

Evaluation of New Postharvest Treatments to Reduce Postharvest Decays and Improve Fruit Quality in Citrus Packinghouse Operations

J. E. Adaskaveg

Plant Pathology, UC/Riverside

In the summer of 2005, fludioxonil and pyrimethanil were registered in California following the federal registration in the fall of 2004 for postharvest use on citrus. The postharvest registration of azoxystrobin is still pending but is expected in the summer of 2006. In Sept. 2005, we successfully placed propiconazole into the federal IR-4 program to establish a residue tolerance for postharvest use, and these studies are planned for 2006.

Thus, we continued our research on developing new fungicides and usage patterns to provide optimum control and prevent the development of resistance in populations of *Penicillium* species on citrus with the use of different mode of action fungicides.

In comparative efficacy studies, in-line drench applications were more effective against green mold than controlled droplet (CDA)-brush or -roller applications, improving efficacy by 23.7% for fludioxonil, 32.8% for azoxystrobin, and 37.5% for pyrimethanil as compared to CDA-roller applications. The CDA-brush application improved efficacy as compared to the CDA-roller application by 22.1% for azoxystrobin and 7.9% for fludioxonil, but not for pyrimethanil. Using this fungicide application method, the same solution is being re-circulated and thus the potential of building up inoculum in it is high unless a sanitizing agent is incorporated.

Fruit inoculation assays showed a reduced efficacy for imazalil and pyrimethanil when sodium hypochlorite was added to the fungicide solution, whereas peroxyacetic acid had no inhibitory effect. Fludioxonil, TBZ, and azoxystrobin were not negatively affected by the sanitizers. These results were supported by gas chromatography analyses studies where aqueous fungicide solutions either alone or amended with sodium hypochlorite, sodium hypochlorite plus sodium bicarbonate, or peroxyacetic acid were analyzed for their stability based on fungicide concentration. Results revealed that imazalil and pyrimethanil were incompatible with sodium hypochlorite or a mixture of sodium hypochlorite and sodium bicarbonate, while fludioxonil and TBZ were stable. In contrast, all fungicides were stable using peroxyacetic acid. Azoxystrobin was not evaluated.

By using the spiral gradient dilution technique, EC_{50} values were obtained for twenty-nine *P. italicum* isolates. EC_{50} values for imazalil ranged from 0.008 to 0.054 ppm. For azoxystrobin, EC_{50} values obtained with incorporation of salicylhydroxamic acid (SHAM) were between 0.010 and 0.057 ppm, while for fludioxonil they ranged between 0.004 and 0.006 ppm, and for pyrimethanil between 0.031 and 0.048 ppm.

Continuing our real-time packinghouse sampling program for monitoring fungicide sensitivities in populations of *Penicillium* species of citrus, we were able to isolate colonies of *P. digitatum* growing at fludioxonil concentrations higher than the baseline isolates. *In vitro* characterization revealed a highly resistant (HR) group growing at 10 ppm of fludioxonil and a moderately resistant (MR) group growing at 0.1 ppm, as compared to the baseline EC_{50} values ranging from 0.009 to 0.072 ppm. Lemon inoculation studies showed differences in pathogenicity (disease incidence) and virulence (sporulation and lesion diameter) among the HR, MR, and baseline isolates. Initial results indicated that the resistant isolates were less fit. Research is underway to further characterize these isolates at molecular and biochemical levels.

Additionally, during the samplings it was noticed that when azoxystrobin-SHAM-amended agar plates were exposed in packinghouses, the predominant colonies at the high fungicide concentrations were those of *P. italicum*. Using molecular primers from other pathogens that had developed resistance to azoxystrobin, we were able to differentiate sensitive from resistant isolates of *P. italicum*. To date, no isolates of *P. digitatum* were less sensitive to azoxystrobin.

Concerning pyrimethanil resistance in *P. digitatum*, laboratory-selected isolates were pathogenic and virulent causing decay of pyrimethanil-treated and non-treated lemon fruit. Decay incidence of treated fruit was up to 80% for some isolates, whereas baseline isolates were nearly completely inhibited by pyrimethanil. Azoxystrobin and fludioxonil were efficacious when applied to fruit inoculated with pyrimethanil-resistant isolates, indicating no multiple-resistance among the new materials. Philabuster[®], a 1:1 premix of imazalil and pyrimethanil, provided excellent disease control to fruit inoculated with pyrimethanil-resistant isolates. Research is in progress to characterize the type of resistance as well as the relative fitness of pyrimethanil-resistant and -sensitive biotypes of *P. digitatum*.

Overall, this research emphasizes the resistance potential of the new fungicides and the use of fungicide mixtures and other integrated pest management practices to prevent resistance from developing to any one of the new materials.

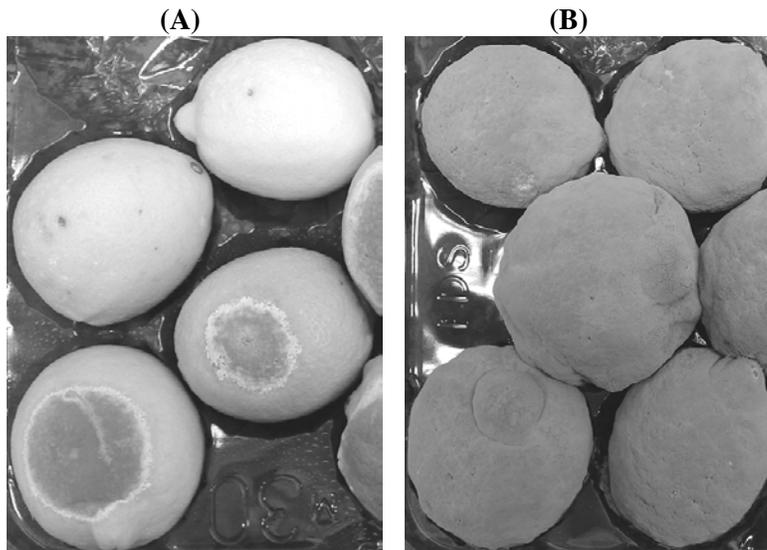


Figure 1. Reduced pathogenicity and virulence of fludioxonil-resistant isolates of *P. digitatum* on fruit treated with a 1,000-ppm solution of the fungicide (A) as compared to baseline isolates inoculated on non-treated fruit (B) and incubated for 8 days at 20 °C.

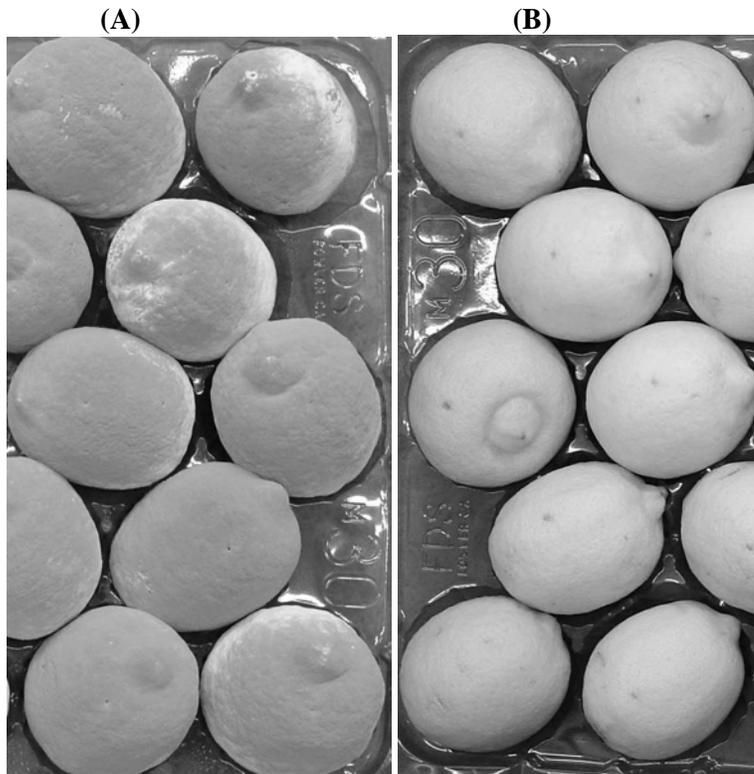


Figure 2. Pathogenicity and virulence of pyrimethanil-resistant isolates of *P. digitatum* was not reduced on fruit treated with a 1,000-ppm solution of the fungicide (A). Pyrimethanil-resistant isolates were controlled with 1:1 mixtures of pyrimethanil and imazalil (Philabuster) (B) or with mixtures of fludioxonil and azoxystrobin demonstrating that multiple resistance does not occur among these fungicides and that mixtures should be used in resistance management practices.

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