ASSESSMENT OF STROBILURIN RESISTANCE IN *CERCOSPORA BETICOLA* ON SUGAR BEET IN MICHIGAN AND NEBRASKA, USA

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ABSTRACT

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* was first confirmed in 2011 from several fields in Michigan and in one field in Nebraska, USA (Kirk *et al.* 2012) following anecdotal reports of reduced fungicide efficacy. In these fields, sugar beet treated with strobilurins had severe CLS and diminished control was also noted in small plot trials in Michigan. Individual leaf spot lesions were sampled from leaves and grown on sugar beet leaf extract agar (SBLEA). A conidial germination bioassay was done on SBLEA 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, the percentage of 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. Effective concentration for 50% reduction in germination (EC_{50}) values for each isolate were calculated by regression analysis of percentage spore germination vs. the log fungicide concentration using Sigmaplot Version 9.01 (Systat Software, Chicago). The EC_{50} for the sensitive isolate was <0.01 µg/ml. Isolates from several counties in Michigan had uninhibited germination and EC_{50} values exceeded the highest concentration tested. Isolates also grew on spiral gradient dilution plates (Förster *et al.*, 2004) amended with the three strobilurins. Two isolates were obtained from Nebraska and each showed a similar response to strobilurin fungicides in amended plate assays. In 2012, widespread strobilurin resistance was recorded in isolates of *C. beticola* collected in Michigan although a few isolates submitted to the program were sensitive.

Pure cultures of a subset of 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 is predicted to lead to a substitution of G143A, which was demonstrated to

confer QoI resistance in several other fungi (Ma & Michailides, 2005). All Michigan isolates with the G143A mutation germinated at 100 μg/ml pyraclostrobin (50% of conidia), while sensitive isolates that lacked the mutation failed to grow. Additional isolates that contained the G143A mutation included representatives from Michigan and Nebraska. A high proportion of isolates (~90%) from the commercial growing region that were screened in 2012 have been found to contain the G143A mutation by PCR-RFLP screening using digestion of the above PCR products. These findings reveal that reduced CLS control in some commercial sugar beet fields may be due to the development of resistance to strobilurins. In 2012, three consecutive applications of pyraclostrobin treatments failed to adequately control *C. beticola* at the Michigan State University (MSU) Saginaw Valley Research and Extension Center.

In addition to the strobilurin sensitivity monitoring, the program at MSU also tests for development of insensitivity in the CLS population to Difenoconazole (Inspire); Tetraconazole (Eminent); Prothioconazole (Proline), Flutriafol (Topguard), Thiophanate-methyl (Topsin) and TPTH (Tin). Some triazole-insensitive isolates have been recovered in MI beet fields over the past 10 years but the proportion is low for all these fungicides, however in 2012, nearly 100% of isolates tested were insensitive to Thiophanate-methyl.

The challenges for the sugarbeet industry with respect to CLS control include a lack of products from diverse FRAC groups; a general increase in inoculum due to the prevalence of CLS susceptible cultivars grown in the Michigan and Ontario sugar beet production regions; an increase in proportion of isolates insensitive to strobilurins (e.g. pyraclostrobin); the industry is inexperienced with tank mixes and has a perceived distrust of TPTH (label restrictions). In addition, to the lack of available chemistries from diverse FRAC groups there is confusion on what to start and end a program with in order to delay the onset of insensitivity to other fungicides. A strobilurin is the mainstay for Rhizoctonia control in MI therefore there is a need for new products with alternative modes of action so that a CLS disease management program starts with a product not used earlier in the season for other disease management.

Acknowledgements

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