Title Determination of natural resistance frequencies in *Penicillium digitatum* using a new air-sampling method and characterization of fludioxonil- and pyrimethanil-resistant isolates

Authors L. Kanetis, H. Förster and J. E. Adaskaveg

Citation Phytopathology 100 (8): 738-746. 2010.

Keywords green mould rot; citrus

Abstract

Fungicide resistance was identified in natural populations of *Penicillium digitatum*, the causal agent of green mold of citrus, to two of three new postharvest fungicides before their commercial use. Using a new air-sampling method where large populations of the pathogen in citrus packinghouses were exposed to agar plates with a continuous, wide-range fungicide concentration gradient, isolates with reduced sensitivity to fludioxonil or pyrimethanil were obtained. Resistance frequencies to fludioxonil and pyrimethanil were calculated as 9.5×10^{-7} to 1.5×10^{-5} and 7.3×10^{-6} to 6.2×10^{-5}, respectively. No isolates resistant to azoxystrobin were detected. Isolates with reduced sensitivity to fludioxonil or pyrimethanil were also obtained in laboratory selection studies, where high concentrations of conidial mixtures of isolates sensitive to the three fungicides were plated onto agar amended with each fungicide at 10 µg/ml. Isolates obtained from fludioxonil selection plates in laboratory and packinghouse experiments were placed into two categories based on mycelial growth: moderately resistant isolates had 50% effective concentration (EC_{50}) values of 0.1 to 0.82 µg/ml and highly resistant isolates had EC_{50} values > 1.5 µg/ml. Isolates resistant to pyrimethanil all had EC_{50} values >8 µg/ml. Representative isolates of the two categories with reduced sensitivity to fludioxonil varied widely in their virulence and sporulation capacity as measured by the incidence of decay and degree of sporulation on inoculated fruit, respectively, whereas pyrimethanil-resistant isolates were mostly similar to the wild-type isolate. Fungicide sensitivity characteristics for isolates from fludioxonil and pyrimethanil selection plates remained stable after passages on nonamended agar, and disease could not be controlled after treatment with the respective fungicides. Types of fungicide resistance were visualized on thiabendazole- (TBZ) and imazalil-amended selection plates that were exposed in packinghouses where resistance to these fungicides was known to occur. The qualitative, single-site resistance to the benzimidazole TBZ was visualized by two distinct subpopulations in regard to fungicide sensitivity, whereas the quantitative, multi-site resistance to the demethylation inhibitor imazalil was apparent as a continuous density gradient of colonies along the fungicide

concentration gradient. Types of resistance could not be assigned to fludioxonil or pyrimethanil because a limited number of resistant colonies was obtained on each plate. Thus, with this new method, we were able to estimate fungicide resistance frequencies as well as characterize and visualize types of resistance within populations of a fungal species. This information will be used to design resistance management strategies for previous and newly registered postharvest fungicides of citrus.