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First Report Of Strobilurin Resistance In *Cercospora Beticola* In Sugar Beet (*Beta Vulgaris*) In Michigan And Nebraska, USA

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New Disease Reports

First report of strobilurin resistance in *Cercospora beticola* in sugar beet (*Beta vulgaris*) in Michigan and Nebraska, USA

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Cercospora leaf spot (CLS) caused by *Cercospora beticola* Sacc. is the most important foliar disease of sugar beet (*Beta vulgaris*) worldwide (Jacobsen & Franc, 2009). CLS is controlled mainly with fungicides, including strobilurins (FRAC group 11). Resistance to strobilurins in *C. beticola* has not been reported in the field (Secor *et al*., 2010) but insensitive mutations have been artificially developed (Malandrakis *et al*., 2011). In 2011, fields from several areas in Michigan, USA treated with strobilurins had severe CLS and diminished control was also noted in small plot trials (Fig. 1). Individual leaf spot lesions were sampled from leaves and grown on sugar beet leaf extract agar (SBLEA). A conidium germination bioassay was done on SBLEA covered with water agar amended with pyraclostrobin, azoxystrobin or trifloxystrobin at 0, 0.001, 0.01, 0.1, 1, 10, or 100 µg/ml, supplemented with salicylhydroxamic acid (SHAM) to block the alternate oxidation pathway (Olaya *et al*., 1998). After 24 h incubation at 22°C, under ambient light, percentage germinated conidia (n = 50) was calculated from three replicates per treatment. Germination was recorded as positive when the germ tube was at least half the length of the conidium. A representative wild type isolate was unable to germinate over the 0.01 µg/ml concentration. EC50 values for each isolate were calculated by regression analysis of percentage growth inhibition vs. the log fungicide concentration using Sigmaplot Version 9.01 (Systat Software, Chicago). The EC50 for the sensitive isolate was <0.01 µg/ml. Isolates from several counties in Michigan had uninhibited germination and EC50 values exceeded the highest concentration tested. Isolates also grew on spiral gradient dilution plates (Förster *et al*., 2004) amended with the three strobilurins (Fig. 2, for illustration of resistance response only). Two isolates were obtained from Nebraska and each showed similar response to strobilurin fungicides in amended plate assays.

Pure cultures of four resistant isolates were grown in potato dextrose broth at 125 rpm, and DNA extracted. A fragment of the cytochrome b (*CYTB*) gene was amplified by PCR using the *C. beticola* primers of Malandrakis *et al*. (2011) to amplify the region of the *CYTB* gene likely to contain resistance mutations (Malandrakis *et al*., 2011). This fragment was sequenced at the Genomics Technology Support Facility (MSU, East Lansing, MI) and showed 99% identity with both the *C. beticola* cytochrome b mRNA, partial sequence (GenBank Accession No. EF176921.1) and the *C. kikuchii* mitochondrial gene for cytochrome b partial sequence (AB231863.1). Sequence results revealed that each resistant isolate contained a change in codon 143 that predicts to a substitution of G143A, which was demonstrated to confer QoI resistance in several other fungi (Ma & Michailides, 2005). All four isolates with the G143A mutation germinated at 100 µg/ml pyraclostrobin (50% of conidia), while sensitive isolates that lacked the mutation failed to grow. Isolates that contained the G143A mutation included representatives from Michigan and Nebraska, USA. These findings reveal that reduced Cercospora leaf spot control in some commercial sugar beet fields may be due to the development of resistance to strobilurins.

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Figure 1

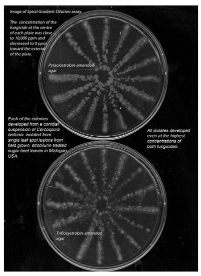

Figure 2

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