First Report of Boscalid and Penthiopyrad-Resistant Isolates of *Alternaria solani*

Causing Early Blight of Potato in Michigan

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Early blight of potato (*Solanum tuberosum*) is caused by *Alternaria solani* and occurs annually in Michigan. If left uncontrolled it can result in yield losses exceeding 20% and impact stored potatoes. The disease is commonly managed using succinate dehydrogenase inhibitor (SDHI) fungicides (1). Unfortunately, recent studies have shown that SDHI resistance has increased dramatically over the past two years in *A. solani* populations (1, 2). To investigate the occurrence of SDHI resistance in Michigan, potato leaves with early blight symptoms were collected from fields in Montcalm County and Ionia County, MI in 2012. To obtain *A. solani* isolates from leaves, small pieces of leaf tissue (5 x 5 mm) were excised from the center of lesions and transferred on to water agar. Plates were incubated at 25°C overnight to allow conidia to germinate. Single germinated *A. solani* conidia were transferred to potato dextrose agar (PDA) and incubated at 25°C for seven days. The identity of cultures was confirmed by colony and conidial morphology (3). Nineteen *A. solani* isolates were obtained and each was screened for sensitivity to the SDHI fungicides boscalid, penthiopyrad, and fluopyram, using a 50 ppm discriminatory dose based on EC₅₀ values previously determined (2).
Mycelial plugs (~5.5 mm) were transferred to amended and non-amended PDA plates that were incubated at 25°C for seven days. An isolate was considered highly resistant if fungal growth relative to control plates exceeded 50%, moderately resistant if it was between 35-50%, and sensitive if it was less than 35% (2). A sensitive A. solani isolate (AS11) from Bonners Ferry, ID was used as a control in these experiments. Of all of the isolates tested, 11% were highly resistant to both boscalid and penthiopyrad and 5% were moderately resistant to both fungicides, 21% were moderately resistant to penthiopyrad alone, and the remaining isolates (84 and 68% respectively) were sensitive to the two fungicides. None of the isolates tested were resistant to fluopyram. Recently, two major mutations H227R in SdhB and H133R in SdhD have been identified in highly resistant A. solani isolates in Idaho (2). Due to the fact that the majority of the identified mutations occur near the 3’ end of each subunit, this region was amplified and sequenced using the following primer sets: SdhB – (5’- TACTGGTGGAAC CAGGAGGAGTA -3’ and 5’-CATACCACTCTAGGTGAAG -3’), SdhC – (5’-CCAAATCACCTGGTACGCCTCG-3’ and 5’-TCATCCGAGGAAGGTGTAGTAAAGGCTG-3’), and SdhD – (5’-CCGACTCTATTCTCTGCACGC-3’ and 5’-CTCGAAAGAGTAGAGGGCAAGACCCA-3’). In this study, all of the isolates that were highly resistant to both boscalid and penthiopyrad were found to contain the H133R mutation in SdhD, which correlated with the strongest resistance phenotype. To our knowledge, this is the first report of resistance to SDHI fungicides in populations of A. solani on potato in Michigan. These data are concerning as they indicate that the highly resistant isolates have already developed cross-resistance between boscalid and penthiopyrad, despite penthiopyrad not yet having regular use in Michigan. Although all
of the isolates tested were sensitive to fluopyram, the discovery of isolates resistant to boscalid and pencyopyrad suggests that all SDHI fungicides should be considered at high risk of resistance development in Michigan.

*Alternaria solani* growth response to 50 ppm of boscalid (a-c), penthionpyrad (d-f), fluopyram (g-i) or non-amended (j-l) potato dextrose agar plates. Three representative isolates are depicted including AS11 from Bonners Ferry, ID (a, d, g, j), M-15 from Montcalm County, MI (b, e, h, k) and M-2 from Montcalm County, MI (c, f, i, l).

127x170mm (300 x 300 DPI)