Comparison of Decay Control Strategies in California Lemon Packinghouses

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ABSTRACT

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Strategies for control of thiabendazole-resistant strains of blue and green mold (*Penicillium italicum* and *P. digitatum*) were evaluated in three lemon packinghouses in central California. Packinghouse layout and sanitation significantly influenced the size of the population of thiabendazole-resistant spores and the level of decay in lemons shipped to East Coast markets. Effective strategies involved isolation of sources of fungicide-resistant strains, disinfestation of contaminated equipment and containers, and a program for monitoring the level of fungicide resistance in the packinghouse.

Postharvest application of the fungicides benomyl, thiabendazole, sodium *o*-phenylphenate tetrahydrate (SOPP), and *sec*-butylamine (2-AB) has provided impressive control of fungicide-sensitive strains of *Penicillium italicum* Wehm. and *P. digitatum* Sacc. on lemons during storage and marketing (6). Widespread and intensive use of these fungicides, however, has resulted in selection and proliferation of *Penicillium* isolates that are resistant to these treatments (3,7,9,11). The problem of fungicide resistance in *Penicillium* spp. has been encountered in citrus fruits grown in all major production areas of the world (8,12,14,16).

The seriousness of the fungicide-resistance problem in a given citrus packinghouse can usually be related to fruit-handling practices and sanitation within the packinghouse. A packinghouse design is based principally on considerations of machine and labor efficiency and on minimizing fruit injury, but little attention is given to dispersal of inoculum of fungi responsible for fruit decays. The layout of many packinghouses is conducive to the movement of airborne spores of *P. digitatum* and *P. italicum* throughout the entire packing operation, presenting the possibility that sound fruit may be inoculated with spores from decayed fruit at several points during washing, waxing, and packing. These spores may arise from fungicide-resistant isolates that develop on fungicide-treated fruits. The buildup of fungicide-resistant strains of *Penicillium* in a packinghouse can greatly increase the level of fruit decay during storage and transportation to market.

The principal strategies that have been advocated to suppress proliferation of fungicide-resistant pathogens are 1) combining two fungicides with different mechanisms of action (4,11,13), 2) rotating two fungicides with different mechanisms of action (7,9,11), or 3) sanitizing packinghouses to reduce the pathogen population, including the fungicide-resistant component (3,5,9). The effectiveness of the first two strategies is reduced in practice because of the limited number of suitable fungicides available for use and the frequency with which *Penicillium* develops isolates resistant to each

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fungicide. Our investigation was undertaken to evaluate the effectiveness of sanitation and control of spore dispersal, combined with approved fungicide treatments, on the incidence of fruit decay in lemons handled in three commercial packinghouses.

MATERIALS AND METHODS

Packinghouse layouts and treatments. Three central California lemon packinghouses, each with a history of excessive decay caused by fungicide-resistant *Penicillium* spp., actively participated in this study during the 1980–1981 lemon season and provided reports on the market condition of their fruit shipments during the 1979–1980 season. The packinghouses were similar in age (about 20 yr old) and size, although plant layouts varied (1). Each plant processed the same type of fruit and followed similar storage practices, eg, length of time fruit held, but varied widely in the amount of fruit processed (Table 1). Each of the cooperating packinghouses followed a sanitation program to reduce the population of *Penicillium* spores in the atmosphere and on fruit-handling equipment. After fruit were dumped onto the packing line, the empty storage bins were sprayed with a solution of a quaternary ammonium compound (800 mg a.i./L) (Cleansan 537, Brogdex Company, Pomona, CA, or Sanitize, F & H Chemical Company, Tulare, CA). All belts and roll conveyors that moved fruit were wiped nightly with a chlorine solution (about 1.6 g/L sodium hypochlorite). In addition, packinghouse 2 sprayed machinery, belts, and rot-handling areas once each week with 50% (v/v) aqueous isopropyl alcohol and packinghouses 1 and 3 were fumigated each week with an aerosol of 3% (w/v) formaldehyde.

Fruit-handling procedures in the three packinghouses for the 1979–1980 and 1980–1981 seasons are outlined in Table 2. Major structural changes were made in packinghouse 1 in an attempt to isolate *Penicillium* spores at one location. The area where the fruit were dumped from the storage boxes was enclosed completely and equipped with a fan to exhaust the spore-laden air from the packinghouse through the roof. This created a negative pressure in the dumping enclosure, minimizing the movement of spores to other areas of the packinghouse. A flume (45.7-cm-diam. PVC pipe) with recirculated water was installed to transport the decayed fruit in an enclosed system from the dump and grading areas to a truck outside the packinghouse. Each morning, a 0.3% solution of SOPP (Freshgard 5) (1 qt/30 gal water) was added to the recycled water in the flume to kill mold spores and reduce odor. Fruit-handling procedures and treatments were not changed in the two seasons covered by our study.

Major changes in fungicide treatments in packinghouse 2 from the 1979–1980 to the 1980–1981 season were the addition of 2% (w/v) potassium sorbate to the wax formulation applied to the fruit before storage and the addition of 0.5% SOPP and 2% potassium sorbate to the water-wax formulation applied to the stored fruit just before packing. Potassium sorbate is effective against *Penicillium* isolates resistant to thiabendazole (15,17). Structural changes for the 1980–1981 season consisted of moving the fruit dump outside the packinghouse and enclosing it. The conveyor belt for decayed fruit, however, remained in the packinghouse, where it was used to transport decayed fruit from the grading table to an open hopper just outside the packinghouse. This conveyor replaced the garbage bin inside the packinghouse that had been used for handling decayed

Table 1. Total cartons packed at each packinghouse for the 1979–1980 and 1980–1981 seasons[a]

	Packinghouse 1		Packinghouse 2		Packinghouse 3	
Month	**1979–1980**	**1980–1981**	**1979–1980**	**1980–1981**	**1979–1980**	**1980–1981**
Nov.	8,452	17,013	2,676	70,047	3,916	35,596
Dec.	44,540	22,060	26,306	71,647	36,819	182,373
Jan.	94,232	39,961	21,622	69,031	39,164	222,374
Feb.	94,377	78,944	24,178	67,087	46,474	188,029
Mar.	80,334	103,804	35,350	18,216	43,768	264,978
April	70,681	89,194	48,817	58,216	35,437	237,471
May	17,949	38,095	53,062	64,078	30,566	288,510
Total	412,565	389,071	212,011	419,095	236,144	1,419,331

[a] About 90% of all cartons packed are shipped to fresh markets; 10% are usually sent to manufacturers for products such as juice.

Table 2. Fruit treatments and sanitation practices in cooperating packinghouses during the 1979–1980 and 1980–1981 seasons[a]

Packinghouse 1		Packinghouse 2		Packinghouse 3
1979–1980	**Changes in 1980–1981**	**1979–1980**	**Changes in 1980–1981**	**1979–1980 and 1980–1981**
Harvest	···	Harvest	···	Harvest
Wet dump[b] (3% sodium carbonate)	···	Dry dump[c]	···	Dry dump[c]
Wash (3% sodium carbonate)	···	Wash (0.5% SOPP)[d]	···	Wash (0.5% SOPP)[d]
Emulsion wax + 2,4-D + thiabendazole	···	Water wax + 2,4-D + thiabendazole	Added 2% potassium sorbate	Emulsion wax + 2,4-D + thiabendazole
Storage 1–4 mo	···	Storage 1–4 mo	···	Storage 1–4 mo
Dry dump[c]	Dump area enclosed and exhausted by fan	Dry dump[c]	Moved outside and enclosed	Dry dump[c]
Rots collected in open bins	Rots transported by water flume to outside the packinghouse	Rots collected in open bins	Rot bin moved outside	Rots collected in open bins
Foam wash (2% SOPP)[d]	···	Foam wash (2% SOPP)[d]	···	Foam wash (2% SOPP)[d]
Water wax + thiabendazole	···	Water wax + thiabendazole	Added 0.5% SOPP and 2% potassium sorbate to water wax	Water wax + thiabendazole
Pack in cartons with biphenyl papers	···	Pack in cartons with biphenyl papers	···	Pack in cartons with biphenyl papers
Ship to market	···	Ship to market	···	Ship to market

[a] Complete diagrams in Bancroft (1).

[b] Lemons dumped from containers into water solution.

[c] Lemons dumped from containers onto a conveyor belt.

[d] Sodium *o*-phenylphenate tetrahydrate

fruit the previous season.

Packinghouse 3 did not adopt any new treatment measures during the 1980–1981 season except for the sanitation program adopted by all three houses.

Assessment of *Penicillium* spore concentration and thiabendazole resistance. The relative concentration of spores of *P. digitatum* and *P. italicum* in the atmosphere of each packinghouse was estimated by exposing plates containing potato-dextrose agar amended with neopeptone (10). Dicloran (3 μg/ml) was added to the medium to suppress expansion of the *Penicillium* colonies and to reduce contamination of the plates with *Rhizopus*. Plates containing the same medium supplemented with thiabendazole (20 μg/ml) were used to determine the percentage of thiabendazole-resistant spores in the *Penicillium* population.

Pairs of petri plates (with and without thiabendazole) were exposed at predetermined sites in the packinghouse. Sites were determined by spore levels

Table 3. Relationship between *Penicillium* spore concentration and packinghouse atmosphere, thiabendazole resistance, and decay of injured lemons

Storage and shipping fungicide treatments[v]	*Penicillium* colonies per plate per minute of exposure: Total	*Penicillium* colonies per plate per minute of exposure: Thiabendazole-resistant	Decayed fruit
Lab. control (TBZ) Mean[w]	1.2 fg	0.0 fh	0.0 fi
Packinghouse 1 (SOPP[x] + TBZ[y]) Mean[w]	6.2 g	2.4 ah	0.6 ai
Packinghouse 2 (SOPP + TBZ + sorbate[z]) Mean[w]	29.4 bg	14.0 bch	4.8 ci
Packinghouse 3 (SOPP + TBZ) Mean[w]	117.6 d	72.0 de	17.2 e

[v] Each treatment was applied to five lots of 25 injured (scratched), but not inoculated, lemons. Total sample size was 125 lemons.

[w] Lowercase letters indicate Duncan's multiple-range groupings of means in each row (a–f) and column (g–i), which do not differ significantly at the 5% level.

[x] Sodium *o*-phenylphenate tetrahydrate (2%) applied in foam wash.

[y] Thiabendazole (1 g/L) applied in wax.

[z] Potassium sorbate (2%) applied in aqueous solution in wax.

Table 4. Relative abundance of *Penicillium* spores in the atmosphere of packinghouses at various locations during the 1980–1981 season and appropriate statistical tests of the mean

Month of packing season		*Penicillium* colonies per plate per minute of exposure[u]										
		Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Total	Mean	DMR[v]	Variance[w]
Packinghouse 1												
Pregrade	T[x]	4	112	4	8	14	38	130	310			
	R	0	0	0	0	0	2	26	28			
Degreening rooms	T	8	36	4	4	32	34	120	238			
	R	0	0	0	0	0	4	60	64			
Sealer area	T	12	138	4	64	84	40	82	424			
	R	0	0	0	0	0	30	40	70			
Wax tank	T	6	164	12	116	110	96	272	776			
	R	0	0	0	0	0	22	68	90			
Total	T	30	450	24	192	240	208	604[y]	1,748[z]	250	a	c
	R	0	0	0	0	0	58	194	252	36	b	
Packinghouse 2												
Pregrade	T	16	22	534	156	56	224	62	1,070			
	R	0	0	80	70	56	34	24	264			
Degreening rooms	T	2	2	12	4	8	4	40	72			
	R	0	0	0	0	8	0	40	48			
Wax tank	T	22	16	90	56	62	302	106	654			
	R	0	0	10	30	62	120	54	276			
Box fillers	T	16	14	230	370	44	176	58	908			
	R	0	0	24	52	44	132	30	282			
Total	T	56	54	866	586	170	706	266	2,704	386	a	...
	R	0	0	114	152	170	286	148	870	124	b	
Packinghouse 3												
Pregrade	T	16	24	90	22	174	38	22	386			
	R	0	0	4	0	26	0	2	32			
Degreening rooms	T	6	4	20	16	70	18	32	166			
	R	0	0	0	0	10	0	4	14			
Pack grade table	T	10	26	426	110	678	118	266	1,634			
	R	0	0	10	16	406	30	80	542			
Box fillers	T	14	30	358	68	854	132	256	1,712			
	R	0	0	108	14	470	38	88	718			
Total	T	46	84	894	216	1,776	306	576	3,898	557	a	...
	R	0	0	122	30	912	68	174	1,306	187	b	

[u] Each value is the sum of colonies on four plates exposed on each of two sampling dates each month.

[v] Lowercase letters indicate Duncan's multiple range (DMR) grouping of means for T and R among packinghouses, which do not differ significantly at the 5% level. Tests using the adjusted values for packinghouse 1 do not change the groupings.

[w] Lowercase letter indicates analysis of variance of the means between T and R within each packinghouse where the *F* distribution is significant at the 5% level.

[x] T = total colonies, R = thiabendazole-resistant colonies.

[y] The first week's assay in May was T = 91 and R = 24.

[z] Assuming the second assay had the same spore level at all locations as the first: total = 1,326 and 106, and mean = 189 and 15 for T and R, respectively.

measured in previous seasons and by analyzing packinghouse wind currents to identify locations where spores would be expected to concentrate. Four pairs of plates were exposed on each sampling date and the packinghouses were sampled twice each month. The exposed petri plates were incubated at 22 C for 5 days. Colonies of *P. digitatum* and *P. italicum* on each plate were counted and the relative concentration of spores in the atmosphere was expressed as the number of colonies per minute of exposure of the plate. The number of colonies on the thiabendazole-supplemented plate times 100, divided by the number on the control plate, gave the percentage of thiabendazole-resistant spores in the *Penicillium* spore population at that site in the packinghouse.

Experimental lemons. All fruit used in this investigation were grown in the southern San Joaquin Valley in order to minimize variability that might exist among fruits produced in different regions. To minimize the impact of careless field handling on fruit decay, samples of fruit were dipped in an aqueous solution of triphenyltetrazolium chloride (1 g/L) to reveal the degree of peel injury (9). Fruit lots arriving from the field were rejected if they had more than two lemons showing more than 5% of their surface area stained.

The relationship between spore concentration in the environment and fruit decay was evaluated in the following manner. At the beginning of the fruit season, lemons were collected at packinghouse 1 before any treatment was applied. The fruit were surface-disinfested by dipping 5 sec in a chlorine solution (200 ppm, pH 7.5) and superficially injured by scratching (1 × 10 mm). Scratched fruit were distributed randomly into 20 sublots of 25 fruit each. Within 3 hr of injury, five sublots were run through each packinghouse (Table 2) and five sublots were retained in the laboratory as a control. The *Penicillium* spore population in the packinghouse was sampled at the same time for concentration and thiabendazole resistance of the spores. Treated fruit were stored at 21 C for 10 days and inspected for decay.

RESULTS AND DISCUSSION

Relation between spore population and fruit decay. In the preliminary experiment with injured (but not inoculated) lemons, decay increased as the concentration of *Penicillium* spores in the packinghouse atmosphere increased, especially if the spore population consisted of a substantial percentage of fungicide-resistant individuals (Table 3). Therefore, a decrease in spore population inside a commercial packinghouse should be reflected by a reduction in fruit decay during storage and marketing.

The concentration of *Penicillium* spores in the atmosphere of the cooperating packinghouses was lowest at the beginning of the 1980–1981 season (Table 4). The highest concentration of spores was found at sampling sites adjacent to or within the packing area. A high concentration of spores was measured in the prestorage wash-line area of packinghouse 2 because of the transfer of spores from the poststorage dump by prevailing air currents. It appears that fruit being prepared for shipment to market were contaminated by the time they were enclosed in the carton. Differences among the total and resistant spores for the season for packinghouses 1, 2, and 3 (1,748 and 252, 2,704 and 870, and 3,898 and 1,306 colonies, respectively) were not statistically significant. Three factors may have influenced the statistical test.

First, biweekly sampling, a standard packinghouse practice, was not sufficient to capture the apparent high variability in spore concentrations within the packinghouse over time. Second, marketing strategies have often resulted in fruit being stored for extended periods, especially during the holiday season (December and January) and the spring marketing season (March through May) in order to receive a higher price at the market. Prolonged storage, however, tends to increase the number of decayed fruit and spores in the packinghouse. Third, the overall level of contamination in packinghouse 1 may have been obscured by a breakdown in sanitation practices during the middle of May. In this episode, a specific lot of fruit with excessive decay was repacked without passing through the enclosed dump and without proper sanitization of the packinghouse after the repacking session. Even adjusting for this factor, however, there appeared to be no statistical significance among packinghouses in the total number of spores trapped (Table 4).

The statistical tests mask several important distinctions among the packinghouses. In packinghouse 1, resistant spores did not develop until April, whereas they appeared in January in the other two houses. The level of resistant spores found in packinghouse 2 was relatively constant each month (100–200 colonies). High levels of resistant spores were found in the packing areas of each house, particularly packinghouse 3. Packinghouse 2 has the additional problem of recycling spores to the prestorage area from the poststorage area where the fruit are dumped and rots removed (Table 2). By reducing the overall spore level in packinghouse 1, a sharp drop in resistant spores in the sealer area where fruit were being packed was observed. Only packinghouse 1 showed a statistically significant difference between the level of total and resistant spores within the packinghouse (Table 4). In comparing these results with the preliminary injured lemon test, the level of decay in storage and market arrivals was expected to be less in packinghouse 1 because the fungicide-resistant spore population in this packinghouse was significantly lower throughout the season than that in the other packinghouses.

Storage and market decay levels. Fruit packed during the 1979–1980 season tended to develop more types of decay, particularly sour rot, than fruit packed during the 1980–1981 season, when Penicillium decay was most prevalent. For the 1979–1980 season, all packinghouses experienced total storage decay levels averaging about 4.4%, whereas packinghouse 1 experienced the highest level of market decay at 4.3% (Table 5). The 1980–1981 crop was harvested in a less mature condition than the previous season's crop and, therefore, the early fruit were more resistant to infection (2). As the season progressed, packinghouse 3 showed no improvement in decay control in stored fruit, whereas packinghouse 2 experienced a 0.9% drop in decay for the seasonal average over the previous season. Packinghouse 1 sharply reduced storage decay to a seasonal average of 2.9%, a marked improvement over 1979–1980.

Although all packinghouses recorded a reduction in the level of market decay, packinghouse 1 showed a substantial reduction of 4.2% between seasonal averages for this type of decay (Table 5). In fact, except for the one week in May, no decay was recorded by packinghouse 1 in market arrivals during 1980–1981. Although some reduction in decay levels can be attributed to the condition of the fruit in 1980–1981, isolation of thiabendazole-resistant spores by packinghouse 1 minimized the possibility for spores to contaminate the packing area where fruit received the final

Table 5. Comparison of the average annual decay levels for fruit stored and shipped from each packinghouse during 1979–1980 and 1980–1981

Location of decayed fruit	Seasonal average decay[a,b] (%) 1979–1980	1980–1981
Packinghouse 1		
Storage decay	4.4	2.9
Market decay	4.3	0.1[c]
Packinghouse 2		
Storage decay	4.3	3.4
Market decay	3.5	2.7
Packinghouse 3		
Storage decay	4.4	4.4
Market decay	3.7	3.4

[a] Data on monthly market decay furnished by FMC, Sunkist Growers, Inc., and packinghouse reports and on storage decay from packinghouse records. Monthly averages can be found in Bancroft (1).

[b] Figures represent total decay including blue and green mold and sour rot. Sour rot was widely prevalent in 1979–1980 but did not appear in 1980–1981. Blue and green mold was most prevalent in 1980–1981.

[c] Decay was 3% for the week of May 15; decay was not observed during the rest of the month.

thiabendazole treatment and were packed. This component of the decay control strategy of packinghouse 1 contributed to the observed reduction in decay in the market.

Market discounts. Wholesalers lower the carton price of fruit, a practice known as "discounting," if an excessive number of decayed fruit is present on arrival at the market. The amount of the discount varies with quantity and size of fruit available at that time. We did not compare the discounts for the 1979–1980 and 1980–1981 seasons because the fruit for the two seasons differed in quality and quantity. Small fruit, which characterized the 1979–1980 crop, sells for a much lower price at the market. Buyers seeking larger fruit were willing to accept large fruit of lower quality (more decay) before discounting. In 1980–1981, fruit size was larger and more fruit was available at better quality. Buyers steadily increased the discount rate (lowered the price) on shipments arriving with decay throughout the season.

Total discounts on fruit from packinghouses 1 and 3, which shipped only to domestic markets during the 1980–1981 season, were obtained from actual market reports and totaled $3,000 and $289,100, respectively. The total season discount (domestic and foreign shipments) for packinghouse 2 was calculated from the actual domestic market discount and an estimate for fruit shipped to foreign markets. Exported fruit comprised 60% of all fruit shipped by packinghouse 2. Assuming all fruit shipped to all markets decayed at the same rate, the $45,200 domestic discount was extrapolated to a total seasonal discount of $113,000 for packinghouse 2. Standardizing on the basis of total cartons shipped, the market discounts for packinghouse 1, 2, and 3 were $9, $300, and $226, respectively, per 1,000 cartons shipped.

The higher return on lemons shipped from packinghouse 1 during the 1980–1981 season compared with that from packinghouses 2 and 3 indicates that sanitation procedures that reduced the population of thiabendazole-resistant spores resulted in a substantial increase in profitability in marketing the fruit. Increased use of fungicides in packinghouse 2 compared with packinghouse 3, however, was not economically justified in this instance in view of the lower returns (about $74/1,000 cartons shipped) earned by packinghouse 2.

Hall and Bice (9) recommended a strategy for control of fungicide-resistant *Penicillium* spp. in Arizona packinghouses but did not provide data on benefits realized in decay reduction or economic returns. Our study with comparatively uniform lemons from a single production area documents the economic benefits of several measures frequently recommended for reducing the negative impact of fungicide-resistant pathogens on disease control (4,7,9,11). We found the most cost-effective measures to be 1) spatial isolation of decay-elimination areas from other packinghouse operations involving sound fruit, 2) sanitation of work areas, and 3) monitoring the magnitude and fungicide resistance of the spore population at frequent intervals during the packing season. These practices minimize cycling of resistant *Penicillium* strains from fungicide-treated decayed fruit and measure the level of resistance in the spore population in the packinghouse, thereby prolonging the useful life of postharvest fungicide treatments. These measures also reduce the concentration of fungicide-sensitive isolates of the pathogen. Although these isolates may be controlled by the fungicide treatment, they are known to increase the infectuousness of the fungicide-resistant isolates (18).

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