Influence of Postharvest Handling and Surfactants on Control of Green Mold of Lemons by Curing

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Supported in part by the California Citrus Research Board and the Richard C. Storkan Plant and Soil Research Foundation. Portion of a thesis submitted by R. R. Stange in partial fulfillment of the requirements for the Ph.D. degree, University of California,

We thank J. Smilanick, P. G. Long, and G. E. Brown for their critical reading of this manuscript. Accepted for publication 7 March 1994.

ABSTRACT

Stange, R. R., Jr., and Eckert, J. W. 1994. Influence of postharvest handling and surfactants on control of green mold of lemons by curing. Phytopathology 84:612-616.

Curing lemons at 32 C in a water-saturated atmosphere was evaluated for control of green mold to determine the effects of curing time, delay of treatment, and surfactants on efficacy. Fruit were inoculated with Penicillium digitatum by wounding with a tool dipped in a suspension of 106 spores per milliliter. For fruit inoculated 18 h prior to treatment, curing 36-48 h was required for optimal disease control. In two of three experiments, curing for 48 h resulted in disease control comparable to dipping in 1 g of imazalil per liter. The curing treatment could be delayed

24 h after inoculation without reducing the effectiveness of the treatment. When fruit inoculated 24 h earlier were immersed in water containing sodium dodecylbenzenesulfonate (SDBS) at 1 g/L and then cured 18 h, decay control was improved by 41-73% compared to the water-dipped cured control. The viability of P. digitatum conidia and germlings on potato-dextrose agar (PDA) was not affected by incubation at 30 C for 24 h or by plating onto PDA containing 40 µg of SDBS/ml. However, the combination of these two treatments resulted in mortalities of 66 and 92% for conidia and germlings, respectively, suggesting that the combination of these treatments was lethal.

Additional keywords: Citrus limon, postharvest decay.

Green mold, caused by Penicillium digitatum Sacc., is generally the most serious postharvest disease of citrus produced in Mediterranean climates (14). Infection occurs through injuries made during picking or handling and results in decay during storage or marketing (14,15). Infected fruit decays rapidly, and, though there is little secondary spread, the surrounding fruit are covered with masses of powdery green spores. This dusting of sound fruit with spores from decayed fruit is termed soilage and is often a greater economic problem in retail cartons than is decayed fruit. Prior to the 1950s soilage was controlled by wrapping fruit individually. The postharvest application of imazalil and/or thiabendazole is now the sole means of controlling soilage in California (14). Lemons may be stored for several months before being regraded and packaged for sale. Because of recent prohibitions on the use of other fungicides, imazalil and thiabendazole treatment of lemons going into storage has intensified (12). Because only resistant biotypes sporulate on treated fruit, fungicide treatment of stored lemons creates an ideal situation for the buildup of resistant biotypes of P. digitatum. Injuries that occur during handling immediately prior to shipment would likely be infected by resistant biotypes, and a packout application of imazalil and/ or thiabendazole may result in poor control of both decay and soilage (11,14). One proposed solution is to avoid use of systemic fungicides on lemons until immediately before shipment (11,12). Although this practice would reduce the buildup of fungicideresistant biotypes, it would also result in a higher incidence of decay in stored fruit. Development of effective and nonselective alternative decay-control measures for stored fruit is the foundation of an integrated program for management of fungicideresistant P. digitatum in lemons.

Curing (i.e., holding plant parts at temperatures and humidities conducive to wound healing and detrimental to pathogen development) has been used to control disease on many plants and plant parts (4,6). In 1927, Fawcett and Barger (16) reported that neither P. digitatum nor P. italicum grew at temperatures above 30 C and that oranges inoculated with either pathogen would not decay at 33 C. Tindale and Fish (24) later proposed curing Australian navel oranges at 34.4 C for 3 days for control of green and blue molds. By the mid-1940s, it was generally recognized in Florida that the standard conditions for degreening oranges with ethylene (30 C and ≥90% relative humidity for 2-3 days) reduced green mold. Hopkins and Loucks (18) confirmed the effectiveness of curing for control of green mold and showed that ethylene was not important for disease reduction. Brown (5) demonstrated the role of temperature and relative humidity in the development of resistance by the injury site. Ben-Yehoshua et al (3) combined individual wrapping of citrus fruit in plastic film with curing at 36 C for 3 days for control of green mold. Most recently, our preliminary studies have indicated that dipping lemons in a surfactant solution prior to curing enhanced decay control (23). Studies to date have not compared curing to a standard fungicide treatment (3,5,18,24).

The purposes of this study were to evaluate curing as a control measure for green mold in lemons, using methods similar to those used in commercial fruit handling, and to further explore the use of surfactants for enhancing control by curing.

MATERIALS AND METHODS

General methods. Tree-ripe yellow lemons, Citrus limon (L.) N.L. Burm. 'Eureka', of uniform size (count size 100-120) were harvested on the first day of the experiment from groves of mature trees on the Citrus Experiment Station, Riverside, CA. Three experiments, however, used commercially harvested yellow lemons from Ventura County, CA. A wild-type isolate (M6-r) of P. digitatum was used in all experiments, except one in which an imazalil-resistant isolate (P-3) was used. Fungi were maintained

on silica gel (25) and grown at 25 C on potato-dextrose agar (PDA) (Difco Laboratories, Detroit). Seven- to 12-day-old cultures were flooded with 0.01% (w/v) Triton X-100 (Sigma Chemical Co., St. Louis) in water, and the suspension was filtered through eight layers of cheesecloth. A tissue homogenizer (30 strokes) was used to break up the chains of conidia and produce a suspension of mostly singular conidia. The turbidity of the suspension was adjusted to 0.1 at A_{425nm} or about 10^6 conidia per milliliter (13). For in vitro studies, this suspension was diluted to the desired concentration. To produce inoculum for decay studies, lemons were surface-disinfested by immersion in 70% (v/v) ethanol/water for 1 min, air-dried, and inoculated by wounding with a tool previously dipped in the conidial suspension. Injuries were about 3 mm deep and 0.5 mm in diameter. Fruit were incubated at 20 C for 7-10 days. Using a sterile technique, spore masses were passed through a 200-mesh screen, placed in vials, and dried over silica gel for 1 wk (5). Dried spores were stored over silica gel at 1 C for up to 1 mo. For decay-control studies, dried spores were suspended in Triton X-100 solution without being homogenized, and their concentration was adjusted to 106/ml by absorbance. Lemons were injury-inoculated once midway between the stem and stylar ends, as described above, and kept in plastic bags at 20 C prior to treatment to insure uniform infection.

Fruit were cured by placing them on a grill raised 2 cm over water in a stainless-steel tray (61 × 43 × 12 cm). Trays were covered with plastic film containing eight 4-mm-diameter holes and placed in a growth chamber maintained at 32 C and maximum relative humidity. Moisture was observed on the fruit throughout the curing period, indicating a saturated or near saturated relative humidity. Imazalil-treated (Janssen Pharmaceutica N.V., Beerse, Belgium; commercial formulation by Decco, Monrovia, CA) fruit were immersed for 45 or 60 s in water containing 1 g of a.i./L. All fruit were placed in paper bags within cardboard boxes after treatments and stored at 15 C in a humidified room (relative humidity ~90% by wet/dry bulb thermometer). This storage temperature, although higher than the usual storage temperature of 10-13 C (14), was chosen to enhance disease development and allow for long-term storage. Fruit were evaluated for disease at weekly intervals, and diseased fruit were removed. Diseased lemons were classified as showing signs of infection by P. digitatum, P. italicum, or Alternaria sp.

All decay experiments used a randomized complete block design, blocking for location in the growth chamber and height within stacks in storage. There were four replicates of 20–35 fruits each (generally \sim 30) per treatment. Analysis of variance (ANOVA) was performed on disease incidence (disease incidence \times 100 = percent decay), using the sin⁻¹ transformation (20).

Curing time. These experiments were designed to evaluate the effects of curing time, utilizing conditions and practices that simulated commercial handling. Lemons were harvested, inoculated without surface-disinfestation, and incubated for 18 h at 20 C. They were immersed for 60 s in water containing active chlorine at 150 mg/L (pH 8), followed by a water rinse (14). Lemons were cured for 12, 24, 36, or 48 h or treated with imazalil. Controls were with or without the chlorine dip. The experiment was repeated at three intervals spanning the 1992 harvest season.

Delay of treatment. These experiments were designed to determine how soon after harvest treatment would have to be initiated. Because fruit could not be surface-disinfested immediately after inoculation, all fruit were dipped in 70% ethanol prior to inoculation. Lemons were incubated at 20 C for 0-48 h after inoculation and cured for 24 h. The uncured control was held at 20 C for 24 h. The experiment was repeated three times.

Effect of surfactants. The first set of experiments used procedures described for fungicide tests (13) (i.e., lemons were surface-disinfested in 70% ethanol prior to inoculation). After 24 h, lemons were immersed in water containing 0, 0.1, 0.5 or 1.0 g of sodium dodecylbenzenesulfonate (SDBS, Sigma) per liter for 30 s and cured for 18 h. Uncured fruit were immersed in water or SDBS solution at the highest concentration. The experiment was repeated three times.

The results of the preceding experiments indicated that pretreatment with SDBS, but not Triton X-100 or Tergitol NP-10 (Union Carbide, Danbury, CT) (data not shown), improved control of green mold by curing. Because of this, SDBS and two additional anionic surfactants were evaluated under conditions simulating commercial handling. Lemons were inoculated without surface-disinfestation; an imazalil-resistant isolate was used in one experiment. Fruit from three of four experiments were commercially harvested in Ventura County, CA, and stored for 2-4 days prior to use at 11 C to retard development of field infections. Twenty-four hours after injury-inoculation fruit were immersed in chlorine and rinsed as above. Fruit were immersed next in water or a 1 g/L solution of SDBS, sodium xylene sulfonate (SXS, Sigma), sodium dodecyl sulfate (SDS, Sigma), or imazalil. Water- and surfactant-dipped fruit were cured for 24 h.

Effect of SDBS in vitro. This experiment was designed to simulate curing and surfactant treatments on P. digitatum. Conidial suspensions were prepared by adding 100 ml of a diluted standardized spore suspension to 100 ml of potato-dextrose broth (Difco). For germlings, a 500-ml flask containing the above mixture was placed on an orbital shaker at 120 cpm for 12 h at 23 C. Solidified medium in petri dishes was uniformly seeded with P. digitatum by spreading 100 μ l of suspension with a glass rod. Two media were used, PDA and PDA containing SDBS at 40 μ g/ml; this concentration caused about a 50% reduction in radial growth of P. digitatum (R. R. Stange, Jr. and J. W.

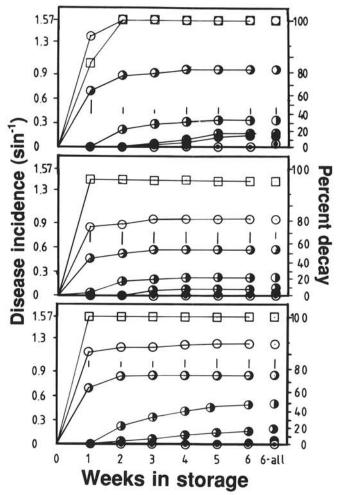


Fig. 1. Cumulative incidence of green mold in lemons during 6 wk of storage at 15 C and the incidence of all decays after 6 wk (6-all). Fruit were injury-inoculated with *Penicillium digitatum* 18 h prior to treatment. Treatments were untreated (\square); chlorine-dipped but not cured (\bigcirc), cured for 12 (\bigcirc), 24 (\bigcirc), 36 (\bigcirc), or 48 h (\bigcirc); or imazalil (\bigcirc) treated. Vertical bars denote LSD (P = 0.05). Experiments were started during 1992 on 17 January (top), 21 March (middle), and 21 April (bottom).

Eckert, unpublished data). A 2% (w/v) solution of SDBS was autoclaved separately and added to the molten agar immediately before pouring. Plates were incubated at 30 C for 0, 1.5, 3, 6, 12, or 24 h and subsequently incubated at 23 C. Colony counts were made at 12-h intervals for 4 days. The experiment was performed three times.

RESULTS

Effect of curing time. All curing treatments (Fig. 1) resulted in a significant reduction in green mold after 6 wk of storage when compared to the chlorine-dipped control. Incremental increases in the curing period resulted in significant reductions in disease for the 12-, 24-, and 36-h curing treatments. In two of three experiments, the final incidence of green mold in fruit cured for 36 h was not significantly different from that in fruit cured for 48 h. Curing fruit for 48 h was as effective as imazalil (1 g a.i./L) in reducing disease in two of three experiments. Essentially the same inferences were made when the cumulative incidences of all postharvest diseases were compared (Fig. 1, week 6-all);

TABLE 1. Effect of delay between inoculation and initiation of curing on control of green mold in lemons

Pretreatment Inoculation period ^a (h)	Experiments, 1992 ^b			
	24 March	6 May	15 May	
Cured for 24 h				
0	0.45	0.36	0.31	
12	0.29	0.21	0.28	
24	0.32	0.30	0.32	
36	0.55	0.70	0.81	
48	0.86	1.04	1.29	
Not cured				
24	1.27	1.37	1.50	
LSD $(P = 0.05)$	0.19	0.12	0.20	
LSD $(P = 0.01)$	0.26	0.16	0.28	

^a Fruit were immersed 1 min in 70% ethanol, injury-inoculated with Penicillium digitatum, and held at 20 C and high humidity for time indicated prior to treatment.

TABLE 2. Effect of immersing lemons in a sodium dodecylbenzenesulfonate (SDBS) solution prior to curing on control of green mold

Treatment SDBS concentration ^a (g/L)	Experiments, 1992 ^b			
	5 April	10 May	24 May	
Cured for 18 h				
0.00 (CK)	0.311	0.963	0.525	
0.1	0.142	0.889	0.270	
0.5	0.182	0.686	0.308	
1.0	0.082	0.508	0.288	
F value ^c	11.5**	7.50**	5.32*	
LSD $(P = 0.05)$	0.091	0.240	0.165	
LSD $(P = 0.01)$	0.131	0.345	0.237	
Not cured ^d				
0.00 (CK)	1.24	1.57	1.50	
1.0	1.06	1.57	1.33	

^a Lemons were surface-sterilized 1 min in 70% ethanol, injury-inoculated with Penicillium digitatum, and incubated for 24 h at 20 C prior to treatment. The fruit were dipped in water or surfactant solution at the given concentration for 30 s before treatment.

curing for 48 h provided disease control comparable to imazalil. Further, curing treatments had no obvious effect on fruit quality or on development of other postharvest diseases. The effect of curing did not seem to vary with fruit harvested during different months. In the first experiment, dipping fruit in chlorine had no effect compared to the undipped control; however, in the next two experiments chlorine treatment alone significantly reduced the incidence of decay.

Changes in disease incidence over time were markedly different in fruit cured for different periods of time. The incidence of disease was maximal in control fruit and fruit cured for 12 h after 2 wk of storage. In contrast, fruit cured for 36 or 48 h was symptomless until week three, with maximal disease development occurring after the fifth or sixth week. An intermediate rate was observed with the 24-h curing treatment. Because of these results, fruit were cured for 18-24 h in the remaining experiments. This promoted a sufficiently high disease incidence for better treatment comparison, and permitted final disease evaluation after only 4 wk of storage.

Effect of delaying treatment. Curing fruit for 24 h resulted in significant ($P \le 0.05$) reductions of green mold over a 4-wk storage period, even if treatment was delayed up to 48 h (Table 1). However, treatment was most effective when fruit were cured within 24 h of inoculation, because significant increases in decay incidence resulted when curing was delayed for 36-48 h. Fruit inoculated 48 h prior to curing had 2.7-4.1 times more diseased fruit than those inoculated 12 h before curing.

Effect of surfactants. Dipping fruit in 1 g of SDBS per liter prior to curing reduced the incidence of green mold by 41-73% compared to the water-dipped cured control (Table 2). Effects of surfactant-dip significantly improved control in all experiments $(P \le 0.05)$. However, the relationship between concentration of SDBS and disease reduction was not consistent. SDBS had no effect on disease incidence of uncured fruit.

In a another set of four experiments, fruit were immersed in a chlorine solution prior to surfactant treatment. In these experiments, dipping in SDBS, SXS, or SDS solutions prior to curing had no significant effect on the incidence of green mold (Table 3). In fruit inoculated with an imazalil-sensitive isolate, imazalil provided the best disease control. However, in the June 25 experiment, which would have had the most-advanced field infections, control with imazalil was not significantly better than any of the curing treatments. When an imazalil-resistant isolate

TABLE 3. Comparison of pretreatment with three anionic surfactants in combination with curing or imazalil for control of green mold in lemons

Treatment Fruit dip ^a	Experiments, 1992 ^b			
	12 June ^{c,d}	24 June ^{d,e}	25 June ^{d,e}	26 June ^{e,f}
Cured for 24 h				
Water	0.180	0.393	0.347	0.418
SXS	0.296	0.316	0.227	0.396
SDS	0.218	0.356	0.225	0.405
SDBS	0.181	0.302	0.237	0.339
Not cured				
Imazalil	0.019	0.065	0.097	0.654
F value ^g	6.48**	8.86**	2.48	3.71*
LSD ($P = 0.05$)	0.121	0.133		0.196
LSD $(P = 0.01)$	0.171	0.187		0.274

^a Fruit injury-inoculated with Penicillium digitatum and held at 20 C for 24 h. All fruit were dipped in active chlorine at 150 mg/L for 1 min and rinsed with water. Fruit were then dipped for 45 s in water or a 1 g/L solution of sodium xylene sulfonate (SXS), sodium dodecyl sulfate (SDS), sodium dodecylbenzenesulfonate (SDBS), or imazalil.

^bSin⁻¹ (incidence of green mold after 4 wk in storage at 15 C). Values are means of four replicates. Dates indicate the day each experiment began.

^bSin⁻¹ (incidence of green mold after 4 wk in storage at 15 C). Values are means of four replicates. Dates indicate the day each experiment

^c Analysis of variance was performed separately on cured fruit. Significance at $P \le 0.05$ or 0.01 indicated by * or **, respectively.

^d The t test was used to compare means, no differences were significant.

^bSin⁻¹ (incidence of green mold after 4 wk in storage at 15 C). Values are means of four replicates. Dates indicate day each experiment began.

Riverside fruit.

d Imazalil-sensitive isolate (M6-r).

^e Ventura County, CA, fruit; picked 22 June 1992 and stored at 11 C prior to start of experiments.

Imazalil-resistant isolate (P-3).

^g and "indicate significance at $P \le 0.05$ and 0.01, respectively.

was used, all curing treatments gave significantly better control than did imazalil.

Effect of SDBS in vitro. The viability of P. digitatum conidia or germlings on PDA at 23 C was not affected by prior incubation at 30 C for up to 24 h or by the presence of 40 μ g of SDBS per milliliter (Fig. 2). However, the viability of conidia seeded onto SDBS-amended PDA and incubated at 30 C for 12 or 24 h was reduced by 12 and 66%, respectively. Germlings showed even greater sensitivity with corresponding reductions of 66 and 92%, respectively. The interaction between SDBS and incubation at 30 C was highly significant, as were all interactions between the main treatments (Table 4).

DISCUSSION

The number of fungicides available for postharvest decay control of California citrus has declined steadily since the 1970s (11,12). Thiabendazole and imazalil are the only postharvest fungicides registered in the United States for citrus, providing both eradicative and antisporulant action (11,12). Intensive use of these compounds has selected for resistant biotypes of *P. digitatum* worldwide (7,11). In 1990, 77% of *P. digitatum* isolates collected from citrus packinghouses in California were resistant to both thiabendazole and imazalil (17). To maintain the usefulness of these fungicides as antisporulants for soilage control

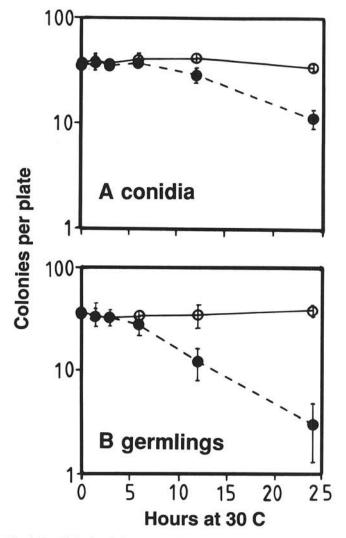


Fig. 2. Survival of conidia and germlings of *Penicillium digitatum* after incubation for different times at 30 C on potato-dextrose agar (PDA) (——) or PDA containing 40 μg of sodium dodecylbenzenesulfonate per milliliter (- - - - - - -). Plates were subsequently incubated at 23 C, and colony counts made every 12 h for 4 days. Each point is the mean of six replicates. Vertical bars indicate standard deviations.

in commercial shipments, management practices minimizing use of imazalil and thiabendazole on stored fruit must be employed (12). Practical alternative treatments must have eradicative activity and, ideally, should face minimal regulatory hurdles for implementation. Biological control of *P. digitatum* on grapefruit and lemons with *Candida guilliermondii* has been reported (9,10,21), but the treatment showed little or no eradicative activity (9). Further, the use of biological control organisms on postharvest commodities faces regulatory requirements similar to synthetic fungicides, because they and their metabolites pose a potential risk to consumers (22).

Earlier workers demonstrated that curing reduced Penicillium decay of oranges and other citrus fruits (3,5,10,24). Because curing faces no regulatory hurdles for implementation, we examined curing for decay control in California lemons. We demonstrated that 18-h-old infections of P. digitatum on lemons could be eradicated by curing for 48 h; in some experiments, decay control by curing for 48 h was equivalent to imazalil, the most effective fungicide registered for postharvest application today. Cured fruit remained essentially free of other diseases and did not develop Diplodia stem end rot at high frequencies, as has been reported in Florida oranges (18). Treatment could be delayed up to 24 h after harvest without compromising effectiveness, allowing considerable flexibility for its implementation. The incidence of disease in fruit cured immediately after inoculation was not the lowest as might be expected. Perhaps the ungerminated conidia were less adversely affected by the treatment than were germlings. In any case, these inoculum are of little practical importance, because they could easily be eradicated by surface-disinfestation (14). Curing, with or without a surfactant pretreatment, also was effective in reducing decay caused by an imazalil-resistant isolate of P. digitatum. Although curing was not always as reliable as imazalil and did not provide antisporulant action, this treatment is relatively simple and immediately available for use in an integrated program to lower selection pressure for fungicide resistance.

The time required for curing, up to 48 h, could be a deterrent to the adoption of this procedure by the California citrus industry. Therefore, we evaluated the use of surfactants that are "generally recognized as safe" (GRAS) in combination with a curing time of 24 h or less (1). Hoy and Ogawa (19) reported that SDBS was inhibitory to Botrytis cinerea, Geotrichum candidum, Phytophthora parasitica, and Rhizopus stolonifer. Carter (8), however, found that dipping grapefruit or oranges in 1 g of SDBS per liter did not reduce green mold on fruit held at 21 C. In our studies too, SDBS in the absence of curing did not reduce decay of lemons infected with P. digitatum. However, dipping fruit in 1 g of SDBS per liter prior to curing significantly improved decay control compared to the water-dipped cured fruit (Table 2).

TABLE 4. Analysis of variance and interactions between treatments for experiment shown in Figure 2, comparing treatments incubated at 30 C for 0 or 24 h^a

Source	df	Mean square	F value ^b
Total	47	7.906	
Treatment	7	7.385	81.0**
SDBS (A) ^c	1	2.22	170**
Incubation at 30 C (B)	1	2.04	157**
Developmental stage			
(conidia vs.			
germlings) (C)	1	0.26	20.0**
$A \times B$	1	1.94	149**
$A \times C$	1	0.36	27.7**
$\mathbf{B} \times \mathbf{C}$	1	0.24	18.7**
$A \times B \times C$	1	0.33	25.0**
Error	40	0.013	

^a Analysis based on log-transformed data.

^b Significance at $P \le 0.01$ indicated by ...

^c Sodium dodecylbenzenesulfonate.

To better understand the interaction between the residual surfactant in injuries and the pathogen during curing, in vitro studies were conducted. The combination of two nonlethal stresses, elevated temperature and SDBS, apparently results in high mortalities of P. digitatum. This reflects the strong interactions between SDBS, a 30 C temperature treatment, and the developmental stage of the fungus. The increased susceptibility of germlings may be due to their higher metabolism. In vitro results are consistent with in vivo results (Table 3); the residual SDBS in injuries reduced decay only when combined with curing.

The effect of surfactants was greatly diminished if fruit were immersed in chlorine prior to curing, as they would be in commercial handling (14). One explanation is that both chlorine and (surfactant plus elevated temperature) treatments are affecting the same inoculum (near the surface of the injury) and that curing alone eradicates P. digitatum that has penetrated more deeply into host tissue. Further investigations into this question are warranted; where possible, test conditions should be similar to those prevalent in commerce. Perhaps a treatment stimulating host defenses would be more effective in improving decay control by curing when infected fruit are surface-disinfested prior to

Practical alternatives to fungicides for control of green mold on stored lemons are needed. Curing lemons for control of green mold has attractive commercial possibilities because it provides effective eradicative action against P. digitatum, is not selective for fungicide-resistant biotypes of the pathogen, and does not leave objectionable residues. Curing has been advocated for control of G. candidum on lemons. However, this pathogen grows well at 30 C, so presumably curing would not eradicate incipient infections but would make injures more resistant to infection (2). Curing offers great potential in an integrated control program; the postharvest fungicides imazalil, thiabendazole, sec-butyl amine, prochloraz, and guazatine have all lost their effectiveness in many instances due to the buildup of resistant biotypes in the packinghouse (11). The additional handling of fruit required by curing will present a challenge to packinghouse management; however, further studies on the combined effects of curing with surfactants, biocontrol agents, or treatments stimulating wound healing may provide means of reducing treatment time and improving efficacy.

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