Impact of cover crops and pH on *Cercospora beticola* growth and survival for management of Cercospora leaf spot of sugar beet, 2022-23

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Objective 1: Evaluate the use of cover crops for management of Cercospora leaf spot in sugar beet.

A laboratory inhibition assay was preformed to evaluate the direct impact of cover crop seedlings on the growth on *C. beticola*. In this study, 'Wheeler' rye, crimson clover, yellow mustard, common oat, and oilseed radish seeds (Johnny's Selected Seeds) underwent sterilization, germination, and placed on to sugarbeet leaf extract agar and soil extract agar. This exercise was replicated four times for each seed and media type in combination with two characterized isolates from the USDA-ARS fungal collection. Observations were recorded and compared to negative control by measuring the isolate radial growth on both the seed-bearing and seedless sides at one-week and two-week intervals. After two weeks, all seedling types except mustard significantly reduced *C. beticola* growth for both media types (Fig. 1). Experiments will be repeated in 2024.

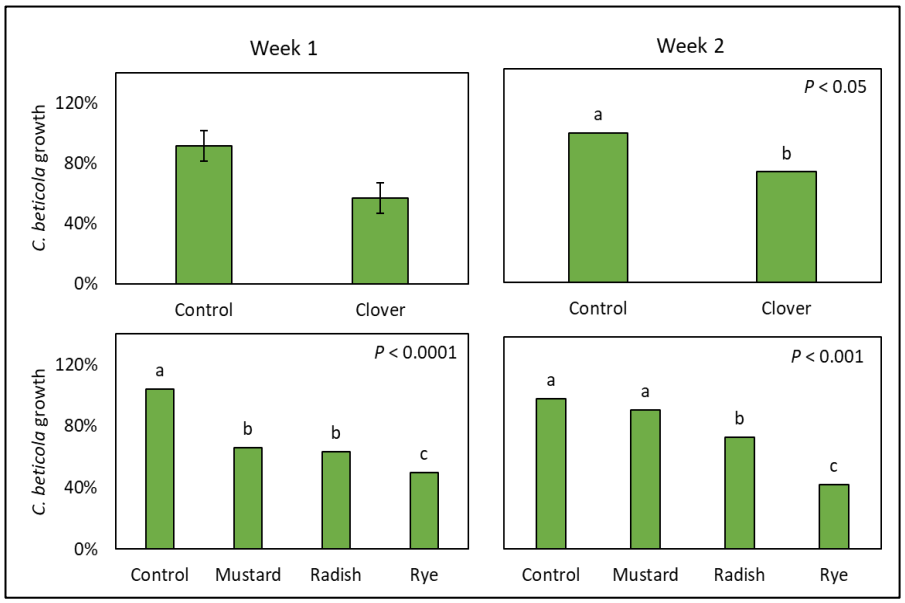

Figure 1. The percentage of *C. beticola* isolate growth on sugar beet leaf extract agar with the cover crop seedling compared to without the seedling after one and two weeks. Bars with the same letter were not significantly different based on Fisher's Protected LSD ($\alpha = 0.05$).

A small-scale greenhouse experiment will be established in the spring of 2024. Subsamples of infected leaf residue will be weighed, placed in mesh bags, and placed under three inches of soil in 8-inch pots. In the top three inches of soil each cover crop treatment will be planted with 4 replicate pots of each seed type. After one month, *C. beticola* spores will be recovered, observed, and tested for viability.

A field-scale cover crop study was established in 2023 at Saginaw Valley Research and Extension Center in Frankenmuth, MI and has been maintained by the Potato and Sugar Beet Pathology program with direction and guidance by the Forages and Cover Crops program. In the first season, the research field was planted with the beet variety SX-1278. The field was inoculated with a $1x10^4$ *C. beticola* conidia/ml suspension on July 3. The sugar beets were topped and harvested in mid-Sept then CLS infected leaf material was incorporated prior to planting of cover crop treatments on Sept 18. The following cover crop treatments were planted: cereal rye (var. Wheeler), an oats and crimson clover mixture, and oilseed radish (var. Defender). The study was arranged in a randomized complete block design with four replicates

surrounded by 10-ft wheat buffer zones. Cover crop establishment was measured on Oct 19 using plant stand densities and biomass dry weights (data not shown). Soil samples will be collected from each plot area and crops will be terminated as advised in the spring of 2024. Following cover crop termination, rotational corn will be planted. Spore levels will be monitored using highly susceptible sentinel beets (Bublitz, McGrath, and Hanson 2021). A final soil sample will be collected at harvest and will be submitted for pH and nutrient testing and fractions may be submitted for assessment of microbial activity.

Objective 2: Investigate the effects of pH on *C. beticola* infection in sugar beet leaves.

The role of pH on *C. beticola* establishment and survival was tested in laboratory and field experiments. CLS lesions were sampled from infected and non-infected sugar beet varieties from a grower field. Eight different varieties were used for this experiment with 20 leaves collected from each of the three replicate variety plots. Twenty symptomatic and asymptomatic leaf areas from at least five different leaves per field replicate were placed into 2 mL of sterile deionized water, ground, and the pH was measured. Change in pH was not significantly different between varieties (Fig. 2). The pH of healthy tissue was not significantly different from the pH of the CLS lesion tissue ($P > 0.05$).

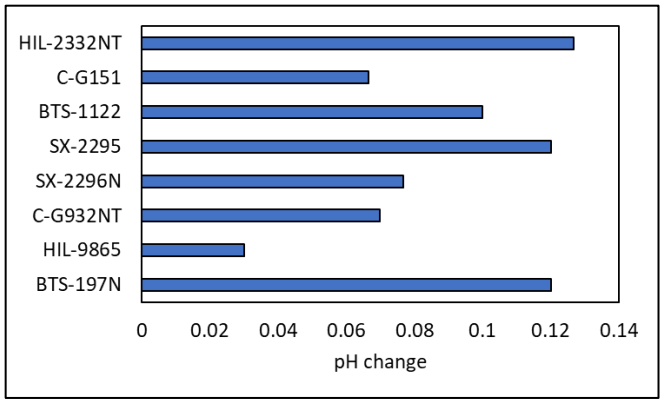

Figure 2. Change in pH of CLS lesion tissue compared to healthy tissue of eight sugar beet varieties from various companies and across CLS resistance levels.

Laboratory experiments were conducted to test the impact of soil and foliar amendments on *C. beticola* isolate growth. Soil extract agar and sugar beet leaf extract agar were amended with Humax and Fulmax (JH Biotech) solutions according to the rates for foliar and soil application for the respective media type. Two *C. beticola* isolates (described above) were placed on amended and non-amended media. The diameter of isolate growth was then measured one and two weeks after inoculation. Humax amendment increased the pH from 6 to 8 and significantly reduced growth of *C. beticola* isolates ($P < 0.001$). Field applications of these products in CLS management will be investigated.

Overall Summary:

- Rye seedlings consistently resulted in the greatest inhibition of *C. beticola* mycelial growth. Mustard, radish, and clover seedlings also inhibited *C. beticola* growth, but to a lesser extent.
- The pH of sugar beet leaf material was consistently greater in CLS-symptomatic tissue compared to healthy tissue. Additionally, *C. beticola* mycelial growth was inhibited for Humax amendments that increased pH in artificial media. Further investigations of the role of pH are necessary.

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