Title Characterization of fludioxonil-resistant and pyrimethanil-resistant phenotypes of *Penicillium expansum* from Apple

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Abstract

Penicillium expansum is the primary cause of blue mold, a major postharvest disease of apple. Fludioxonil and pyrimethanil are two newly registered postharvest fungicides for pome fruit in the United States. To evaluate the potential risk of resistance development in *P. expansum* to the new postharvest fungicides, one isolate of each of thiabendazole-resistant (TBZ-R) and -sensitive (TBZ-S) *P. expansum* was exposed to UV radiation to generate fungicide-resistant mutants. Four fludioxonil highly-resistant mutants (EC_{50} > 1,000 μg/ml) and four pyrimethanil-resistant mutants (EC_{50} > 10 μg/ml) were tested for sensitivities to thiabendazole, fludioxonil, and pyrimethanil, and fitness parameters including mycelial growth, sporulation on potato dextrose agar (PDA), sensitivity to osmotic stress, and pathogenicity and sporulation on apple fruit. The stability of resistance of the mutants was tested on PDA and apple fruit. Efficacy of the three fungicides to control blue mold incited by the mutants was evaluated on apple fruit. Six fungicide-resistant phenotypes were identified among the parental wild-type isolates and their mutants based upon their resistance levels. All four fludioxonil highly-resistant mutants were sensitive to pyrimethanil and retained the same phenotypes of resistance to TBZ as the parental isolates. All four pyrimethanil-resistant mutants had a low level of resistance to fludioxonil with a resistance factor >15. The two pyrimethanil-resistant mutants derived from a TBZ-S isolate became resistant to TBZ at 5 μg/ml. After 20 successive generations on PDA and four generations on apple fruit, the mutants retained the same phenotypes as the original generations. All mutants were pathogenic on apple fruit at both 0 and 20°C, but fludioxonil highly-resistant mutants were less virulent and produced fewer conidia on apple fruit than pyrimethanil-resistant mutants and their parental wild-type isolates. Compared with the parental isolates, all four fludioxonil highly-resistant mutants had an increased sensitivity to osmotic stress on PDA amended with NaCl, while the pyrimethanil-resistant mutants did not. Pyrimethanil was effective against blue mold caused by fludioxonil-resistant mutants at both 0 and 20°C. Pyrimethanil and fludioxonil reduced blue mold incited by pyrimethanil-resistant mutants during 12-week storage at 0°C but

were not effective at 20°C. TBZ was not effective against pyrimethanil-resistant mutants derived from TBZ-S wild-type isolates at room temperature but provided some control at 0°C. The results indicate that: (i) a fitness cost was associated with fludioxonil highly resistant mutants of *P. expansum* in both saprophytic and pathogenic phases of the pathogen but not pyrimethanil-resistant mutants; (ii) pyrimethanil possessed a higher risk than fludioxonil in the development of resistance in *P. expansum*; and (iii) triple resistance to the three apple-postharvest fungicides could emerge and become a practical problem if resistance to pyrimethanil develops in *P. expansum* populations.