Effect of Heated Solutions on Decay Control and Residues of Imazalil in Lemons

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Freshly harvested lemons [(*Citrus limon* (L.) Burm)] were dipped 3 min in water with and without imazalil (IMZ) at 50, 100, and 200 ppm at 50 °C and at 1000 ppm IMZ at 20 °C. Following treatments fruit were kept at 9 °C and 90%−95% relative humidity (RH) for 13 weeks and an additional week at 21 °C and *ca*. 75% RH, to simulate a marketing period (SMP). No decay control was observed with fruit dipped in water at 50 °C. In contrast, IMZ treatments provided 90%−96% control of *Penicillium* rots during cold storage and SMP. Fungi other than *Penicillium* spp. were also found in all samples as differences among treatments were negligible. IMZ treatment caused some external damage to the fruit (peel browning), and the percentage of damaged fruit was related to the amount of active ingredient (AI) present in it. Dipping in 200 or 1000 ppm IMZ promoted off-flavor development after 10 weeks of storage, and fruit were judged to be unacceptable for consumption after 13 weeks of cold storage. After 1000 ppm IMZ dipping at 20 °C, residue concentration in fruit was 8.20 ppm; this value doubled that found in a previous investigation on lemons treated with comparable IMZ levels. Residue concentrations in fruit after treatment at 50 °C was strictly related to the amount of fungicide employed. After 13 weeks AI residues in fruit decreased to average *ca*. 35% of the initial values. During the 1 week SMP, residue levels decreased by a further *ca*. 25%. It was concluded that it is possible to achieve significant control of decay in lemons during long-term storage by dipping fruit in 50 ppm IMZ mixtures at 50 °C. Such treatment should be advised to remarkably reduce potential pollution in the environment due to packinghouse wastewater disposal.

Keywords: *Citrus limon; postharvest; heat treatments; imazalil*

INTRODUCTION

Over the last decade, the ever growing fear of risks coming from chemical residue contamination of horticultural crops and the public environmental concern on wastewater disposal after treatments have led to the implementation of studies and the improvement of postharvest technologies aimed at replacing or reducing the use of agrochemicals. Namely, postharvest heat treatments (high-temperature conditioning, hot water dipping) have been shown to reduce decay development, enhance fruit resistance to chilling injury, and greatly increase fungicide efficacy in various horticultural crops (Couey, 1989; Barkai-Golan and Phillips, 1991).

The enhanced efficacy of fungicides against rots when applied in combination with hot water had been related to the effect exerted by heat in increasing coverage and penetration of the active ingredient (AI) into the fruit. It has been shown (Schirra et al., 1996) that dipping lemons in IMZ mixtures with concentrations ranging from 250 to 1000 ppm at 50 °C produced a 4.5-fold increase in fungicide residue levels in comparison with treatments performed at room temperature. A total control of decay was achieved by 1500 ppm IMZ treatment at room temperature, but when the fungicide was applied at 50 °C rot development was suppressed with 250 ppm of AI.

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Results reported herein provide additional information concerning IMZ potential to control storage decay in lemons, AI residue levels, and degradation patterns when very low rates of fungicide mixtures are applied at 50 °C in comparison to conventional treatments at room temperature.

MATERIALS AND METHODS

Plant Material. The experiment was carried out on Di Massa lemons [(*Citrus limon* (L.) Burm)] harvested at an advanced stage of maturity in the second week of June 1996 from a single lot of 12 trees growing in the National Research Council experimental orchard located at Oristano, Italy (central western Sardinia, 39° 55′ north latitude).

Fruit Sampling. Fruits were delivered to the laboratory immediately after harvest, sorted to eliminate those with defects, selected for uniform size, placed in plastic boxes (48 boxes, 50 fruits per box), and grouped into eight treatment groups (six boxes per group, 2 boxes × 3 replicates), corresponding to the 3-min water dip treatments with and without imazalil (IMZ) at 50, 100, and 200 ppm IMZ at 50 °C and 1000 ppm IMZ at 20 °C and untreated fruit. IMZ mixtures in water were prepared with commercially available Fungazil 500 EC (44.66% AI, Janssen Pharmaceutica N.V. Belgium).

Treatments and Storage Conditions. Fruit were dipped for 3 min in a bath fitted with 3.96 kW/h heating elements and an electronic recirculation pump (22 L/min water flow). Ambient temperature before treatment was 22 °C. Two hundred liters of water or fungicide mixtures were used for the treatments dipping one box of fruit per run. Bath temperature was constantly maintained within ±0.5 °C of the required temperature by an electronic thermostat (OEM/HT, Carel, France) and probe (PTC 40, Carel, France). Following treatment, the fruit were left to dry at room temperature for *ca*. 5 h. Each treatment group was then divided into two subgroups of three boxes each. The first subgroup was used for the evaluation of rot incidence, treatment damage, and freshness rating of fruit. The fruit of the second subgroup were used for chemical analyses.

Finally, fruit were moved to a storage room and kept for 13 weeks at 9 °C and 90%−95% relative humidity (RH) with a complete air change every hour. At the end of storage they were kept at 21 °C and *ca*. 75% RH to simulate a 1 week marketing period (SMP).

Visual Assessment of Fruit. After 5, 10, and 13 weeks of storage and the additional week of SMP, fruit were inspected for physical damage, decay percentage, freshness (overall visual quality), firmness, flavor, and taste. Decay was identified as rots caused by blue and green molds (*Penicillium italicum* Wehmer and *P. digitatum* Sacc.), Alternaria rot (*Alternaria citri* Ell. & Pierce), gray mold (*Botrytis cinerea* Pers. ex Fr.), or as miscellaneous rots of unidentified fungi, and total percentage of rotted fruit was calculated. Overall visual quality was rated subjectively from 9 (excellent) to 0 (poor) by an informal panel of three people familiar with lemons.

Chemical Analyses of Fruit. Three replicates of 12 healthy fruit were randomly selected prior to treatments and after SMP for assessment of internal quality attributes. These included total soluble solid concentration (SSC) as °Brix, titratable acidity (expressed as % of citric acid), and ethanol concentration by juice head space GC analysis (Schirra and Mulas, 1995).

Imazalil Analysis. *Chemicals.* Imazalil was analytical standard (Ehrenstorfer, Augsburg, Germany). Triphenyl phosphate (99%) was used as the internal standard (IS) and was analytical grade (Janssen, Geel, Belgium). Acetone was HPLC grade, and petroleum ether was pesticide grade (Carlo Erba, Milan, Italy). Anhydrous sodium sulfate and sodium chloride were analytical grade (Carlo Erba). A stock standard solution of imazalil (*ca*. 500 ppm) was prepared in acetone. Working standard solutions containing 0.3 ppm IS were obtained by dilution with the extract from untreated flavedo. Extraction solution: acetone/petroleum ether mixture (1:1, v/v) containing 0.3 ppm IS.

Apparatus and Chromatography. An HRGC Mega 5160 gas chromatograph (Carlo Erba, Milan, Italy) was employed. It was fitted with an NPD-40 nitrogen phosphorus detector, an AS 550 autosampler (Carlo Erba), and a split−splitless injector. It was connected to an HP 3396-II reporting integrator (Hewlett-Packard, Avondale, PA). A Durabond fused silica column (30 m × 0.25 mm i.d.) (J&W Scientific, Folsom, CA) was employed, with DB 5 (5% phenylmethylpolysiloxane) liquid phase (film thickness 0.25 μm). The injector and detector were operated at 250 and 280 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 110 °C for 1 min, raised to 280 °C (20 °C/min), and held for 6 min. Helium was the carrier and makeup gas at 120 and 130 kPa, respectively. The calibration graph was drawn with the IS method by measuring peak heights *vs* concentrations. A good linearity was obtained in the 0−25 ppm range (r = 0.9995).

Sample Preparation. Three fruits per replication were weighed, and their flavedo was removed with a vegetable peeler. It was weighed, and its percentage with respect to the whole fruit was calculated. It was then triturated with a mincing knife and homogenized. The samples were stored in a freezer at −20 °C until analysis.

Extraction Procedure. A 2.5-g aliquot of homogenized sample (1 g for sample at highest concentrations) was weighed in a 30-mL screw-capped tube; 2 g of sodium chloride and 10 mL of an acetone/petroleum ether mixture (1:1, v/v) containing triphenyl phosphate as the IS were added, and the tube was shaken in a rotary shaker (GFL, Germany) for 15 min. The phases were allowed to separate, and the organic layer was poured into another flask containing 1 g of anhydrous sodium sulfate and was then injected for GC analysis.

Table 1. Influence of Postharvest Treatments on Decay in Di Massa Lemons during 13 Weeks of Storage at 9 °C and One Subsequent Week at 21 °C

treatments[a]	storage duration (weeks) 5	10	13	13 + 1
Penicillium spp. (%)				
control	6.6a[b]	40.4a	50.3a	66.7a
water at 50 °C	3.4b	37.9a	51.1a	62.7a
50 ppm IMZ at 50 °C	0.0c	0.0b	1.3b	7.3b
100 ppm IMZ at 50 °C	0.0c	1.3b	3.8b	5.7b
200 ppm IMZ at 50 °C	0.6c	0.6b	6.6b	10.6b
1000 ppm IMZ at 20 °C	0.7c	0.7b	1.3b	4.3b
Miscellaneous Rots (%)				
control	2.0a	6.5[ns]	9.0[ns]	10.3[ns]
water at 50 °C	0.0b	0.7	2.7	7.4
50 ppm IMZ at 50 °C	0.0b	6.0	8.6	13.9
100 ppm IMZ at 50 °C	3.2a	7.1	10.4	10.4
200 ppm IMZ at 50 °C	0.0b	4.6	9.2	10.5
1000 ppm IMZ at 20 °C	0.0b	5.4	10.2	10.9

[a] Three-minute dipping, air-dried. [b] Mean separation within column groups at $P \leq 0.05$ by Duncan's multiple range test. ns, nonsignificant.

Recovery Assays. Untreated flavedo samples were fortified with 1, 10, and 25 ppm imazalil and processed according to the procedure described above. Recoveries from four replicates showed values ranging from 90% to 105%.

Data Analysis. Analysis of variance (ANOVA) and mean separation within each inspection time were calculated, where applicable, by Duncan's multiple range test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Rot Percentage. After 5 weeks of storage *ca*. 7% of the untreated fruit exhibited *Penicillium* decay (mainly *P. digitatum*) as compared with *ca*. 3% of fruit submerged in water at 50 °C (Table 1). As storage time increased, development of *Penicillium* rots augmented rapidly to *ca*. 67% in control fruit and *ca*. 63% in fruit treated with hot water after the simulated marketing period (SMP), with no significant differences between these two treatments. Therefore hot water treatment only gave some measure of control of *Penicillium* decay during the first 5 weeks of storage but had no significant effect during prolonged periods. Conversely, IMZ treatments resulted in almost complete control of green mold over 10 weeks at 9 °C and was highly effective in controlling fungus development during the 13 weeks of cold storage and the additional week of SMP, giving a protection against the fungus ranging from 90% to 96%. Besides *Penicillium* spp. gray mold, *Alternaria* rot, Black pit (*Pseudomonas syringae* van Hall), and miscellaneous rots of unidentified fungi developed to various extents, differences among treatments being not significant.

The occurrence of severe rotting as well as development of fungi other than *Penicillium* spp. on lemons can be associated with long-term storage and especially with abnormal climatic conditions, such as prolonged rainy weather late in the spring (Smoot and Johnson, 1983).

Imazalil Analysis. After 1000 ppm IMZ dipping at 20 °C, residue concentration in fruit was 8.20 ppm (Table 2). This value doubled that found in a previous investigation on lemons treated with comparable IMZ levels (Schirra et al., 1996). Such differences could be explained by the different stages of maturity between fruit in the two experiments which are known to affect the AI diffusion mechanism across the plant cuticle (Riederer and Schreiber, 1995). As far as citrus fruit are concerned, during growth and ripening the epicuticular wax layer undergoes a change from the crystal-

Table 2. Residue Levels of IMZ Fungicide (ppm, Whole Fruit Basis) in Di Massa Lemons Following Postharvest Dipping and after 5, 10, and 13 Weeks of Storage at 9 °C and One Subsequent Week at 21 °C

	storage duration (weeks)			
treatments[a]	0	5	13	13 + 1
50 ppm IMZ at 50 °C	2.00c[b]	1.50c	1.27b	0.83b
100 ppm IMZ at 50 °C	2.93c	2.60c	1.87b	1.53b
200 ppm IMZ at 50 °C	5.88b	5.91b	4.67a	3.33a
1000 ppm IMZ at 20 °C	8.20a	7.60a	5.43a	4.03a

[a] Three-minute dipping, air-dried. [b] Mean separation within column groups at $P \leq 0.05$ by Duncan's multiple range test.

line to the amorphous state, with the appearance of rough and granular wax surface structures with numerous deep "cracks" which become more pronounced with fruit maturation (Freeman et al., 1979; El-Otmani et al., 1989). Therefore, we believe that when IMZ is applied at room temperature its penetration is likely to be positively related to the stage of maturity. Conversely, previous studies (Norman et al. 1971) showed that in green peel Eureka lemons and Valencia oranges diphenyl uptake was higher than that measured in fully coloured fruit. Differences in AI uptake between IMZ and diphenyl in relation to ripening stage may be due to the different molecular structures of fungicides.

A linear relationship ($r = 0.98$; $P \leq 0.001$) was found between residue concentrations in fruit after treatment at 50 °C and amount of fungicide employed. Residue concentrations in fruit following treatment at 50 °C with comparable IMZ levels (calculated values) were similar to that found previously (Schirra et al., 1996), suggesting that AI deposition following IMZ dips at 50 °C is not dependent on maturity stage of fruit. Early studies showed that heat treatment induced a partial melting and remodelling of the wax layer, resulting in the disappearance of cracks in the wax layer (Schirra and D'hallewin, 1997) and in the trapping of the AI. Therefore, the deposition of the fungicide when this is supplied in combination with hot water seems not to be dependent on the stage of maturity but rather on its increased mobility in the epicuticular wax during heat treatment. Studies are under way at our laboratory to shed more light on this topic.

IMZ residues showed great persistence during fruit storage at 9 °C, since after 13 weeks AI residues in fruit decreased to average *ca.* 35% of the initial values. A higher degradation rate was found on removal of fruit to SMP because in just 1 week at 21 °C the residue fungicide decreased by a further *ca.* 25%. Therefore, residue reduction totaled *ca.* 60%.

Treatment Damage. No heat damage was observed on fruit after dipping in hot water (Table 3). In contrast, IMZ treatment caused external damage to the peel consisting of browning of the rind to various extents, depending on the amount of fungicide applied. In lemons treated with 50−100 ppm IMZ the percentage of damaged fruit was lower than 3%, whereas 1000 ppm IMZ treatment caused a 13% damage. Intermediate percentages were found following treatment at 200 ppm IMZ. The percentage of fruit with treatment damage resulted to be strictly related ($r = 0.92$; $P \leq 0.001$) to AI deposition in the fruit after treatment.

Juice Analysis. As far as the internal quality attributes are concerned, SSC and titratable acidity of juice were not affected by treatments. After SMP, ethanol concentration in the juice increased in all samples, especially in untreated fruit, and to a greater extent in fruit dipped in 200 ppm IMZ at 50 °C or in 1000 ppm IMZ at 20 °C.

Table 3. Influence of Postharvest Treatments on Percentage of Treatment-Damaged Fruit, SSC, Titratable Acidity, and Ethanol Amount in the Juice of Di Massa Lemons after 13 Weeks of Storage at 9 °C Plus 1 Week at 21 °C

treatments[a]	treatment damage (%)	SSC (°Brix)	titratable acidity (%)	ethanol (mg × 100 mL^{-1})
	At Harvest			
	−	5.9	5.8	3.0
	13 Weeks at 9 °C + 1 Week at 21 °C[b]			
control	0.0c	6.1[ns]	5.9[ns]	13.9b
water at 50 °C	0.0c	5.7	5.8	6.7c
50 ppm IMZ at 50 °C	0.7c	6.0	5.6	9.7c
100 ppm IMZ at 50 °C	2.7bc	6.0	5.5	6.6c
200 ppm IMZ at 50 °C	6.6b	5.9	5.3	17.4a
1000 ppm IMZ at 20 °C	12.7a	6.1	5.3	21.3a

[a] Three-minute dipping, air-dried. [b] Mean separation within column groups at $P \leq 0.05$ by Duncan's multiple range test. ns, nonsignificant.

Overall Visual Quality, Flavor, and Taste. After 10 weeks of storage, fruit dipped in 200 or 1000 ppm IMZ appeared old, developed off-flavors, and further became unacceptable for consumption after 13 weeks of cold storage. Visual quality of fruit untreated or treated with 50−100 ppm IMZ was judged to be fairly good, and no off-flavors were found in the juice (data not shown).

CONCLUSIONS

Studies on Shamouti oranges (Barkai-Golan and Apelbaum, 1991) showed that sodium *o*-phenylphenate (SOPP) fungicide mixtures in combination with water at 45 °C gave positive synergistic effects against green and blue mold. The increased efficacy of hot fungicides is believed to be the result of their better distribution and penetration into the fruit because of their enhanced mobility in the epicuticular wax (Wells and Harvey, 1996). Previous investigations with lemons revealed that 250 ppm IMZ dipping at 50 °C enables fruit to be protected against *Penicillium* decay during 13 weeks of cold storage at 8 °C plus one additional week of SMP, to an extent comparable with 1500 ppm IMZ dipping at room temperature. Because there was a linear relationship between the amount of fungicide employed and AI deposition in fruit, it was forecast that 50 ppm IMZ dipping at 50 °C should be sufficient to reasonably control *Penicillium* rots. The present study corroborates this hypothesis as no significant differences in decay control were found following dipping in 50 ppm IMZ at 50 °C as compared to treatments with fungicide at higher levels.

In the U.S. the imazalil residue tolerance limit for citrus fruit is 10 ppm, which is double that fixed by EC regulation (5 ppm). Therefore, from a regulatory standpoint 1000 ppm IMZ dipping at 20 °C resulted in an accumulation which would be legal in the U.S. but not in the EC.

It was concluded that when IMZ is applied in combination with water at 50 °C very low concentrations of fungicide are enough to achieve a reasonable control of rot development. In addition, in view of public concern due to wastewater disposal after treatments in commercial packinghouses, this approach should be strongly recommended to minimize potential pollution to the environment.

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LITERATURE CITED

Barkai-Golan, R.; Apelbaum, A. Synergistic effects of heat and sodium *o*-phenylphenate treatments to inactivate *Penicillium* spores and suppress decay in citrus fruits. *Trop. Sci.* **1991**, *31*, 229−230.

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

El-Otmani, M.; Arpaia, M. L.; Coggins, Ch. W., Jr.; Pehrson, J. E.; O'Connell, N. W. Developmental changes in 'Valencia' orange fruit, epicuticular wax in relation to position on the tree. *Sci. Hortic.* **1989**, *41*, 69−81.

Freeman, B.; Albrigo, L. G.; Biggs, R. H. Ultrastructure and chemistry of epicuticular waxes of developing citrus leaves and fruits. *J. Am. Soc. Hort. Sci.* **1979**, *104*, 801−808.

Norman, S; Craft, C. C.; Fouse, D. C. Biphenyl absorption of citrus fruit: The effect of variety, color class, and injury by freezing, peeling, and lack of oxygen. U.S. Department of Agriculture, Market Research Report No. 896; USDA, Washington, DC, 1971.

Riederer, M.; Schreiber, L. Waxes: The transport barriers of plant cuticles. In *Waxes: Chemistry, molecular biology and functions*; Hamilton, R. J., Ed.; The Oily Press: Dundee, Scotland, 1995; Vol. 6, pp 131−156.

Schirra, M.; Cabras, P; Angioni, A.; Melis, M. Residue level of imazalil fungicide in lemons following prestorage dip treatment at 20 and 50 °C. *J. Agric. Food Chem.* **1996**, *44*, 2865−2869.

Schirra, M.; D'hallewin, G. Storage performance of Fortune mandarins following hot water dips. *Postharvest Biol. Technol.* **1997**, *10*, 229−238.

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

Smoot, J. J.; Houck, L. G.; Johnson H. B. *Market Diseases of Citrus and Other Subtropical Fruits*; Agricultural Handbook No. 398; USDA, Agricultural Research Service: Washington, DC, 1983.

Wells, J. M.; Harvey, J. M. Combination heat and 2,6-dichloro-4-nitroaniline treatments for control of *Rhizopus* and brown rot of peaches and nectarines. *Phytopathol.* **1970**, *60*, 116−120.

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