First report of QoI resistance in Alternaria spp. infecting sugar beet (Beta vulgaris)

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Accession Nos. MF422130-MF422138. Pure cultures of conidial suspensions were prepared. Isolate sensitivity expressed in mg a.i./l was determined by estimation of the EC50 (effective control of 50% of germinating conidia) on water agar amended with pyraclostrobin (technical grade) at 0, 0.01, 0.1, 1, 10, or 100 mg/l, with and without salicylhydroxamic acid (SHAM) at 100 mg/l (to determine toxicity of SHAM). Isolates were incubated for 24 hours, at 24°C in the dark (two replications). It was determined that SHAM was toxic to germinating conidia for all isolates tested, thus EC50 values were based on relative germination assays of fungicide-only amended-agar. The EC50 value for a sensitive, intermediate-resistant and resistant isolate was 0.38, 5.32 and 22.19 mg/l, respectively. Isolates showed a similar response based on the spiroplasm dilution method and a relative growth assay (Figs. 1–3, only for illustration of dose-response with SHAM).

Genomic DNA was extracted from sensitive, intermediate-resistant and resistant isolates. PCR amplification targeted the Alternaria spp. cytochrome b (cytb) gene using the previously described Alternaria-specific primer pair AF and AR (Ma et al., 2003). Partial sequences of the cytb (227 bp) gene (MFX001498-MFX001504) shared 99% identity when compared to QoI-sensitive (AY263408) and -resistant (AY263409) reference isolates of

Alternaria spp. Sequence analysis revealed that all intermediate and resistant isolates contained a substitution of G143A in the cytb gene (DQ209283), which confers QoI resistance in Alternaria alternata (Grasso et al., 2006). The differential sensitivity response of G143A mutants is worth noting, and studies to determine its biological significance are currently underway. Taken together with results from sensitivity testing, this may suggest that an increased incidence and severity of ALS in some commercial sugar beet production areas in Michigan, and populations of Alternaria spp. dominated by QoI resistant isolates (~92%), may be contributing to recent disease control issues.

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References


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