Disease Notes

First Report of *Penicillium expansum* Isolates Resistant to Pyrimethanil from Stored Apple Fruit in Pennsylvania

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Abstract

Apples in the United States are stored in low-temperature controlled atmospheres for 9 to 12 months and are highly susceptible to blue mold decay. *Penicillium* spp. cause significant economic losses worldwide and produce mycotoxins that contaminate processed apple products. Blue mold is managed by a combination of cultural practices and the application of fungicides. In 2004, a new postharvest fungicide, pyrimethanil (Penbotec 400 SC, Janseen PMP, Beerse, Belgium) was registered for use in the United States to control blue mold on pome fruits (1). In this study, 10 blue mold symptomatic 'Red Delicious' apples were collected in May 2011 from wooden bins at a commercial facility located in Pennsylvania. These fruit had been treated with Penbotec prior to controlled atmosphere storage. Ten single-spore *Penicillium* spp. isolates were analyzed for growth using 96-well microtiter plates

containing Richards minimal medium amended with a range of technical grade pyrimethanil from 0 to 500 µg/ml. Conidial suspensions adjusted to 1×10^5 conidia/ml were added to three 96-well plates for each experiment; all experiments were repeated three times. Nine resistant isolates had prolific mycelial growth at 500 µg/ml, which is 1,000 times the discriminatory dose that inhibited baseline sensit *P. expansum* isolates from Washington State (1). However, one isolate (R13) had limited conidial germination and no mycelial proliferation at 0.5 µg/ml and was categorized as sensitive. One resistant (R22) and one sensitive (R13) isolate were selected on the basis of their different sensitivities to pyrimethanil. Both isolates were identified as *P. expansum* via conventional PCR using β-tubulin gene-specific primers according to Sholberg et al. (2). Analysis of the 2X consensus amplicon sequences from R13 and R22 matched perfectly (100% identity and 0.0 E value) with other *P. expansum* accessions in GenBank including JN872743.1, which was isolated from decayed apple fruit from Washington State. To determine if pyrimethanil applied at the labeled rate of 500 µg/ml would control R13 or R22 in vivo, organic 'Gala' apple fruit were wounded, inoculated with 50 µl of a conidial suspension (1×10^4 conidia/ml) of either isolate, dipped in Penbotec fungicide or sterile water, and stored at 25°C for 7 days. Twenty fruit composed a replicate within a treatment and the experiment was performed twice. Non-inoculated water-only controls were symptomless, while water-dipped inoculated fruit had 100% decay with mean lesion diameters of 36.8 ± 2.68 mm for R22 and 38.5 ± 2.61 mm for R13. The R22 isolate caused 30% decay with 21.6 ± 5.44 mm lesions when inoculated onto Penbotec-treated apples, while the R13 isolate had 7.5% decay incidence with mean lesion diameters of 23.1 ± 3.41 mm. The results from this study demonstrate that *P. expansum* pyrimethanil-resistant strains are virulent on Penbotec-treated apple fruit and have the potential to manifest in decay during storage. To the best of our knowledge, this is the first report of pyrimethanil resistance in *P. expansum* from Pennsylvania, a major apple growing region for the United States. Moreover, these results illuminate the need to develop additional chemical, cultural, and biological methods to control this fungus.

References: (1) H. X. Li and C. L. Xiao. Phytopathology 98:427, 2008. (2) P. L. Sholberg et al. Postharvest Biol. Technol. 36:41, 2005.

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