Detection of QoI fungicide resistant *Cercospora beticola* airborne inoculum using quantitative PCR

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Cercospora leaf spot (CLS) caused by the fungus *Cercospora beticola* Sacc. is a devastating foliar disease of sugar beet (*Beta vulgaris* L.) worldwide. Under favorable conditions CLS could result in losses up to 40% or greater, and 30% losses in recoverable sucrose due to CLS are common under moderated disease conditions (Khan et al., 2001). This disease is considered the most important constraint for sugar beet production in North Dakota and Minnesota, and warrants multiple fungicide applications per growing season. Despite multiple fungicide applications, unsatisfactory control of the disease has been noticed in some production areas. Development of fungicide resistance in pathogen populations is considered the reason for this. Thus, monitoring of the pathogen population for prevalence of fungicide resistance is essential for effective management of the disease using fungicides.

Spore traps coupled with DNA amplification methods have been used successfully to monitor (detect and quantify pathogen spores) several airborne pathogens including *Peronospora effuse* (Klosterman et al., 2014), *Leptosphaeria maculans* (Calderon et al., 2002) , *L. biglobosa, Pyrenopezzia brassicae* (Calderon et al., 2002), *Botrytis squamosa* (Carisse et al., 2009), *Erysiphe necator* (Falacy et al., 2007), *Sclerotinia sclerotiorum* (Rogers et al., 2009), and *Fusarium circinatum* (Schweigkofler et al., 2004). Previous research has showed that *C. beticola* conidia can be collected in sugarbeet fields using spore traps (Khan et al. 2009). Molecular assays to detect and quantify QoI resistance (G143A) in *C. beticola* are available (Bolton et al. 2013).

The objective of this research was to assess the possibility of using qPCR methods to monitor QoI-resistant *C. beticola* in airborne inoculum trapped using spore samplers placed in sugarbeet fields so that the best time to apply appropriate fungicides could be recommended for growers.

Materials and Methods

In 2016, preliminary studies were conducted in Foxhome, MN and Hickson, ND using Burkard volumetric spore samplers to study the feasibility of extracting DNA from trapped spores on adhesive coated tapes and its usability for subsequent molecular detection assays. In 2017, experiments were conducted in Foxhome, MN using two types of spore samplers (Burkard's volumetric and cyclone sampler) to compare for their ease of DNA extraction from trapped spores and sensitivity of detection of *C. beticola* airborne inoculum.

DNA extraction and quantitative real-time PCR assays

In 2016, pieces of tapes representing each day (≈ 48 mm) were cut into 4-6 equal sized smaller sections, and DNA from the airborne inocula trapped on the tapes were extracted following the method described in Rogers et al. 2009 or using Qiagen DNeasy plant mini kit with some

modifications. In 2017, tapes were processed similar to the previous year, except that the tapes representing each day were cut into two halves; one portion was used for microscopic observation and the other half was used for DNA extraction. DNA from both the tapes and micro-centrifuge tubes were extracted using the Qiagen kit. Real time PCR assay to detect G143A mutation was conducted as described in Bolton et al. 2013.

Results and Discussion

In 2016, results showed that DNA can be successfully isolated from the trapped spores on adhesive tapes, following either of the two extraction methods. Further, qPCR assays have successfully detected the presence the *C. beticola* QoI sensitive and resistant isolates in both Foxhome, MN (Table 1) and Hickson, ND (data not presented).

Table 1. Detection of *C. beticola* QoI fungicide resistance mutations using a qPCR assay from spores trapped on adhesive tapes collected from Foxhome, MN.

Tape	Sensitive Isolates	Resistant isolates
1	Y	Y
2	N	Y
3	N	Y
4	N	Y

In 2017, microscopic observations showed that the daily number of *C. beticola* conidia trapped on tapes varied during the sampling period (Fig. 1).

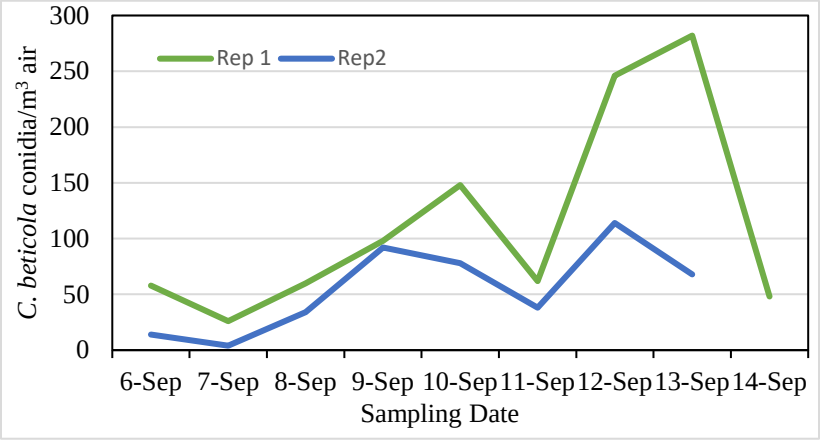

Figure 1. Daily number of *C. beticola* conidia collected with volumetric spore sampler in 2017 season at Foxhome, MN.

Similar to 2016, DNA was successfully isolated from *C. beticola* spores trapped either on tapes or micro-centrifuge tubes. Compared to tapes, DNA extraction from the tubes was found to be easy and efficient. Proportion of QoI resistant *C. beticola* spores varied within the sampling dates (Fig. 2). The qPCR data showed consistency between the volumetric sampler and cyclonic samplers in detection of QoI resistant *C. beticola.*

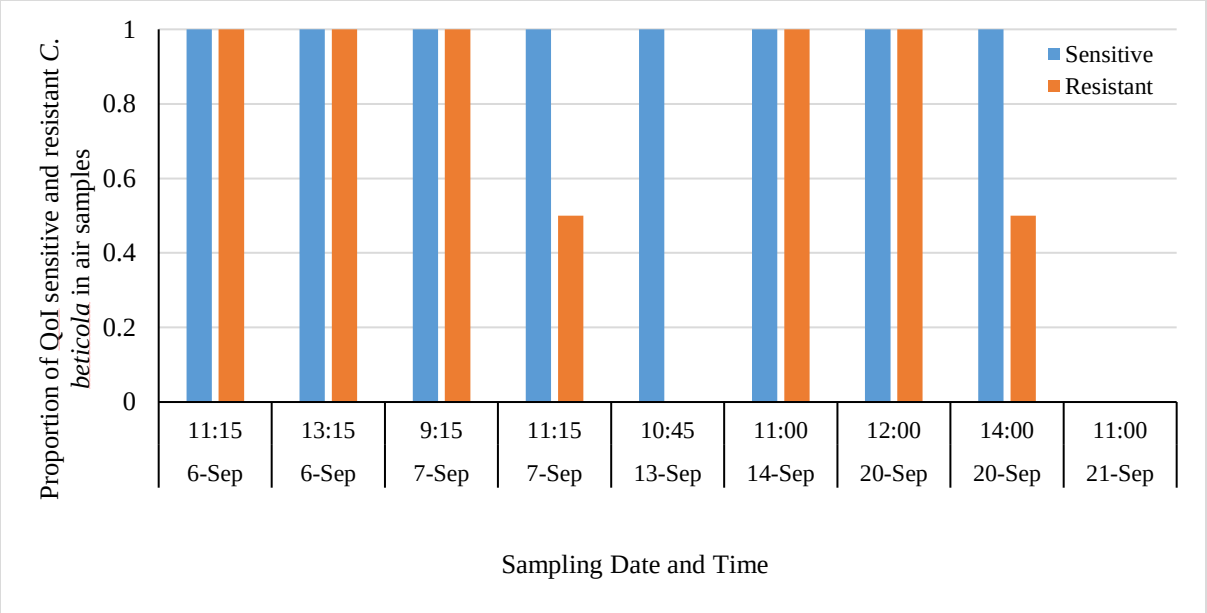

Figure 2. Proportion of QoI sensitive and resistant *C. beticola* in air samples collected using cyclonic sampler. Proportions are calculated as the ratio between the number of replications with positive detections to total number of replications at each time point.

Results from this study suggest that by using cyclonic samplers and qPCR assays, *C. beticola* can be monitored for QoI resistance in real time. Further, molecular detection assays can be extended for other fungicide classes as they become available.

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