Title Resistance to pyraclostrobin and boscalid in populations of *Botrytis cinerea* from stored apples in Washington State

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Citation Plant Disease 94 (5): 604-612. 2010.

Keywords apple; gray mold

Abstract

Gray mold caused by *Botrytis cinerea* is a major postharvest disease of apple. Pristine, a formulated mixture of pyraclostrobin and boscalid, was recently registered for use on apple. Pristine applied within 2 weeks before harvest is effective in controlling gray mold in stored apple fruit. To determine the baseline sensitivity of *B. cinerea* populations to these fungicides, 40 isolates from organic and 80 from conventional apple orchards where Pristine had not been used were tested for mycelial growth or conidial germination on fungicide-amended media. To monitor fungicide resistance, gray-mold-decayed apple fruit originating from orchards in which Pristine had been used were sampled from a fruit packinghouse. Isolates of *B. cinerea* recovered from the fruit were tested for resistance to the two fungicides. In the in vivo study in the orchards, Pristine was applied to fruit 1 day before harvest. Fruit were then harvested, wounded, and inoculated with isolates exhibiting different fungicide-resistance phenotypes. Fruit were stored at 0°C for 8 weeks for decay development. The effective concentration that inhibits mycelial growth by 50% relative to the control (EC_{50}) values for sensitive isolates ranged from 0.008 to 0.132 µg/ml (mean = 0.043, n = 116) for pyraclostrobin and from 0.003 to 0.183 µg/ml (mean = 0.075, n = 117) for Pristine in a mycelial growth assay on potato dextrose agar. The EC_{50} values of boscalid for sensitive isolates ranged from 0.065 to 1.538 µg/ml (mean = 0.631, n = 29) in a conidial germination assay on water agar. Four isolates were resistant to pyraclostrobin, with resistance factors (RFs) ranging from 12 to 4,193. Of the four pyraclostrobin-resistant isolates, one also was resistant to boscalid (RF = 14) and Pristine (RF = 373), and two exhibited reduced sensitivity to Pristine (RF = 16 and 17). The minimum inhibitory concentration for conidial germination (for boscalid) or mycelial growth (for pyraclostrobin and Pristine) of sensitive isolates was 5 µg/ml, which is thus recommended as a discriminatory concentration for phenotyping isolates for resistance to these fungicides. Of the 56 isolates obtained from decayed apple fruit that had been exposed to Pristine, 11 (approximately 20%) were resistant to

both pyraclostrobin and boscalid and 1 was resistant only to pyraclostrobin. Of the additional 43 isolates obtained from decayed apple fruit originating from an organic orchard, 3 were resistant only to pyraclostrobin, 2 were resistant only to boscalid, and 2 were resistant to both fungicides. It appeared that there was no cross resistance between pyraclostrobin and boscalid because of the existence of isolates resistant only to either pyraclostrobin or boscalid. Pristine applied at label rate in the orchard failed to control gray mold on apple fruit inoculated with the Pristine-resistant isolates. This is the first report of multiple resistance to pyraclostrobin, boscalid, and Pristine in field populations of *B. cinerea*. Our results suggest that the development of dual resistance to pyraclostrobin and boscalid in *B. cinerea* populations could result in the failure to control gray mold with Pristine.