New Disease Reports

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

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Received: *12 Jun 2017.* ***Published:*** *02 Aug 2017.* ***Keywords:*** *Alternaria leaf spot, fungicide resistance, strobilurins*

Alternaria leaf spot (ALS) of sugar beet (*Beta vulgaris*), caused by *Alternaria* spp. in the *A. alternata* and *tenuis* species-group, is common wherever sugar beet is grown (Franc, 2009). Historically, *Alternaria* spp. infection and disease management has been a minor issue in the USA due to its opportunistic or secondary nature, and normally, ALS does not significantly affect yield (Franc, 2009). The occurrence of ALS is a concern in some sugar beet production areas outside the USA including parts of Europe (Özgönen & Kiliç, 2009), as the reduction of photosynthetic area from leaf spot infection (starting at 5%) results in significant reductions in sugar yield (Wolf & Verreet, 2002).

Recently, increased incidence and severity of ALS has been observed by some growers in Michigan, USA at levels of infection that caused yield loss. Additionally, in 2015 and 2016 *Alternaria* spp. with insensitivity to many classes of fungicides, including quinone outside inhibitor (QoI) fungicides (FRAC group 11) were recovered from fields in Michigan. Mono-conidial isolates of *Alternaria* spp. from individual ALS lesions were obtained to determine sensitivity to the QoI pyraclostrobin. Isolates were identified to species based on conidial morphology and molecular based methods (Woudenberg *et al*., 2013) by DNA sequencing (GenBank 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 spiral gradient 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 (MF001498-MF001504) 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.

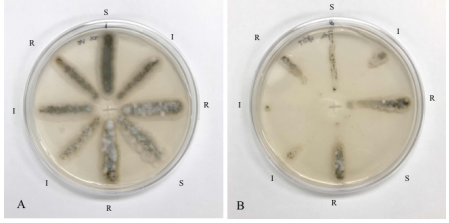

Figure 1

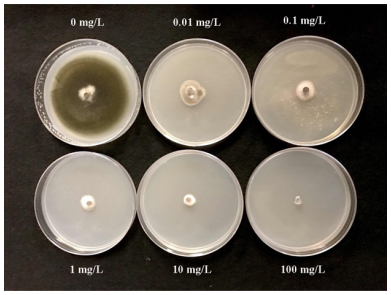

Figure 2

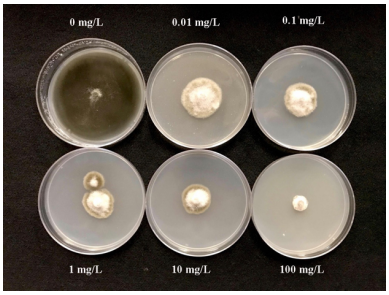

Figure 3

Acknowledgements

The authors wish to thank all the agriculturists from Michigan Sugar Company that assisted in collection of samples. This project was supported by the Michigan Sugar Company Research & Education Advisory Council.

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To cite this report: Rosenzweig N, Hanson LE, Pratt D, Stewart J, Somohano P, 2017. First report of QoI resistance in *Alternaria* spp. infecting sugar beet (*Beta vulgaris*). *New Disease Reports* **36**, 5. http://dx.doi.org/10.5197/j.2044-0588.2017.036.005

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