Effect of Heat Treatment on Green Mold Infection in Tangerine Fruit cv. Sai Num Pung

Sirisopha Inkha[1*], Danai Boonyakiat[2] and Sombat Srichuwong[3]

[1]Postharvest Technology Institute, Chiang Mai University, Chiang Mai 50200, Thailand

[2]Department of Horticulture, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

[3]Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

**Corresponding author. E-mail: sirisophai@yahoo.com*

ABSTRACT

The effect of heat treatment on Penicillium digitatum infection which caused green mold rot in tangerine fruit cv. Sai Num Pung was studied. Fungus was dipped in hot water at 45±2, 50±2 and 55±2°C for 0.5, 1, 2 and 3 minutes/ each treatment. The results showed that hot water dip at 55±2°C for 3 minutes was the best in delaying P. digitatum spore germination when incubated fungus at 25±2°C in darkness for 48 hours. Dipping tangerine fruit in hot water at the temperature and time mentioned above before and after inoculation compared with the control fruits that were inoculated by fungus and without hot water dip and uninoculated fruit reduced disease severity (lesion diameter) from 9.68 cm. to 0.32 cm. Dipping tangerine fruit in hot water at 50±2°C for 3 minutes and 55±2°C for 2 and 3 minutes after inoculation was able to delay disease incidence and severity and reduced sporulation index of P. digitatum when stored at 24±2°C and 90±5% relative humidity for 5 days.

Key words: Tangerine fruit, Citrus fruit, Green mold rot, Hot water treatment.

INTRODUCTION

Tangerine fruit is the most economically important citrus crop in Chiang Mai (Thailand). The most widely grown cultivar is Sai Num Pung. Owing to the fact that tangerines are smaller than other citrus fruits and the peel structure is thinner, so they can be easily damaged (SARDI Citrus Information, 2007). Damage in terms of injuries which occurs during harvest and subsequent handling allows the entry of wound pathogens, including *Penicillium digitatum* Sacc., the causal agent of green mold rot. This pathogen is prevalent in almost all regions of the world where citrus is grown, and causes serious postharvest losses annually (Palou et al., 2001; Obagwu and Korsten, 2003). Postharvest chemical treatments are very effective in controlling decay and are widely used on citrus. Recently,

there has been an increased demand for fresh horticultural commodities with less or no chemical residues. A number of fungicides are no longer registered for use on fresh citrus, including those that were used to effectively control postharvest diseases. There are only three fungicides (imazalil, thiabendazole and sodium o-phenylphenate) currently registered for postharvest use on citrus and there are problems like development of resistant pathogenic strains and environmental concerns in disposing the chemicals (Wilson et al., 1994; Ben-Yehoshua et al., 1997). Several chemical-free technologies to extend the storage and shelf life of fresh produce are being investigated. Heat treatment appears to be one of the most promising means for postharvest control of decay (Fallik et al., 2001). Many fresh horticultural products can tolerate temperatures of 50°C to 60°C for up to 10 minutes, but shorter exposure at this temperature can control many postharvest diseases (Barkai-Golan and Phillips, 1991). Prestorage heat treatments to control decay are often applied for a relatively short time (minutes) because the target pathogens are usually found on the surface or in the first few cell layers under the skin of the fruit. Heat treatments against decay-causing agents may be applied to fruits in several ways: by hot water (HW) dips, by vapor heat or by hot dry air (Klein and Lurie, 1991). Hot water treatments (HWT) were originally used to control fungal development, but their use has been extended to disinfection of insects (Lurie, 1998). The benefits of HWT for control of decay caused by *Penicillium* spp. on citrus have been reported (Schirra and D'hallewin, 1997; Porat et al., 2000; Palou et al., 2001). Fawcett (1922) conducted the first studies using HWT and first reported the control of decay in oranges. For citrus, HW dips at 50-53°C for 2-3 minutes were shown to be as effective as curing at 36°C for 72 hours in controlling postharvest decay and chilling injury in various citrus fruits and are much less expensive, mainly because of shorter treatment duration (Rodov et al., 1995). Dipping grapefruit in water at 53°C for 3 minutes resulted in about 50% reduction in decay (Rodov et al., 1995). Ben-Yehoshua et al., (2000) reported that the effective temperature range for 2 minutes of grapefruit dip treatments was between 51 and 54°C, temperatures above 54°C caused brown discoloration of the peel and temperatures below 51°C were not effective in reducing decay. However, their efficacy against green mold has not been evaluated on tangerine fruits. In the present study, we examined the optimum temperatures and exposure periods required to control green mold rot on artificially-inoculated tangerine fruits.

MATERIALS AND METHODS

Plant material:

Sai Num Pung tangerine fruits (*Citrus reticulata* Blanco) were obtained from a commercial orchard in Fang, Chiang Mai province, Thailand and harvested at 9 month after full bloom. Fruits were harvested in November, 2006 when commercially mature. After being harvested, the fruits were selected by hand from field bins before any commercial postharvest treatment was imposed. Samples of blemish-free fruits of uniform size and appearance were washed with water at room temperature (20±2°C), air-dried and placed at random in plastic baskets.

Postharvest HWT and storage conditions:

HWT at 45±2, 50±2 and 55±2°C for 0.5, 1, 2 and 3 minutes for each treatment were applied for tangerine fruit dipping. After treatments, the fruits were incubated at 24±2°C and 90±5% relative humidity (RH) for 5 days. Each treatment comprised of three replicated boxes, each containing 10 fruits.

Fungal cultures:

P. digitatum was obtained from an infected tangerine fruit and cultured on potato dextrose agar. Spore suspensions were prepared by removing the spores from the sporulation edges of a 1-2-week-old culture with a bacteriological loop, and suspending them in sterile distilled water. Spore concentration was determined by a haemacytometer and adjusted to 10^5 spores ml^{-1}.

Effect of HWT on *P. digitatum* spore germination *in vitro*:

Tests on conidial viability after exposure to heat treatments were carried out. Glass tubes containing 1.2 ml of distilled water were placed in water bath at 45, 50 and 55°C. When water in the tubes reached the temperature (measured by thermometer), 0.8 ml of a concentrated *P. digitatum* spore suspension was added to the tubes to achieve a final concentration of $2x10^5$ spores ml^{-1}. After 0.5, 1, 2 and 3 minutes, the tubes were immediately cooled in ice water. The control tubes were placed in water bath at 20°C for 1 minute. Aliquots (50 µl) of the spore suspensions were transferred to glass tubes containing 450 µl of 10% potato dextrose broth. Samples of these solutions (30 µl drops) were placed on ethanol-washed microscope slides (three drops per slide), kept in Petri dishes padded with moistened filter paper and incubated for 24 or 48 hours at 25±2°C in darkness. Spore germination was measured in three microscope fields, each containing 180-200 spores, under a light microscope.

Effect of HWT on infection of *P. digitatum in vivo*:

Tangerine fruits were wound-inoculated with a dissecting needle (2 mm long and 1 mm wide) that had been dipped into a spore suspension (10^5 spores ml^{-1}) of *P. digitatum* at two sites midway between the stem and stylar end. Fruits were kept for 3 hours after inoculation at room temperature to simulate actual conditions between harvest and application of control treatments. After 3 hours, fruits were dipped in water bath at 45±2, 50±2 and 55±2°C for 0.5, 1, 2 and 3 minutes/each treatment. Afterwards, fruits were removed from the water bath and drenched with tap water (20±2°C), air-dried and placed in boxes. A second batch of fruits was dipped in hot water at the temperature and time mentioned above and left for 45 minutes before inoculation. The control treatment was divided into 2 groups, first was inoculated by fungus without hot-water-dipped fruits (untreated fruits) and second was inoculated by sterile water without hot-water-dipped fruits (uninoculated fruits). Disease incidence, severity (lesion diameter) and sporulation index describes the percentage of the fruit surface covered with green mold spores where 0 = no sporulation on the surface of the fruit; 1 = 1-20%; 2 = 21-40%; 3 = 41-60%; 4 = 61-80% and 5 = > 80%, as well as external disease appearance,

were recorded daily after inoculation at 24±2°C and 90±5% RH for 5 days. The experiment was conducted in completely randomized design (CRD) with 26 treatments. Each treatment comprised three replicated boxes, each containing 10 fruits (total of 60 wounds), and the experiment was repeated twice.

Data analysis:

Analysis of variance (ANOVA) and mean separation within each inspection time was calculated where applicable, using the least-significant difference (LSD) test together with Duncan's multiple range test at P=0.05.

RESULTS

To establish a postharvest HWT that is efficient in disinfecting tangerine fruits, we first examined the effect of various heating periods on the *in vitro* spore germination of the green mold pathogen *P. digitatum*. Short exposure of 0.5 minute at 55±2°C resulted in spore germination of 81.00% but a longer exposure of 2 and 3 minutes at 50±2°C could inhibit spore germination of 53.33% and 18.50% after 24 hours, respectively. Heating at 55±2°C was more effective in inhibiting *P. digitatum* spore germination than heating at 45±2 and 50±2°C while longer exposures of 1, 2 and 3 minutes at 55±2°C only delayed spore germination. The exposure of 1 minute at 55±2°C, spore germinated 7.00% after 24 hours and 15.00% after 48 hours, for 2 minutes exposure, spore germinated 1.00% after 24 hours and 8.67% after 48 hours and for 3 minutes exposure, spore germinated 0.00% after 24 hours and 1.67% after 48 hours (Figures 1 and 2).

To evaluate the effects of HWT on eradication of established infections, Sai Num Pung tangerine fruits were wound-inoculated with *P. digitatum* spore suspension. The results showed that hot water dips at 50±2°C for 3 minutes and 55±2°C for 2 and 3 minutes after inoculation were more effective in reducing the development of green mold, and were significantly different from control untreated fruit. Disease incidence on the control treatment inoculated by fungus without hot-water-dipped fruits reached 96.67% which was higher than the incidence of green molds on fruit treated with hot water dips at 50±2°C for 3 minutes and 55±2°C for 2 and 3 minutes after inoculation which were 41.67, 6.67 and 7.00%, respectively. Moreover, dipping at the temperature and time mentioned above after inoculation reduced disease severity from 9.68 cm. of control to 2.61, 0.32 and 1.62 cm. of hot water dips at 50±2°C for 3 minutes and 55±2°C for 2 and 3 minutes, respectively, and reduced sporulation index level from 4.36 of control to 0.28, 0.07 and 0.36 of hot water dips at 50±2°C for 3 minutes and 55±2°C for 2 and 3 minutes, respectively, when stored at 24±2°C and 90±5% relative humidity for 5 days (Table 1).

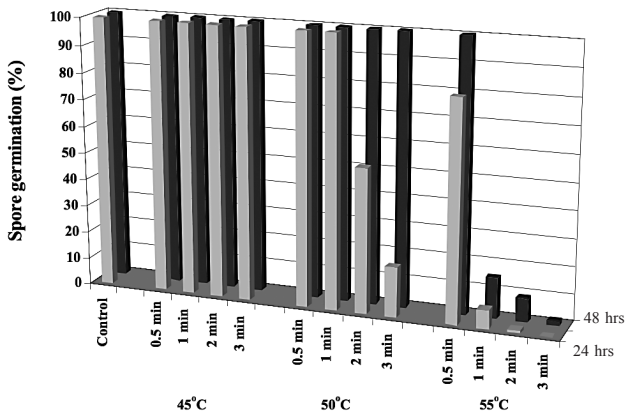

Figure 1. Effect of various heat exposures on *Penicillium digitatum* spore germination *in vitro*. *P. digitatum* spore suspensions were dipped in hot water for various periods, and the percentage of germination was measured after 24 and 48 hours at 25±2°C. Values are means of three replications per treatment, each containing 180-200 spores.

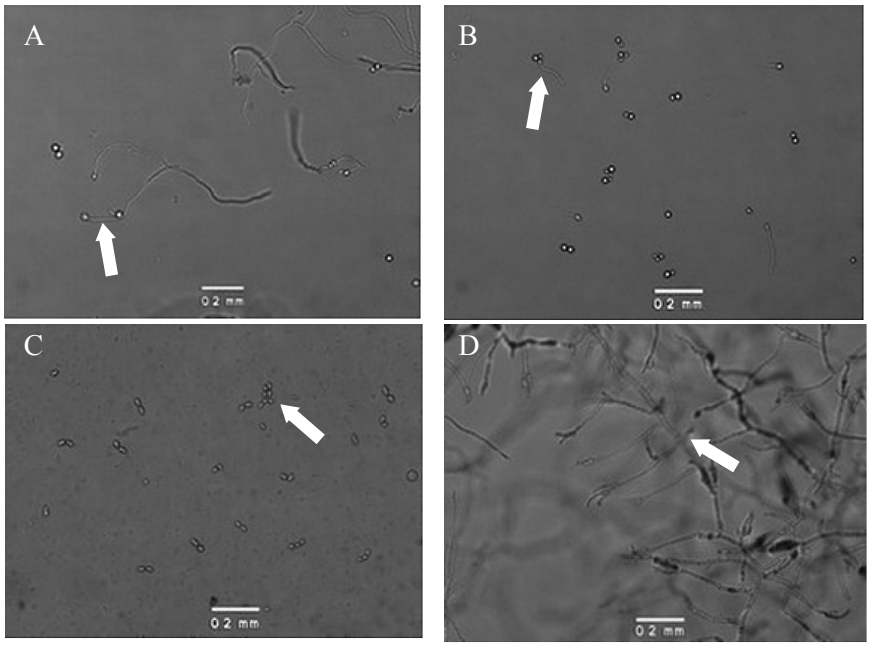

Figure 2. Effect of hot water treatment at 55±2°C for various minutes on spore germination of *Penicillium digitatum* when stored after 48 hours at 25±2°C by a light microscope (x20).
A = dipped for 1 minute; B = dipped for 2 minutes; C = dipped for 3 minutes; D = control

Table 1. Effect of hot water treatments on green mold rot disease incidence, severity and sporulation index on artificially-inoculated tangerine fruits when stored at 24±2°C and 90±5% relative humidity for 5 days.

Treatments	Disease incidence (%)	Disease severity (cm)	Sporulation index
Inoculation before hot water treatments			
45°C 0.5 min	92.00±10.58^{ab1}	8.41±3.68^{bcde2}	4.28±0.47^{a2}
45°C 1 min	95.00± 8.66^{ab}	8.80±3.34^{bcde}	4.28±0.47^{a}
45°C 2 min	84.33± 4.04^{abc}	7.01±3.89^{cdef}	3.36±1.08^{b}
45°C 3 min	72.67±20.03^{bcd}	6.21±4.34^{efg}	2.43±1.34^{c}
50°C 0.5 min	76.00±26.23^{abcd}	6.46±3.88^{defg}	2.43±1.60^{c}
50°C 1 min	88.00±10.58^{ab}	7.36±3.41^{bcdef}	2.43±0.85^{c}
50°C 2 min	62.33±19.40^{cde}	4.55±3.13^{gh}	0.93±0.83^{de}
50°C 3 min	41.67± 2.89^{e}	2.61±2.72^{hi}	0.28±0.61^{f}
55°C 0.5 min	60.00±34.64^{de}	5.30±4.05^{fg}	1.86±1.17^{c}
55°C 1 min	63.00±22.52^{cde}	4.36±3.68^{gh}	1.21±1.19^{d}
55°C 2 min	6.67±11.55^{f}	0.32±1.19^{j}	0.07±0.27^{f}
55°C 3 min	7.00±17.52^{f}	1.62±3.50^{ij}	0.36±0.63^{ef}
Inoculation after hot water treatments			
45°C 0.5 min	95.00± 8.66^{ab}	8.95±2.85^{bcd}	3.86±0.86^{ab}
45°C 1 min	93.33±11.55^{ab}	8.66±2.24b^{cde}	4.28±0.82^{a}
45°C 2 min	87.33±15.53^{ab}	8.56±2.69^{bcde}	4.21±1.05^{a}
45°C 3 min	93.33±11.55^{ab}	9.28±2.51^{bc}	4.28±0.82^{a}
50°C 0.5 min	95.00±8.66^{ab}	8.78±2.18^{bcde}	4.36±0.84^{a}
50°C 1 min	98.33±2.89^{a}	9.45±3.58^{bc}	4.50±0.52^{a}
50°C 2 min	93.33±11.55^{ab}	11.78±4.52^{a}	4.21±0.42^{a}
50°C 3 min	90.67±10.07^{ab}	8.36±3.98^{bcde}	3.78±0.80^{ab}
55°C 0.5 min	83.33± 5.77^{abcd}	8.70±2.88^{bcde}	4.00±0.68^{ab}
55°C 1 min	92.00±10.58^{ab}	8.81±1.96^{bcde}	4.14±0.36^{a}
55°C 2 min	93.33±11.55^{ab}	9.22±2.31^{bc}	4.14±0.36^{a}
55°C 3 min	93.33±11.55^{ab}	8.62±1.89^{bcde}	4.14±0.36^{a}
Inoculation without			
hot water treatments	96.67±5.77^{a}	9.68±2.26^{ab}	4.36±0.50^{a}
Uninoculated	0.00±0.00^{f}	0.00±0.00^{j}	0.00±0.00^{f}
$LSD_{0.05}$	23.37	-	-
C.V. (%)	18.87	-	-

[1] = Mean separation within column groups is by least-significant difference (LSD) test at P = 0.05.
[2] = Mean separation within column groups is by Duncan's multiple range test at P = 0.05.

DISCUSSION AND CONCLUSION

Postharvest heat treatments have been used for many years to control fungal disease in fruits and vegetables (Barkai-Golan and Phillips, 1991; Lurie, 1998). During the last few years, heat treatments have attracted increasing interest as a result of the growing demand to reduce the postharvest use of chemical fungicides. With citrus fruits, hot-water-dip treatments were reported to control postharvest decay several decades ago (Rodov et al., 1995; Schirra and Mulas, 1995). Several recent studies have confirmed that hot-water-dips, usually at 53°C for 2-3 minutes, with or without the addition of fungicides, were capable of reducing decay in a wide range of citrus cultivars (Schirra and D'hallewin, 1997; Ben-Yehoshua, 2003).

In the present study, we found that HWT at 55±2°C for 1, 2 and 3 minutes reduced spore germination, 48 hours after inoculation with *P. digitatum* spore suspension, by about 85-95% as compared with control (Figures 1 and 2). Schirra et al., (2000) reported that heat treatments have a direct effect on fungal pathogens by slowing germ tube elongation or by inactivating or killing the germinating spores. Moreover, HWT 50±2°C for 3 minutes and 55±2°C for 2 and 3 minutes after inoculation were more effective in reducing green mold rot development in Sai Num Pung tangerine fruits to 45-90% as compared with control untreated fruits (Table 1). The beneficial effects of HWT in reducing green mold rot develop-ment in Sai Num Pung tangerine fruits are consistent with those of previous studies on a wide variety of citrus fruit (Rodov et al., 1995; Gonzalez-Aguilar et al., 1997; Schirra and D'hallewin, 1997; Schirra et al., 2004). The significant reduction in decay development in postharvest citrus fruit treated with HWT is considered to be mainly due to the host-pathogen interactions modulated by the treatments and partly to the reduction in the epiphytic microorganism population, compared to untreated fruit (Porat et al., 2000; Schirra et al., 2000). The primary postharvest pathogen of citrus fruit in many places is *P. digitatum*, a wound pathogen. Wounds are mostly made and inoculated when the fruit are harvested and HWT affects the control of the pathogen inside these wounds. Hot-water-dipping reportedly had a transient inhibitory effect on *P. digitatum,* arresting its growth for 24-48 hours. During this lag period when the pathogen was arrested, the combined effects of the pathogen and the hot-water-dip induced the build up of resistance in the peel (Ben-Yehoshua, 2003). The effect of HWT on citrus fruit may be associated with melting and redistribution of natural epicuticular wax on the fruit surface, plugging numerous microscopic cuticular cracks and stomata to improve physical barriers to pathogen penetration (e.g., Botrytis cinerea whose spores can germinate and penetrate the surface of fruit) (Porat et al., 2000). In fact, natural openings and barely visible cracks in the epidermis of treated fruit were partially or entirely sealed with rearranged natural wax components present on the cuticle, thus limiting sites of fungal penetration into the fruit (Rodov et al., 1995; Schirra and D'hallewin, 1997). This mechanism can prevent the development of decay in fruit.

In conclusion, the results demonstrate that a postharvest HWT at temperatures 50±2°C for 3 minutes and 55±2°C for 2 and 3 minutes significantly reduced green mold rot in Sai Num Pung tangerine fruits. There are a narrow range of

temperatures and duration that can delay green mold rot development and the suitability of this treatment in Thai citrus packinghouse operations. The advantages of HWT are that it also cleans the fruit and improves its general appearance. In addition, it is simple to apply in the citrus industry since it can be incorporated into the packinghouse sorting line and does not require any special handling. Besides, marketing of hot-water-treated fruit is not encumbered by fungicide residues, and it can qualify for specialty classifications such as 'organic', which command much higher prices. However, up-scaling of the hot dip method from the laboratory to packinghouse scale demands additional technical solutions to maintain a desired treatment regime with large masses of fruit.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support from Postharvest Technology Institute, Chiang Mai University and the Graduate School, Chiang Mai University, Ministry of Education, Thailand.

REFERENCES

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

Ben-Yehoshua, S., V. Rodov, and J. Peretz. 1997. The constitutive and induced resistance of citrus fruit against pathogens. pp. 78-92. In G.I. Johnson, E. Highly and D.C. Joyce (eds) Disease resistance in fruit. ACIAR Proc. No. 80, Canberra, Australia.

Ben-Yehoshua, S., J. Peretz, V. Rodov, and B. Nafussi. 2000. Postharvest application of hot water treatment in citrus fruits: The road from laboratory to the packing-house. Acta Hort. 518: 19-28.

Ben-Yehoshua, S. 2003. Effect of postharvest heat and UV applications on decay, chilling injury and resistance against pathogens of citrus and other fruits and vegetable. Acta Hort. 599: 159-173.

Fallik, E., S. Tuvia-Alaklai, A. Copel, A. Wiseblum, and R. Regev. 2001. A short hot water rinse and brushes: a technology to reduce postharvest losses-4 years of research. Acta Hort. 553: 413-416.

Fawcett, H.S. 1922. Packing house control of brown rot. Citrograph 7: 232-234.

Gonzalez-Aguilar, G.A., L. Zacarias, M. Mulas, and M.T. Lafuente. 1997. Temperature and duration of water dips influence chilling injury, decay and polyamine content in 'Fortune' mandarins. Postharvest Biol. Technol. 12: 61-69.

Klein, J. D., and S. Lurie. 1991. Postharvest heat treatment and fruit quality. Postharvest News Info. 2: 15-19.

Lurie, S. 1998. Postharvest heat treatments of horticultural crops. Hort. Rev. 22: 91-121.

Obagwu, J., and L. Korsten. 2003. Integrated control of citrus green and blue molds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. Postharvest Biol. Technol. 28: 187-194.

Palou, L., J. L. Smilanick, J. Usall, and I. Vihas. 2001. Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Dis. 85 (4): 371-376.

Porat, R., A. Daus, B.Weiss, L. Cohen, E. Fallik, and S. Droby. 2000. Reduction of postharvest decay in organic citrus fruit by a short hot water brushing treatment. Postharvest Biol. Technol. 18: 151-157.

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

SARDI Citrus Information. 2007. Postharvest Handling of Citrus: Mandarin Packing Line Recommendations. [Online]. Available:http://www.sardi.sa.gov.au/hort/cit_page/hand_app.html. (November 7, 2007).

Schirra, M., and M. Mulas. 1995. Improving storability of 'Tarocco' oranges by postharvest hot-dip fungicide treatments. Postharvest Biol. Technol. 6: 129-138.

Schirra, M., and G. D'hallewin. 1997. Storage performance of fortune mandarins following hot water dips. Postharvest Biol. Technol. 10: 229-238.

Schirra, M., G. D'hallewin, S. Ben-Yehoshua, and E. Fallik. 2000. Host-pathogen interactions modulated by heat treatment. Postharvest Biol. Technol. 21: 71-85.

Schirra, M., M. Mulas, A. Fadda, and E. Cauli. 2004. Cold quarantine responses of blood oranges to postharvest hot water and hot air treatments. Postharvest Biol. Technol. 32: 191-200.

Wilson, C.L., A. El Ghaouth, E. Chalutz, S. Droby, C. Stevens, J.Y. Lu, V. Khan, and J. Arul. 1994. Potential of induced resistance to control postharvest diseases of fruits and vegetables. Plant Dis. 78: 837-844.

none