

2012

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

W.W. Kirk  
Michigan State University, kirkw@msu.edu

L.E. Hanson  
USDA-ARS

G.D. Franc  
University of Wyoming

W.L. Stump  
University of Wyoming

E. Gachango  
Michigan State University

*See next page for additional authors*

Follow this and additional works at: <http://digitalcommons.unl.edu/usdaarsfacpub>

 Part of the [Agricultural Science Commons](#)

Kirk, W.W.; Hanson, L.E.; Franc, G.D.; Stump, W.L.; Gachango, E.; Clark, G.; and Stewart, J., "First Report Of Strobilurin Resistance In *Cercospora Beticola* In Sugar Beet (*Beta Vulgaris*) In Michigan And Nebraska, USA" (2012). *Publications from USDA-ARS / UNL Faculty*. 987.

<http://digitalcommons.unl.edu/usdaarsfacpub/987>

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

---

**Authors**

W.W. Kirk, L.E. Hanson, G.D. Franc, W.L. Stump, E. Gachango, G. Clark, and J. Stewart



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

W.W. Kirk<sup>1\*</sup>, L.E. Hanson<sup>2</sup>, G.D. Franc<sup>3</sup>, W.L. Stump<sup>3</sup>, E. Gachango<sup>1</sup>, G. Clark<sup>4</sup> and J. Stewart<sup>4</sup>

<sup>1</sup> Department of Plant Pathology, Michigan State University, 612, Wilson Road, 35 Plant Biology Building, East Lansing, MI 48824, USA; <sup>2</sup> USDA-ARS, 1066 Bogue Street, Room 494, East Lansing, MI 48824, USA; <sup>3</sup> Department of Plant Sciences, University of Wyoming, Laramie, WY 82071, USA; <sup>4</sup> Michigan Sugar Company, Euclid Road, Bay City, MI 48706, USA

\*E-mail: kirkw@msu.edu

**Received:** 30 Apr 2012. **Published:** 31 Jul 2012. **Keywords:** *Cercospora* leaf spot, fungal plant disease, fungicide resistance, QoI

*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.

## Acknowledgements

The authors would like to thank Tom Goodwill for his excellent technical assistance.

## References

- Förster H, Kanetis L, Adaskaveg JE, 2004. Spiral gradient dilution, a rapid method for determining growth responses and 50% effective concentration values in fungus-fungicide interactions. *Phytopathology* **94**,163-170. [http://dx.doi.org/10.1094/PHYTO.2004.94.2.163]
- Jacobsen BJ, Franc GD, 2009. *Cercospora* leaf spot. In: Harveson RM, Hanson LE, Hein GL, eds. *Compendium of Beet Diseases and Pests*, 2nd edn. St. Paul, MN, USA: American Phytopathological Society, 7-10.
- Ma Z, Michailides TJ, 2005. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Protection* **24**, 853-863. [http://dx.doi.org/10.1016/j.cropro.2005.01.011]
- Malandrakis AA, Markoglou AN, Nikou DC, Vontas JG, Ziogas BN, 2011. Molecular diagnostic for detecting the cytochrome b G143S - QoI resistance mutation in *Cercospora beticola*. *Pesticide Biochemistry and Physiology*, 87-92. [http://dx.doi.org/10.1016/j.pestbp.2011.02.011]
- Olaya G, Zheng D, Köller W, 1998. Differential responses of germinating *Venturia inaequalis* conidia to kresoxim-methyl. *Pesticide Science* **54**, 230-236. [http://dx.doi.org/10.1002/(SICI)1096-9063(1998110)54:33.0.CO:2-O]
- Secor GA, Rivera VV, Khan MFR, Gudmestad NC, 2010. Monitoring fungicide sensitivity of *Cercospora beticola* of sugar beet for disease management decisions. *Plant Disease* **94**, 1272-1282. [http://dx.doi.org/10.1094/PDIS-07-09-0471]



Figure 1

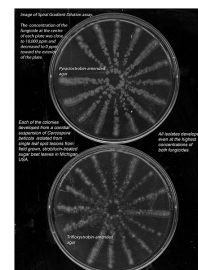


Figure 2

**To cite this report:** Kirk WW, Hanson LE, Franc GD, Stump WL, Gachango E, Clark G, Stewart J, 2012. First report of strobilurin resistance in *Cercospora beticola* in sugar beet (*Beta vulgaris*) in Michigan and Nebraska, USA. *New Disease Reports* **26**, 3. [http://dx.doi.org/10.5197/j.2044-0588.2012.026.003]

©2012 The Authors

This report was published on-line at [www.ndrs.org.uk](http://www.ndrs.org.uk) where high quality versions of the figures can be found.