Title First report of occurrence of pyrimethanil resistance in *Penicillium expansum* from stored apples in Washington State

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Citation Plant Disease 95 (1): 72. 2011.

Keywords apple; fruit rot

Abstract

Blue mold caused by *Penicillium expansum* is a major postharvest fruit rot disease of apples (*Malus domestica*) worldwide. Pyrimethanil was registered in late 2004 in the United States for postharvest use on apples. Since then, pyrimethanil has been increasingly used in Washington State as a postharvest drench treatment for control of blue mold and other postharvest diseases in apples. Baseline sensitivity to pyrimethanil in *P. expansum* populations from apples in Washington State has been established and all isolates in the baseline population were sensitive to pyrimethanil (1). To monitor resistance to pyrimethanil in *P. expansum* populations, blue mold-like decayed apple fruit were sampled from May to August 2009 from the fruit that had been drenched with pyrimethanil prior to storage from fruit packinghouses. Isolation of *Penicillium* species from decayed fruit was attempted. Isolates of *Penicillium* species were identified to species according to the descriptions by Pitt (2). In total, 186 *P. expansum* isolates were collected and tested for resistance to pyrimethanil in a conidial germination assay on an agar medium amended with pyrimethanil at the discriminatory concentration of 0.5 μg ml^{-1} (1). Isolates that were able to germinate were considered resistant to pyrimethanil. Of the 186 isolates tested, one was resistant to pyrimethanil. EC_{50} (the effective concentration that inhibits fungal growth by 50% relative to the control) of pyrimethanil for the resistant isolate was determined according to a method described previously (1) and the test was done twice. EC_{50} values of pyrimethanil on mycelial growth and conidial germination for the resistant isolate were 9.9 and 3.1 μg/ml, respectively, which were 7.4-fold and 16.5-fold higher than the means of the baseline population (1). To evaluate whether pyrimethanil at label rate is still able to control this resistant isolate, 'Fuji' apples were wounded, inoculated with conidial suspensions (1×10^4 conidia ml^{-1}) of either the resistant isolate or a pyrimethanil-sensitive isolate, treated with either pyrimethanil or sterile water as controls, and stored at 20°C for 10 days following a method described previously (1). There were four 20-fruit replicates for each treatment. The experiment was performed twice. All inoculated fruit in the nontreated controls were decayed.

Pyrimethanil applied at label rate completely controlled blue mold incited by a pyrimethanil-sensitive isolate, but 75% of the fruit that were inoculated with the resistant isolate and treated with pyrimethanil developed blue mold. To our knowledge, this is the first report of pyrimethanil resistance in *P. expansum* from decayed apple fruit collected from commercial packing houses. The pyrimethanil-resistant isolate was obtained from a packing house in which pyrimethanil had been used as a postharvest drench treatment in each of four consecutive years, suggesting that pyrimethanil-resistant individuals are emerging in *P. expansum* populations in Washington State after repeated use of pyrimethanil. Our results also indicate that pyrimethanil resistance in *P. expansum* reported in this study can result in failure of blue mold control in apples with pyrimethanil.