* 59:922-924.
5. Barkai-Golan, R., Kopeliovitch, E., and Brady, C. J. 1986. Detection of polygalacturonase enzymes in fruits of both a normal tomato and its nonripening *Nor* mutant infected with *Rhizopus stolonifer*. *Phytopathology* 76:42-45.
6. Barkai-Golan, R., Lavy-Meir, G., and Kopeliovitch, E. 1989. Stimulation of fruit ethylene production by wounding and by *Botrytis cinerea* and *Geotrichum candidum* infection in normal and non-ripening tomatoes. *J. Phytopathol.* 125:148-156.
7. Cheng, T. S., and Shewfelt, R. L. 1988. Effect of chilling exposure of tomatoes during subsequent ripening. *J. Food Sci.* 53:1160-1162.
8. Couey, H. M. 1989. Heat treatment for control of postharvest diseases and insect pest of fruits. *HortScience* 24:198-202.
9. Couey, H. M., Alvarez, A. M., and Nelson, M. G. 1984. Comparison of hot-water spray and immersion treatments for control of postharvest decay of papaya. *Plant Dis.* 68:436-437.
10. Couey, H. M., and Follstad, M. N. 1966. Heat pasteurization for control of postharvest decay of fresh strawberries. *Phytopathology* 56:1345-1347.
11. Dennis, C. 1983. Salad crops. Pages 157-177 in: *Post-Harvest Pathology of Fruits and Vegetables*. C. Dennis, ed. Academic Press, London.
12. Fallik, E., Grinberg, S., Davidson, H., Lomanic, E., Lurie, S., Klein, J., Lalazar, A., and Basker, D. 1993. Postharvest heat treatment of tomatoes to reduce rots while maintaining fruit quality. (In Hebrew, with English summary.) *Hassadeh* 73:973-975.
13. Klein, J. D., and Lurie, S. 1991. Postharvest heat treatment and fruit quality. *Postharvest News Inf.* 2:15-19.
14. Lavy-Meir, G., Barkai-Golan, R., and Kopeliovitch, E. 1989. Resistance of tomato ripening mutants and their hybrids to *Botrytis cinerea*. *Plant Dis.* 73:976-978.
15. Lurie, S., and 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.
16. Lurie, S., and Klein, J. D. 1992. Ripening characteristics of tomatoes stored at 12 C and 2 C following a prestorage heat treatment. *Sci. Hortic.* 51:55-64.
17. Mitchell, F. G. 1986. Influence of cooling and temperature maintenance on stone fruit quality. *Decid. Fruit Grower* 36:205-211.
18. Saltveit, M. E., and Cabrera, R. M. 1987. Tomato fruit temperature before chilling influences ripening after chilling. *HortScience* 22:452-454.
19. Sommer, N. F., Fortlage, R. J., Buckley, P. M., and Maxie, E. C. 1967. Radiation-heat synergism for inactivation of market disease fungi of stone fruits. *Phytopathology* 57:428-433.
20. Spalding, D. H., and Reeder, W. F. 1986. Decay and susceptibility of mangos treated with combinations of hot water, imazalil, and γ -radiation. *Plant Dis.* 70:1149-1151.
21. Spotts, R. A., and Chen, P. M. 1987. Prestorage heat treatment for control of decay of pear fruit. *Phytopathology* 77:1578-1582.
22. Stadman, F. H., Buhlert, J. E., and Marsh, G. L. 1977. Titratable acidity of tomato juice as affected by break procedure. *J. Food Sci.* 42:379-382.
23. Teitel, D. C., Barkai-Golan, R., Aharoni, Y., Copel, A., and Davidson, H. 1991. Toward a practical postharvest heat treatment for 'Galia' melons. *Sci. Hortic.* 45:339-344.