Title Development of new postharvest fungicides for integrated management of citrus green mold caused by *Penicillium digitatum*

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Abstract

Green mold, caused by the fungus *Penicillium digitatum* (Pers.:Fr.) Sacc., is the most important postharvest disease of citrus. Crop losses caused by the disease can have significant economic impact on citrus growers and packers. Currently, two fungicides thiabendazole (TBZ) and imazalil comprise the core component of the management programs against postharvest decays of citrus. Their extensive and continuous usage for over thirty and twenty years respectively, has resulted in selection and proliferation of resistant individuals of *P. digitatum,* causing significant crop losses. During this dissertation we developed three new, reduced-risk fungicides, azoxystrobin, fludioxonil, and pyrimethanil, all belonging to different chemical classes. The new materials were found to be highly effective in controlling green mold. Mixture applications were found to be more efficacious that single fungicide treatments. The effect on fungicide efficacies were also studied in relevance to co-applications with storage and pack waxes. In time-course studies to evaluate post-infection activity of the new materials fludioxonil and azoxystrobin performed best when applied 12 h after fruit inoculations, while pyrimethanil provided excellent control even after 21 h. Efficacy data on decay incidence and sporulation control from this work supported registration labels for all three materials for management of citrus green mold on citrus fruit in the United States. We also developed a new, rapid, spiral gradient dilution method for determining 50% effective concentration values and fungus-fungicide interactions. Baseline sensitivities of *P. digitatum* populations to these fungicides were established before their commercial use. Sixty-seven single spore isolates of the pathogen were assayed using the spiral gradient dilution (SGD) method. No cross-resistance was detected between imazalil or TBZ and the new materials. EC_{50} values obtained using the SGD method, were very similar to those obtained using the serial dilution method. In-line re-circulated drench applications provided excellent control, as compared to control droplet applications (CDA) over roller and brush beds. To prevent pathogen contamination of drench solutions, two sanitizing agents were evaluated. A hydrogen dioxide-based product was compatible and efficacious with all fungicides tested. Sodium hypochlorite was incompatible with imazalil and pyrimethanil, but not with azoxystrobin, fludioxonil and TBZ. Thus,

fungicide-sanitizer mixtures and in-line drenches were effective as decay control treatments and as anti-resistance strategies. Additionally, heated treatments of fludioxonil to 50°C and its combined application with 3% sodium bicarbonate significantly improved efficacy against green mold. Knowing the mechanism of resistance to a fungicide could be helpful in developing new approaches to tackle future fungicide resistance problems. Thus, the mechanisms of fludioxonil and pyrimethanil resistance in field isolates of *P. digitatum* were studied. Fludioxonil-sensitive isolates were found to be more sensitive to iprodione than the moderately resistant (MR) and highly resistant (HR) isolates, with no consistent relationship between the HR and MR isolates and sensitivity to iprodione and osmotic stress. An increase in phosphorylated Pdos-2 protein was observed for sensitive and resistant isolates after exposure to fludioxonil. Interestingly, glycerol concentrations were significantly increased in the sensitive and MR isolates, but not in the HR isolate. In addition, reduced growth and sporulation of the MR and HR isolates as compared to the sensitive isolates was evident. Therefore, it is indicated that in *P. digitatum* glycerol synthesis is affected by fludioxonil and most likely the fungicide functions as a suppressor of the mitogen-activated protein kinase cascade in resistant isolates. EC_{50} values for mycelial growth of *Botrytis cinerea* and *P. digitatum* were determined for cyprodinil and pyrimethanil using a defined culture medium without and with the addition of selected amino acids and homocysteine. The addition of amino acids resulted in a reduced toxicity of the two AP fungicides in both fungi, but the effect of each additive was significantly lower for *P. digitatum* than for *B. cinerea.* This suggests that methionine biosynthesis is not the primary target site of APs in *P. digitatum.*