EARLY DETECTION OF CERCOSPORA BETICOLA ASYMPTOMATIC INFECTION IN COMMERCIAL SUGARBEET FIELDS IN 2023

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Cercospora leaf spot (CLS), caused by the fungus *Cercospora beticola*, (*Cb*) is the most important leaf spot disease of sugar beet and is endemic in sugarbeet fields in the Red River Valley (RRV). CLS severity varies yearly and causes serious economic losses if not managed. CLS is managed using a combination of crop rotation, cultural practices, resistant cultivars, and timely fungicide applications. *Cb* from the RRV has developed decreased sensitivity at varying levels to all fungicides used, including organotin compounds, strobilurin fungicides such as Priaxore and Headline, benzimidazoles such as Topsin, and triazole fungicides that include Proline, Inspire, and Provysol.

Timing of fungicide applications, especially the first application, is highly variable. For example, first applications can be based on calendar date, first appearance of CLS symptoms, or first observation CLS in the area. Subsequent fungicide applications are often based on daily infection values (DIVs) calculated from relative humidity and temperature in the region. As DIVs increase, disease favorability increases, and fungicide applications are recommended when a threshold is reached. Conditions typically indicative of CLS symptom development are high relative humidity and temperature.

Recent results from field surveys of asymptomatic leaf samples from commercial sugarbeet fields have shown that CLS infection is occurring earlier and at wider prevalence than previously thought. To investigate the occurrence, prevalence, and fungicide resistance profile of early CLS infection, we utilized molecular assays to detect known fungicide resistance mutations for detection of latent CLS infection and fungicide resistance mutations. Results of this work indicate that the latent phase of CLS infection occurs earlier than previously reported and opens numerous new avenues of research into the utility of early fungicide applications, molecular and genomic epidemiology, and pathogen basic biology.

OBJECTIVES

1) Detect the onset of CLS asymptomatic infection across the entire RRV growing region.

2) Determine fungicide resistance profiles for CLS asymptomatic samples.

METHODS AND MATERIALS

In 2021, 2022, and 2023, with financial support of the Sugarbeet Research and Extension Board of MN and ND, we tested samples collected for 5-6 weeks from 280 commercial sugarbeet fields in MN and ND. We asked the Agriculturalist staff from the region to collect five leaf samples from seven fields weekly to be mailed or dropped off to the USDA-ARS Sugarbeet and Potato Research Unit located in Fargo, ND.

Upon sample arrival, leaves are hole punched for a total of 10 leaf disks from each of the five leaves submitted per field location. These leaf punches are batch processed as a single sample for DNA extraction using a KingFisher™ Flex Purification System (ThermoFisher: 5400630) with the sbeadex™ plant nucleic acid purification kit (LGC: NAP41620) after freeze drying samples. Sample DNA is then subjected to qPCR assays designed to detect the G143A mutation associated with Strobilurin fungicide resistance (Bolton et al. 2013), The E170 and L144F mutations associated with Triazole fungicide resistance (Spanner et al. 2021, Shrestha et al. 2022), and the E198A mutation associated with Benzimidazole fungicide resistance. A probe designed to detect the wild type at the G143A locus is also incorporated to ensure that *C. beticola* DNA is detected in either of the two forms this mutation is present as. Results from each weekly sample set and assay batches are compiled into weekly reports and distributed back to the regional sugar cooperatives.

RESULTS AND DISCUSSION

Detection of latent CLS infection steadily rose as the sampling season progressed (Figure 1). In each of 2021, 2022, and 2023 the frequency of latent CLS detected in submitted samples approached 100% during the first week of July, corresponding to row closure events.

Assays to detect the G143A fungicide resistance mutations showed that during the initial detection of latent infection, samples primarily contain the sensitive allele for Strobilurin fungicides but as the weeks progress, detection of resistant mutations rises. This may be due to management practices used in sugarbeet cultivation such as in-furrow fungicide treatments using strobilurin fungicides, but this hypothesis has yet to be examined. Another potential cause of the rise of resistance detection is observation that strobilurin resistant isolates produce lower spore numbers and need higher temperatures to sporulate in laboratory experiments. This would create a natural lag in resistance detection due to the delay in spore production and subsequent infection due to environmental conditions in the early growing season (**Figure 2**).

Fungicide resistance mutations for triazole fungicides show similar patterns to strobilurin fungicides, beginning at low frequency and trending upwards as the latent infection progresses. As of yet we do not know if there are fitness penalties associated with triazole fungicide resistance in terms of spore production but results from radial growth assays show that triazole resistant isolates grow faster than their sensitive counterparts. This increase in growth may be the reason we see increased sample detection as the latent infection progresses as the resistant isolates out pace sensitive isolates growth in the sugarbeet host. Additional experiments are necessary to confirm this observation (**Figure 3**).

Last, benzimidazole resistance mutation detection during the latent infection progression steadily increased as was observed with triazole fungicides though additional work needs to be done to better understand the affects of this resistance mutation on the fitness of the pathogen (**Figure 4**).

SUMMARY

Across three sampling years, a consistent pattern of latent CLS progression has been observed, leading to near 100% prevalence of CLS detection just prior to or at sugarbeet row closure. These results have implications for the initial timing of fungicide applications for CLS management. Control of primary infection is important to mitigate the exponential increase in inoculum levels that can occur when CLS symptoms begin to arise. Comparative examination of early season fungicide resistance profiles and late season fungicide sensitivity assays show that the prevalence of fungicide resistance is higher at the end of the year than the beginning of CLS infection. This makes sense as isolates collected at the end of the year have undergone significant selection though fungicide applications. These results indicate that fungicide chemistries previously considered ineffective due to widespread resistance may have utility if deployed properly in the growing season.

REFERENCES

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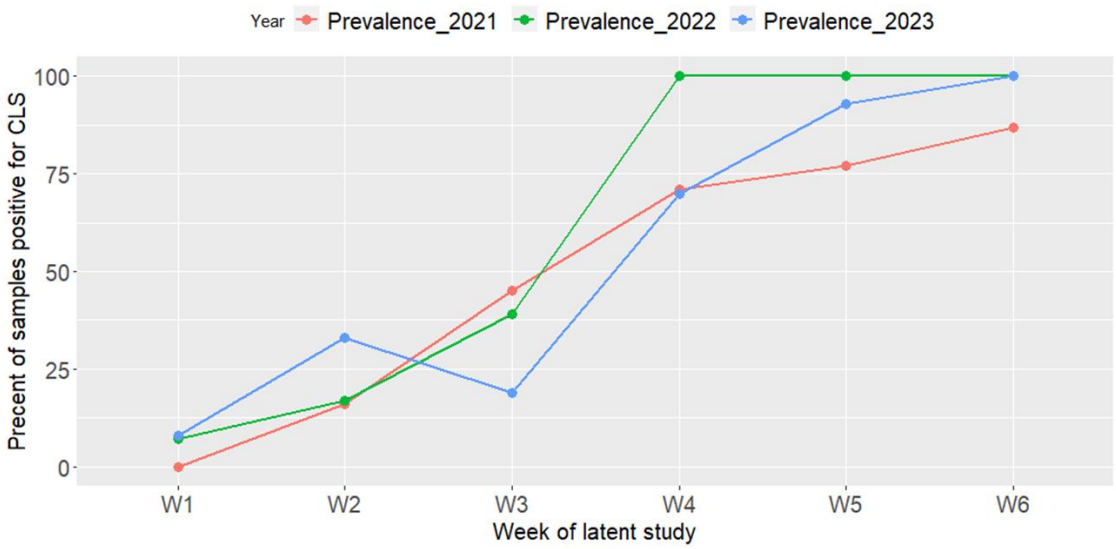

Figure 1: Prevalence of latent CLS detection in years 2021, 2022, and 2023 across sampling weeks. Sampling week 5 (W5) corresponds to the first week in July.

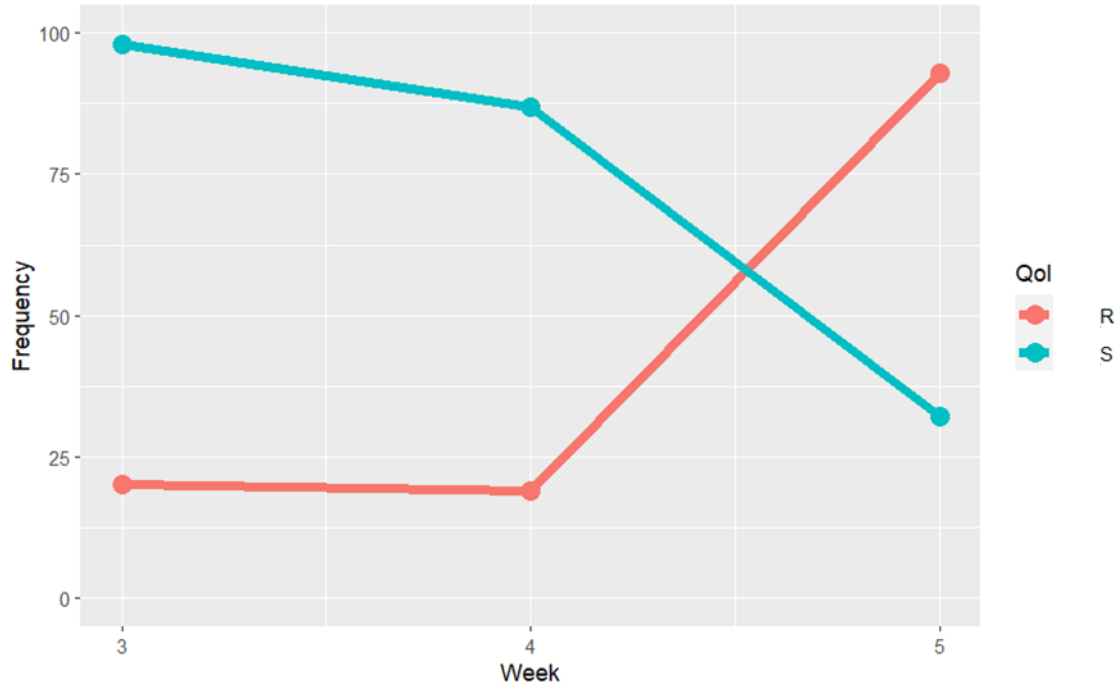

Figure 2: Strobilurin resistance and sensitivity mutation prevalence in CLS latent infection samples during weeks 3, 4, and 5 of the 2023 Latent CLS survey. Week 5 corresponds to the first week of July 2023. The blue line denotes the prevalence of the G143A strobilurin sensitivity allele and the red line denotes the prevalence of the G143A strobilurin resistance allele.

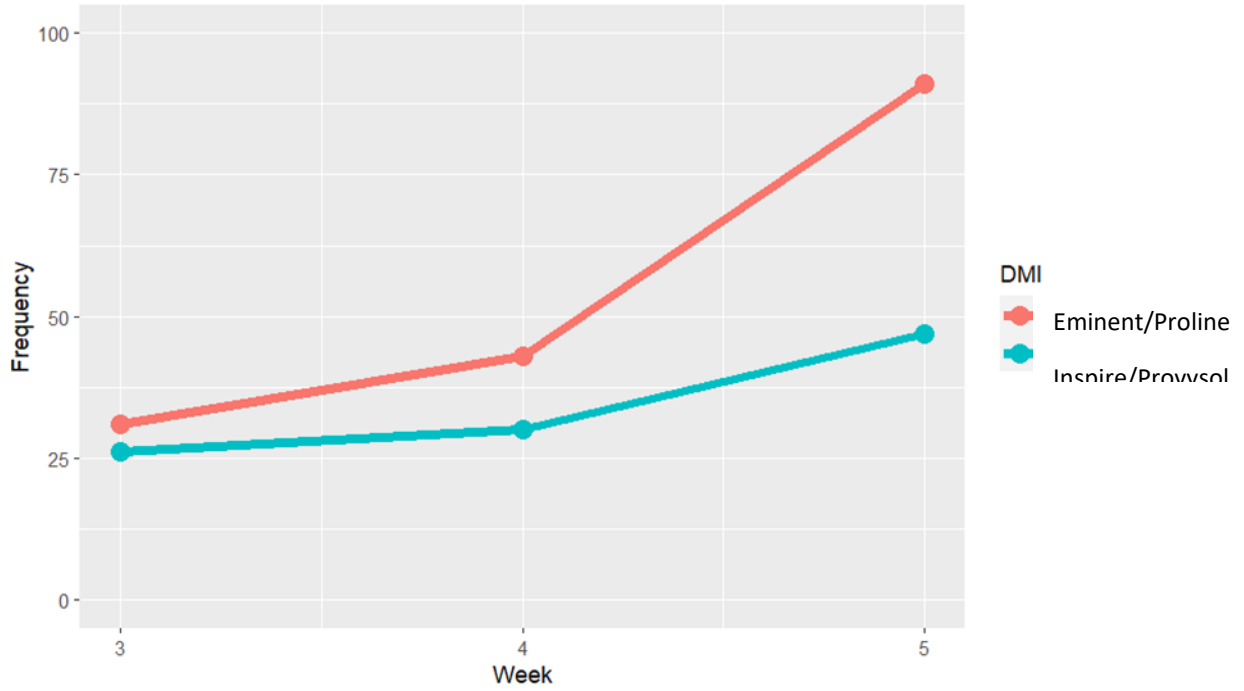

Figure 3: Triazole resistance mutation prevalence in CLS latent infection samples during weeks 3, 4, and 5 of the 2023 Latent CLS survey. Week 5 corresponds to the first week of July 2023. The red line denotes the prevalence of the E170 triazole resistance allele associated with resistance for the triazoles Tetraconazole and Prothioconazole and the blue line denotes the prevalence of the L144F triazole resistance prevalence for the triazoles Difenoconazole and Mefentrifluconazole.

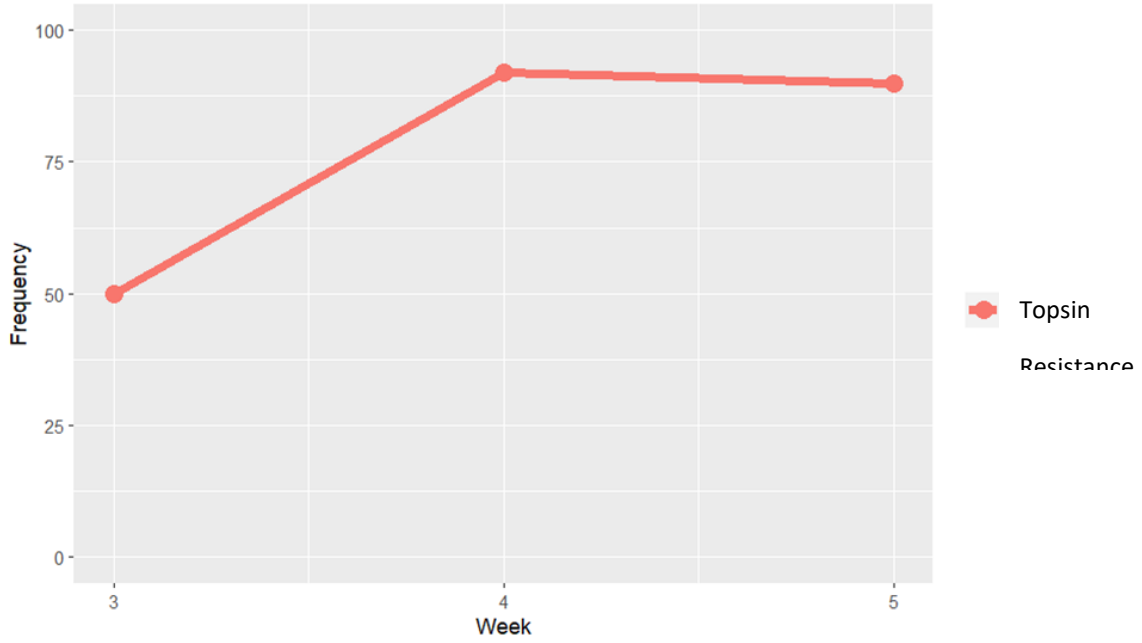

Figure 3: Benzimidazole resistance mutation prevalence in CLS latent infection samples during weeks 3, 4, and 5 of the 2023 Latent CLS survey. Week 5 corresponds to the first week of July 2023. The red line denotes the prevalence of the E198A benzimidazole resistance mutation.