# Residue Level of Imazalil Fungicide in Lemons following Prestorage Dip Treatment at 20 and 50 °C

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Commercially mature Di Massa lemons [*Citrus limon* (L.) Burm.] were submerged for 3 min in water at room temperature (20 °C) or in hot water (50 °C) with or without imazalil (IMZ) fungicide solution at concentrations ranging from 250 to 1500 ppm. They were then stored at 9 °C for 13 weeks plus 1 additional week at 20 °C to simulate shelf life. After simulated shelf life, rot percentage was 28.9% in fruit submerged in water at 20 °C and 15.0% in fruit treated with hot water, a decrease of about 50%. IMZ applied at 20 °C was highly effective in suppressing rot development. The fungicide provided complete control of decay at a concentration of 1500 ppm. When applied in combination with hot water, the complete control of decay was achieved at 250 ppm. The residual level of IMZ in lemons dipped at 20 °C was related to the amount of fungicide used in postharvest treatment. The mean values (on a whole fruit basis) ranged from 1.47 to 5.05 ppm when used at 250 or 1500 ppm, respectively. The combination of IMZ and hot water produced a residue level 4.59-fold higher compared to corresponding treatments performed at room temperature. It was concluded that IMZ solution applied at 50 °C greatly reduces the dose of fungicide needed to control postharvest decay of citrus fruit when the treatment is applied at room temperature and offers environmentally interesting prospects owing to the smaller amounts of pesticide in citrus packinghouse wastewater following treatment.

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

## INTRODUCTION

Extensive work has been performed on citrus fruit in attempts to control storage decay, minimize chilling injury in sensitive cultivars, and maintain fruit quality as long as possible (Grierson and Ben-Yehoshua, 1986). Postharvest technologies are mainly based on temperature and humidity management, air change in the refrigerated rooms, and prophylactic use of fungicides (Kader, 1985). Sodium *o*-phenylphenate (SOPP), thiabendazole (TBZ), and imazalil (IMZ) are currently applied to control mold decay in citrus fruit (Eckert and Eaks, 1988). However, the widespread use of these fungicides in commercial packinghouses has led to the proliferation of resistant strains of *Penicillium* (Holmes and Eckert, 1992), thus reducing their efficacy. If one considers the high cost of synthesis and experimentation and the difficulties in regulating new synthetic chemicals and in maintaining the register of postharvest fungicides that have been used commercially for years (Eckert, 1995), the effort being made by scientists to develop and/or improve nonchemical methods for the postharvest defense of horticultural crops is justified also from the public health and environmental viewpoints.

Among the several prestorage technologies developed to extend the marketing period of horticultural crops (Watada, 1994), heat treatments are currently recognized as possible future prestorage strategies to follow in reducing postharvest losses (Couey, 1989; Klein and Lurie, 1992). Studies on citrus fruit have shown that prestorage fruit dipping in hot water inhibits or reduces pathogen development (Barkai-Golan and Phillips, 1991), improves fruit resistance to chilling injury (McDonald et al., 1991; Wild, 1993; Rodov et al., 1994), reduces fruit weight loss in kumquats (Schirra et al., 1995a), and greatly enhances the efficacy of fungicides applied in postharvest treatments (Sharma and Kaul, 1990; Barkai-Golan and Apelbaum, 1991). These approaches may therefore be useful in minimizing the use of selective synthetic fungicides.

However, no specific investigations have been performed to determine the relationship between the amount of fungicide residue remaining in citrus fruit following hot-dip treatment and its efficacy against pathogens.

This study was therefore undertaken to assess the keeping quality of lemon fruit during and after long-term storage following prestorage dipping in imazalil solutions at 50 or 20 °C, as well as to quantify pesticide residue, pesticide degradation rate, and the lowest concentration of IMZ that would ensure reasonable control of postharvest decay.

## MATERIALS AND METHODS

**General.** Mature, light yellow lemons [*Citrus limon* (L.) Burm. cv. Di Massa] were obtained from a single lot of 12 trees growing in the National Research Council experimental orchard located in Oristano (central western Sardinia: 39° 55′ north latitude). Freshly harvested fruits were sorted to eliminate those with defects and selected for uniform size, placed in plastic boxes (50 fruits per box), and grouped into 12 treatment lots of 6 boxes each (3 replications of 2 boxes), corresponding to the 3-min water dip treatment at 20 or 50 °C with and without imazalil at 250, 500, 750, 1000, or 1500 ppm. Water mixture of imazalil were prepared with Decozil EC W2 (50% active ingredient) (Decco Italia, Catania, Italy).

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**Table 1. Residue Levels of IMZ Fungicide in Di Massa Lemons following Prestorage Dip Treatments at 20 or 50 °C**

| treatment variables[a] | | residue level[b] (ppm) dip temp of | |
|---|---|---|---|
| IMZ concn (ppm) | storage condition | 20 °C | 50 °C |
| 250 | before storage | 1.47 ± 0.23 | 8.50 ± 0.74 |
| | 5 weeks at 9 °C | 1.34 ± 0.28 | 7.37 ± 0.65 |
| | 13 weeks at 9 °C | 1.16 ± 0.01 | 5.52 ± 1.18 |
| | 13 weeks at 9 °C + 1 week at 20 °C | 1.35 ± 0.26 | 4.47 ± 0.98 |
| 500 | before storage | 2.72 ± 0.18 | 11.03 ± 1.41 |
| | 5 weeks at 9 °C | 2.49 ± 0.21 | 11.15 ± 0.91 |
| | 13 weeks at 9 °C | 1.53 ± 0.35 | 8.17 ± 0.88 |
| | 13 weeks at 9 °C + 1 week at 20 °C | 1.62 ± 0.32 | 7.76 ± 0.98 |
| 750 | before storage | 3.94 ± 0.67 | 15.09 ± 1.13 |
| | 5 weeks at 9 °C | 3.96 ± 0.50 | 11.26 ± 1.01 |
| | 13 weeks at 9 °C | 2.94 ± 0.50 | 7.93 ± 0.95 |
| | 13 weeks at 9 °C + 1 week at 20 °C | 2.32 ± 0.22 | 7.72 ± 1.28 |
| 1000 | before storage | 3.91 ± 0.35 | 19.05 ± 1.87 |
| | 5 weeks at 9 °C | 3.49 ± 0.32 | 18.44 ± 1.32 |
| | 13 weeks at 9 °C | 2.27 ± 0.57 | 9.36 ± 0.79 |
| | 13 weeks at 9 °C + 1 week at 20 °C | 2.37 ± 0.18 | 8.77 ± 0.49 |
| 1500 | before storage | 5.07 ± 0.77 | 19.06 ± 0.35 |
| | 5 weeks at 9 °C | 4.00 ± 0.67 | 16.97 ± 0.98 |
| | 13 weeks at 9 °C | 3.07 ± 0.16 | 8.85 ± 1.68 |
| | 13 weeks at 9 °C + 1 week at 20 °C | 3.86 ± 0.50 | 8.23 ± 1.15 |

[a] Three-minute dip treatment, air-dried. [b] On the whole fruit basis.

Dip treatment was performed in a 200-L bath fitted with an electronic recirculation pump and thermostat. The ambient temperature before treatment was 23 ± 1 °C. The bath treated one box of fruit per run. During each treatment the bath temperature was constantly maintained within ±0.5 °C of the established value by means of an electronic device. The fruits were then left to dry for 6 h at room temperature and stored at 9 °C and 90−95% relative humidity (RH), with a complete change of air every hour. After 13 weeks of storage, fruits were kept at 20 °C and about 75% RH to simulate shelf life conditions.

**IMZ Analysis.** *Chemicals.* The imazalil used was an analytical standard purchased from Ehrenstorfer (Augsburg, Germany). Triphenyl phosphate (99%) was used as the internal standard (i.s.) and was of analytical grade (Janssen, Geel, Belgium). Acetone and ethanol were of HPLC grade, while petroleum ether was of a special reagent for pesticide determination (Carlo Erba, Milan, Italy); anhydrous sodium sulfate and sodium chloride were of analytical grade (Carlo Erba). A stock standard solution of imazalil (ca. 1000 ppm) was prepared in acetone. Working standard solutions, containing the i.s. at 0.3 ppm, were obtained by dilution with the extract from untreated flavedo without interfering peaks.

*Apparatus and Chromatography.* An HRGC Mega 5160 gas chromatograph (Carlo Erba) 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-210 (50% trifluoropropylsilicone, 50% methylsilicone) liquid phase (film thickness 0.25 μm). The injector and detector were operated at 250 and 260 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 150 °C for 1 min, raised to 250 °C (10 °C/min), and held for 20 min. Helium was the carrier and makeup gas at 120 and 130 kPa, respectively. The calibration graph was constructed with the i.s. method by measuring peak heights vs concentrations. Good linearity was achieved in the range 0−25 ppm, with a correlation coefficient of 0.9992.

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

*Extraction Procedure.* A 5-g aliquot of homogenized sample (1 g for samples treated at 50 °C) 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 i.s. 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 2 g of anhydrous sodium sulfate and then injected for gas chromatographic analysis.

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

*Visual Assessment of Fruit.* Fruits were inspected after 5 weeks, immediately upon removal from cold storage, and after shelf life to assess the percentage of fruit showing mold decay and chilling injury (CI). Symptoms of CI in each fruit were evaluated subjectively, on the basis of the extent of damage to the rind, from 0 (= no injury) to 4 (= severe injury), when fruit would be rejected. Finally, a weighted average from a chilling index was calculated (Schirra and Mulas, 1995a). External quality (marketability) was evaluated on fruit without visible infection or CI by an informal taste panel of three technicians using a scale from 9 (= excellent) to 0 (= poor; not fresh, when fruit would be rejected), and an average visual score was calculated.

*Juice Analysis.* Three replications of 10 healthy fruits were randomly selected immediately after harvest and after simulated shelf life for internal quality attributes. These included total soluble solid content (TSS), as °Brix; titratable acidity, as citric acid %; and ethanol and acetaldehyde concentrations in the juice determined by juice headspace analysis by gas chromatography (Schirra and Mulas, 1995a).

*Statistical Analysis.* Analysis of variance (ANOVA) was performed by MSTAT-C software using a completely randomized plot design. Mean comparisons were performed by Tukey's honestly significant difference test at $P = 0.01$.

## RESULTS

**Residue Level of IMZ.** Mean values of IMZ residue in fruit following treatment at 50 °C (Table 1) were found to be 4.5 (±0.9)-fold higher compared to treatments at 20 °C and were closely related ($r = 0.98$) to fungicide solution concentration.

When applied at 50 °C, solutions containing 250 ppm of IMZ led to higher deposition of pesticide (8.50 ppm) with respect to treatment with 1500 ppm of fungicide

**Table 2. Influence of Postharvest Dip Treatments on Titratable Acidity, Total Soluble Solids (TSS), and Acetaldehyde and Ethanol Amount, in the Juice of Di Massa Lemons after 1 Week at 20 °C following 13 Weeks of Storage at 9 °C[a]**

| treatments | titratable acidity (% citric acid) | TSS (°Brix) | acetaldehyde (mg/100 mL) | ethanol (mg/100 mL) |
|---|---|---|---|---|
| harvest[c] | 6.53 | 7.70 | 0.11 | 0.84 |
| 13 weeks at 9 °C + 1 week at 20 °C | | | | |
| water at 20 °C | 6.34a | 7.60a | 0.12b | 1.66c |
| 250 ppm IMZ at 20 °C | 6.34a | 7.67a | 0.18b | 2.82c |
| 500 ppm IMZ at 20 °C | 5.89b | 7.53a | 0.19b | 5.28c |
| 750 ppm IMZ at 20 °C | 5.54bc | 7.50a | 0.14b | 2.13c |
| 1000 ppm IMZ at 20 °C | 5.70bc | 7.53a | 0.14b | 2.21c |
| 1500 ppm IMZ at 20 °C | 5.63bc | 7.23ab | 0.13b | 2.22c |
| water at 50 °C | 5.66bc | 7.43ab | 0.13b | 1.77c |
| 250 ppm IMZ at 50 °C | 5.44cd | 7.27ab | 0.32ab | 24.79ab |
| 500 ppm IMZ at 50 °C | 5.70bc | 7.37ab | 0.55a | 26.65ab |
| 750 ppm IMZ at 50 °C | 4.96e | 6.73b | 0.55a | 22.43b |
| 1000 ppm IMZ at 50 °C | 5.15de | 7.13ab | 0.60a | 30.57a |
| 1500 ppm IMZ at 50 °C | 5.02e | 7.03ab | 0.66a | 30.78a |

[a] Means in each column group followed by a common letter are not significantly different by Tukey's honestly significant difference test at $P = 0.01$. [b] Three-minute dip treatment, air-dried. [c] Harvest data are only included to provide a comparison to the other measurements.

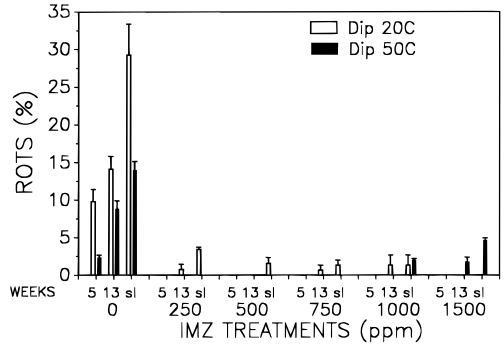


**Figure 1.** Influence of postharvest dip treatments at 20 and at 50 °C on percentage of fruit showing rots in Di Massa lemons after 5 and 13 weeks of storage at 9 °C and after 1 additional week of simulated shelf life at 20 °C.

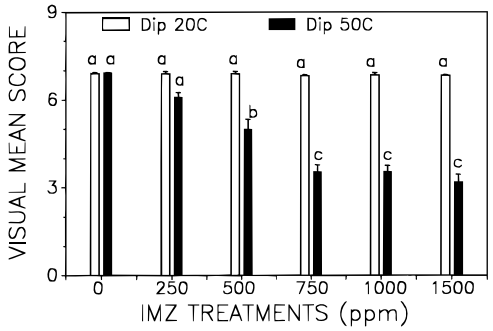


**Figure 2.** Effect of postharvest dip treatments at 20 and at 50 °C on visual mean score in Di Massa lemons after 13 weeks of storage at 9 °C plus 1 week of simulated shelf life at 20 °C.

at 20 °C (5.07 ppm). However, differences in IMZ residue were reduced after shelf life: 4.47 ppm in fruit dipped in hot IMZ at 250 ppm and 3.86 ppm in those treated with 1500 ppm of fungicide at room temperature.

**Decay.** Water dip treatment at 50 °C consistently suppressed pathogen development in lemons (Figure 1). In fruit dipped in water at 20 °C, decay incidence was 9.8% and 14%, respectively, after 5 and 13 weeks of cold storage; in fruit dipped in hot water it was 2.3% and 8.5%, respectively. By the end of shelf life, rot percentage was 28.9% in control fruit and 15.0% in fruit treated with hot water, a decrease of about 50%. Treatments with IMZ were highly effective in controlling the development of rots. When used at room temperature, the fungicide solution at 250 ppm led to 0.7% decay by the end of cold storage and 3.47% decay after shelf life. Concentrations of pesticide ranging from 500 to 750 ppm gave better results, with no decay during storage and less than 2% rot after shelf life. At 1000 or 1500 ppm, IMZ conferred complete decay control both during storage and after shelf life. The combination of water at 50 °C and IMZ with concentrations ranging from 250 to 750 ppm was as effective as 1500 ppm of IMZ at 20 °C, but concentrations of 1000 or 1500 ppm exhibited 1.4% and 4.7% decay after shelf life, respectively.

**Chilling Injury.** Lacking visible CI symptoms, the results of this study did not allow evaluation of the influence of heat and IMZ on CI. Moreover, the fruit was kept at 9 °C, which is a nonchilling temperature for Di Massa lemons (Schirra and Mulas, 1995b).

**Visual Quality of Fruit.** No important differences occurred with the different treatments during 13 weeks of storage at 9 °C (Figure 2). By the end of shelf life, fruits with no pathological disorders were rated as good in all treatments at 20 °C (mean score 6.9) and in hot IMZ at 250 ppm (mean score 6.1) and fairly good in hot IMZ at 500 ppm. Fruit treated with higher concentrations of IMZ was judged to be poor (score below 4). In this case the fruit peel surface showed very small, reddish pitting and appeared to be older when compared to other treatments.

**Internal Quality Attributes.** Treatment by dipping in water at 20 °C with or without IMZ led to retention of the highest juice acidity values (Table 2). IMZ solution at 50 °C at concentrations from 750 to 1500 ppm produced the lowest values, while intermediate juice acidity values were detected in the other cases. Soluble solids content was only slightly affected by treatments. Acetaldehyde and ethanol levels in the juice were significantly higher when fruit was treated with hot water in combination with IMZ.

## DISCUSSION

The recommended doses of IMZ fungicide in postharvest treatments of citrus fruit range from 1000 to 2000 ppm when applied in a water mixture or from 2000 to 4000 ppm or above when used in combination with a water wax coating of the fruit (Cohen et al., 1992). Such

levels of IMZ would ensure best protection against the fungis *Penicillium digitatum* and *P. italicum*, the main cause of postharvest decay in citrus fruit. Studies on Tarocco oranges (Schirra et al., 1992) indicate that IMZ in a water solution at 1500 ppm provided almost complete control of decay during 3 months of storage at 8 °C plus 1 week of simulated shelf life at 20 °C; the efficacy of IMZ appeared only slightly reduced when used in combination with a water wax solution. Studies on oranges and grapefruits (Cohen et al., 1992) indicate that a reduction of IMZ concentration from 1000 to 250 ppm in postharvest treatments at room temperature did not significantly reduce the efficacy of the fungicide in controlling natural mold infection. Results of this study suggest that these levels may be further reduced when IMZ is applied in combination with hot water.

The residue level of fungicide following treatment at 250 ppm of IMZ at 50 °C was higher than it was with treatment at a concentration of 1500 ppm applied at room temperature, but the different degradation rate during storage led to a very similar residue level after shelf life. In light of these occurrences, and taking into account the low residue values found in these experiments, it may be postulated that the lowest threshold of residue in the fruit capable to control pathogen development is on the order of 3 ppm. Inexplicably, the fruit treated at 50 °C with higher concentrations of IMZ (1000 and 1500 ppm), with consequently higher amounts of residues, were subjected to fungus attack during shelf life, with an incidence of decay that increased with the increase in the concentrations of the solution, going from 1.4% to 4.7% for concentrations of 1000 and 1500 ppm, respectively. At present, we can offer no explanation for this apparently contradictory phenomenon. However, one hypothesis that needs experimental confirmation can be advanced. The IMZ initially present on the fruit at high concentrations (>3 ppm) suppresses fungus development since it inhibits the biosynthesis of ergosterol (Tomlin, 1994). During storage, the active ingredient degrades and forms degradation products, which are structurally similar but lack an inhibiting action. As degradation proceeds, the concentration of the active ingredient decreases while that of the degradation products increases. When the latter reach high concentrations (which occurs only when initial levels of IMZ are quite high), they can compete with the active ingredient and decrease its efficacy. Finally, the higher deterioration rate of fruit treated with IMZ at concentrations ranging from 750 to 1500 ppm at 50 °C, as evaluated by external and internal quality attributes, appeared to be the result of excessive accumulation of fungicide residues, which may have caused the phytotoxic effects noticed in earlier studies on lemons (Schirra and Mulas, 1995b) and grapefruits (Schirra et al., 1995b).

## CONCLUSIONS

The combination of IMZ and hot water made it possible to use a significantly lower amount of fungicide without impairing its efficacy in controlling postharvest decay in lemons than when the treatment was performed at room temperature. In fact, to supply the lowest amount of IMZ residue in fruit (about 3 ppm) still capable of providing a reasonable defense against decay, the concentration of fungicide to be used should be about 50 ppm, a value that can be indicated thanks to the linear relationships found between fungicide concentration used in treatment and the amount of residue found at the end of the test.

The increased fungicide efficacy was related in part to the effect of the heat and in part to the increased chemical activity due to the enhanced penetration and coverage of active ingredient on fruit (Wells and Harvey, 1970), probably for its higher mobility into the epicuticular wax.

In addition, on taking into account the toxicity of IMZ for humans and animals, this approach provides the opportunity of reducing the amounts of fungicides in citrus packinghouse wastewater after treatments. These results have considerable practical importance, since savings deriving from the use of smaller amounts of fungicides would partially compensate for the higher costs of heating the solutions.

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