

heated solutions to the conventional industry practice of spraying imazalil in wax on fruit for the control of green mold; and to determine if an imazalil-resistant isolate of *P. digitatum* could be adequately controlled by applying heated imazalil solutions.

MATERIALS AND METHODS

Fruit and packing line configuration. Sweet orange (*Citrus sinensis* [L.] Osbeck) cultivars Valencia and Washington and lemon (*C. lemon* [L.] N. L. Burm.) cultivar Eureka grown in the San Joaquin Valley of California were commercially harvested 1 to 2 days before they were selected by hand from field bins and randomized. When inoculated, they were inoculated once each about 24 (± 2) h before treatment. The internal temperature of the fruit was 18–20°C. All treatments were applied on a commercial-scale packing line to four replicates of 60 oranges or 75 lemons each. A fifth replicate of 30 to 50 uninoculated fruit was included in some tests for residue analysis and later inoculation in sporulation tests. The temperatures of fruit were monitored with 1-mm-diameter, copper-constantan probes (Gaffney Engineering, Gainesville, FL) attached to a thermocouple thermometer (model HH11, Omega Engineering, Stamford, CT). The temperatures of air or water were monitored with a 3-mm-diameter by 30-cm-long corrosion-resistant, copper-constantan probe (model TJ48, Omega Engineering) attached to the same thermocouple thermometer. The temperatures of water or imazalil solutions reported are $\pm 0.5^\circ\text{C}$.

Each replicate was placed on the packing line just before a high-pressure washer. The residence time in the high-pressure washer, operating at 1,500 Newtons pressure, was 12 s. The water in the pressure washer contained 10 to 50 $\mu\text{g}\cdot\text{ml}^{-1}$ sodium hypochlorite at pH 7.2. After the high-pressure washer, the fruit passed for 15 to 20 s over rotating brushes through a dryer operating at 32.2°C to a moving belt sorting table, where decayed or damaged fruit were removed, and passed into a 2,400-L

capacity tank containing water or imazalil. The concentration of imazalil in the tank was determined periodically. The pH of water and imazalil solutions was 7.2. The residence time of the fruit in the tank was 15, 30, 60, or 90 s. After treatment in the tank, the fruit were dried for 15–20 s through a dryer operating at 32.2°C (first stage of drying), wax was applied, and the fruit were dried again for 15–20 s through a dryer operating at 32.2°C (second stage of drying). Two waxes were used—a high solids content, shellac-based organic finishing wax was applied in all tests with fruit that were dried in the first and second dryers and a water-emulsion lemon storage wax with a 2% solids content was applied on lemons that were dried in the first stage dryer and air-dried after wax treatment.

Green mold control. An imazalil-sensitive isolate M6R of *P. digitatum* (a gift of J. W. Eckert, University of California-Riverside) and an imazalil-resistant isolate (151, a gift G. R. Holmes, University of California-Riverside) were used in tests where fruit were inoculated. To determine the minimum imazalil concentration that was inhibitory to these isolates, 2-mm-diameter mycelial plugs from 1-week-old potato dextrose agar (PDA) cultures of each isolate were transferred to imazalil-amended PDA followed by 3 days incubation at 25°C. To inoculate the fruit, a steel rod with a 2-mm-long by 1-mm-wide tip was dipped into a water suspension of 10^6 spores $\cdot\text{ml}^{-1}$, then the tip of the rod was used to puncture the fruit. Inoculated fruit were stored at 10°C in cartons lined with perforated plastic liners and the incidence of green mold was recorded after 3 weeks.

Sporulation tests. To determine the influence of treatments on sporulation, the method of Eckert and Brown (7) was used. Fruit were inoculated by the injection of 0.10 ml volume of water containing of 10^6 spores $\cdot\text{ml}^{-1}$ of imazalil-sensitive isolate M6R about 1 cm below the rind surface, then the abundance of sporulation was recorded after incubation for 21 days at 20°C. A sporulation index that described

the percentage of the fruit surface covered with green mold spores was used where 5 = 100%; 4 = 90–99%; 3 = 50–89%; 2 = 11–49%; 1 = 1–10%; 0 = 0%. Each value represented the mean of sporulation ratings for 12 to 15 fruit per treatment.

Imazalil analyses. Imazalil residues on fruit are expressed as $\mu\text{g}\cdot\text{g}^{-1}$ fresh weight. Because the surface to mass ratio will change with fruit size, and presumably influence the quantity of residue per gram of fruit weight, the orange and lemons selected for analysis were of uniform size and represented common commercial size classes. The oranges were 7 cm in diameter. The lemons were 7.5 cm in length and 5.5 cm wide. Imazalil residues in fruit were determined from two replicates of eight fruit each. Each fruit was sliced into eight portions, and one portion from each of the eight fruit was combined before analysis to comprise one replicate. To the combined fruit portions, a water volume one-half the weight of the fruit sample was added, blended at high speed in a blender, and an aliquot extracted with 10% (w/v) sodium hydroxide saturated with sodium chloride, followed by the addition of ethyl acetate. The suspension was briefly macerated in a homogenizer, mixed by rotation for 30 min, centrifuged, an aliquot of the supernatant (ethyl acetate layer) was then removed. After 0.05 N H_2SO_4 was added to this aliquot, it was shaken vigorously for 2 min, the ethyl acetate layer was discarded, and the aqueous layer was adjusted to pH 9 to 10 with sodium hydroxide and trisodium phosphate buffer. An aliquot of ethyl acetate was added and shaken vigorously. The ethyl acetate layer was removed, dried with anhydrous sodium sulfate, and analyzed by gas chromatography with an electron capture detector. Recovery of imazalil from fruit portions spiked with 1 to 5 $\mu\text{g}\cdot\text{g}^{-1}$ imazalil was about 95%. The imazalil content of water or wax solutions was determined by analysis. Water or wax solutions of imazalil were extracted with ethyl acetate, dried with sodium sulfate, and analyzed by gas chromatography.

Influence of fungicide concentration, temperature, and immersion period on imazalil residues. To determine the influence of imazalil (Fungaflor 500EC, 44.6% a.i.; Janssen Pharmaceutica, Titusville, NJ) concentration, tank temperature, and immersion time on imazalil residues on Valencia oranges, the fruit were pressure washed with water and immersed in 0, 153, 244, or 335 $\mu\text{g}\cdot\text{ml}^{-1}$ imazalil at 32.2, 37.8, or 43.3°C for 15, 30, or 60 s. After treatment, the oranges were dried in the first stage of the packing line dryer as previously described; waxed with a high solids content, shellac-based organic finishing wax on the packing line; and dried in the second stage of packing line dryer as previously described. To determine the influence of imazalil concentration, tank temperature, and immersion time on residues

Table 1. Influence of imazalil concentration, temperature, and immersion period on imazalil residues ($\mu\text{g}\cdot\text{g}^{-1}$) on Valencia oranges

Imazalil ($\mu\text{g}\cdot\text{ml}^{-1}$)	Temperature($^\circ\text{C}$)	Immersion time (s)		
		15	30	60
153	32.2	1.04 (1.18) ^a	1.21 (1.30)	1.29 (1.55)
153	37.8	1.09 (0.90)	1.45 (1.36)	2.41 (2.29)
153	43.3	1.26 (1.66)	2.85 (2.46)	4.20 (4.06)
244	32.2	1.24 (1.38)	2.02 (1.59)	2.95 (2.03)
244	37.8	1.39 (1.12)	1.54 (1.87)	2.24 (3.38)
244	43.3	2.17 (1.90)	2.82 (3.19)	5.85 (5.76)
335	32.2	2.05 (2.57)	2.94 (2.88)	3.26 (3.50)
335	37.8	2.88 (2.33)	3.78 (3.38)	5.35 (5.47)
335	43.3	2.84 (3.14)	4.71 (4.91)	8.84 (8.45)

^a Each value ($\mu\text{g}\cdot\text{g}^{-1}$) is the mean of two analyses. Values in parentheses are predicted by a second order surface model. Imazalil residues = $25.74 - 0.0117 (IC) - 1.25 (T) - 0.000345 (IC * T) - 0.00662 (t) - 0.000761 (IC * t) + 0.000134 (T * t) + 0.0000258 (IC * T * t) + 0.0000604 (IC)^2 + 0.0170 (T)^2$. IC = aqueous imazalil concentration ($\mu\text{g}\cdot\text{ml}^{-1}$); T = aqueous imazalil solution temperature ($^\circ\text{C}$); and t = immersion time (s) in aqueous imazalil.