**Epidemiological studies of Cercospora leaf spot of sugar beet for improved management**

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**Background:** Cercospora leaf spot (CLS), caused by *Cercospora beticola*, is the most important foliar disease of sugar beets in Michigan (Harveson et al. 2009; Lartey et al. 2010). Increased inoculum due to overwintering has posed a larger problem when managing fields with high disease pressure. In recent years, BEETcast thresholds advising the initial spray occurred around the time spots were forming. Since CLS lesions were observed 7 to 10 days *after* infection, and are difficult to manage once the initial infection has taken place, these recommendations were delayed. Therefore, accurate prediction of the initial infection, based on a better understanding of *C. beticola* spore presence and abundance, is crucial. Short-term and long-term management methods are required for sustainable management of CLS. In this research, strategies were investigated to improve prediction models, reduce inoculum survival, and manage fungicide resistance development.

**Methods:**

**Objective 1. Monitored *C. beticola* spore presence and abundance using spore traps and sentinel beets and used this information to refine existing predictive modeling tools.** Environmental factors, such as temperature, humidity, leaf wetness, and soil conditions were monitored using on-site or MSU Enviroweather stations and were tested for correlations to spore abundance. Stepwise regression analyses were conducted to assess the accuracy of the model variables separately and together. R-squared values were used to evaluate each variable and variable combination and a preliminary weather-based model predicting spore abundance was determined.

**Objective 2. Assessed potential management strategies, targeting in-season and end-of-season treatments, with the aim to reduce inoculum levels, and therefore disease.** Treatments included a nontreated control, plowing immediately post-harvest, burning prior to defoliation at-harvest, and applying a desiccant (saflufenacil) seven days pre-harvest. Four replicates of each treatment were included in a randomized complete block design (RCBD). Four leaf samples were collected from each plot: one sample was collected at harvest for destructive sampling and three samples were placed in mesh bags and left in the field to be evaluated 45, 90, and 135 days post-harvest. Samples were processed by counting the number of CLS lesions, then placed in moist chambers for three days. Leaves were then re-assessed for percentage of sporulating lesions. Fifteen representative lesions from each treatment replicate were surface disinfested and plated on clarified V8 juice or water agar media to detect viable *C. beticola*.

**Objective 3. Determined fungicide sensitivity of *C. beticola* populations recovered from resistance management tactic efficacy trials.** Treatment programs were evaluated at the SVREC included: 1) a nontreated control; 2) a mixed application, where both high-risk (Headline; pyraclostrobin) and low-risk (Manzate Max; mancozeb) fungicides were applied at each spray timing; 3) high-low, where alternate sprays of pyraclostrobin and mancozeb were applied, with pyraclostrobin sprayed first; and 4) low-high, which is similar to the previous treatment but with low-risk applied first. Treatments were replicated four times and arranged in a RCBD. Leaves with symptoms of CLS were sampled from field trials mid-season in July (after three treatments) and end-of-season in September (after all six treatments). Mono-conidial *C. beticola* isolates were then tested for *in vitro* pyraclostrobin sensitivity. A spiral gradient dilution method was used to find the effective concentration inhibiting growth by 50% ($EC_{50}$) for at least 15 isolates per treatment replicate.

For all objectives, statistical analyses (analysis of variance and simple and mixed linear regression) were conducted in SAS v. 9.4 and evaluated at the $\alpha$=0.05 significance level. Fisher's protected Least Significance Difference was used for mean comparisons.

**Results & Conclusions:**

**Objective 1**: The abundance of spores was not significantly correlated with the number of spots on the sentinel beets for the corresponding week ($P > 0.05$). However, sentinel beet studies were generally comparable to spore traps in the initial spore detections. The strongest correlations to spore count were with wind speed ($r = 0.38$, $P < 0.0001$), minimum relative humidity ($r = 0.24$, $P < 0.01$), maximum soil temperature ($r = -0.24$, $P < 0.01$), and precipitation ($r = 0.22$, $P < 0.05$). The model with the best fit included all variables stated above ($R^2 = 0.23$, $P < 0.0001$). The model proposed for spore number is: $CB = 9.53 * PC + 0.70 * RH_{min} - 1.21 * ST_{max} + 2.43 * WS_{max}$, where *CB* is daily *C. beticola* spore abundance, *PC* is daily total precipitation (in), $RH_{min}$ is minimum daily relative humidity, $ST_{max}$ is maximum daily soil temperature (°F), and *WS* is maximum daily wind speed (mph).

Initial detections and general trends of abundance were identified using both mechanical spore trap and sentinel beet methods. A preliminary spore abundance model was determined and will be further validated to improve existing prediction tools.

**Objective 2**: For samples collected at harvest (N=133 leaves and 240 lesions), significant treatment differences were detected in percentages of lesion sporulation (Fig. 1A, $P < 0.001$). Treatment also significantly affected percentages of lesion viability (Fig. 1B, $P < 0.05$). Remaining leaf samples from inoculum overwintering studies will continue to be evaluated as described 45, 90, and 135 days post-harvest.

Novel management strategies, particularly the use of a foliar burner at-harvest, have the potential to significantly reduce inoculum overwintering and aid in long-term CLS control. In 2020, early-season spore presence and abundance, weekly disease ratings, and final yield and sugar data will be collected to assess the long-term efficacy of inoculum reduction strategies.

**Objective 3**: No significant differences were found in mean $EC_{50}$ values for isolates treated with pyraclostrobin in any of the fungicide treatment programs for mid-season samples taken in July ($P > 0.05$). These samples did not receive the entire fungicide program; the remaining full-season treatment samples will be processed. All programs resulted in similar yields ($P < 0.001$) and relative area under the disease progress curves (RAUDPC; $P < 0.01$) and performed better than the non-treated control (Table 1.).

All isolates tested were sensitive to pyraclostrobin concentrations below label rates (1,200-1,500µg $ml^{-1}$) (Fig. 2). Resistance management tactics were found to have little effect on mid-season populations of *C. beticola*. Testing of end-of-season *C. beticola* populations is in progress and will continue.

**Table 1.** Mean yield and RAUDPC value for fungicide resistance management programs.

| Treatment | Description | Yield (T/A) | | RAUDPC[w] (%) | |
|---|---|---|---|---|---|
| **1** | Control | 13.6 | b[x] | 39.9 | a |
| **2** | Headline + Manzate Max ABCDEF | 20.8 | a | 22.3 | b |
| **3** | Headline[y] ACE[z] + Manzate Max BDF | 19.4 | a | 27.4 | b |
| **4** | Manzate Max ACE + Headline BDF | 19.9 | a | 25.3 | b |

[w] Area under the disease progress curve (AUDPC) was determined from four disease severity ratings (25 Jul, 6 Aug, 19 Aug, 11 Sep) with a 0 to 10 scale. Relative AUDPC (RAUDPC) was calculated by dividing the mean for each treatment by the maximum possible AUDCP.

[x] Column values followed by the same letter were not significantly different based on Fisher's Protected LSD (α=0.05); if no letter, then the effect was not significant

[y] Headline was applied at 12 fl oz/A and Manzate Max was applied at 1.6 qt/A. MasterLock was added to all tank mixes at a rate of 0.25 % v/v.

[z] Application letters code for the following dates: A=26 Jun, B=8 Jul, C=22 Jul, D=31 Jul, E=14 Aug, F=23 Aug.

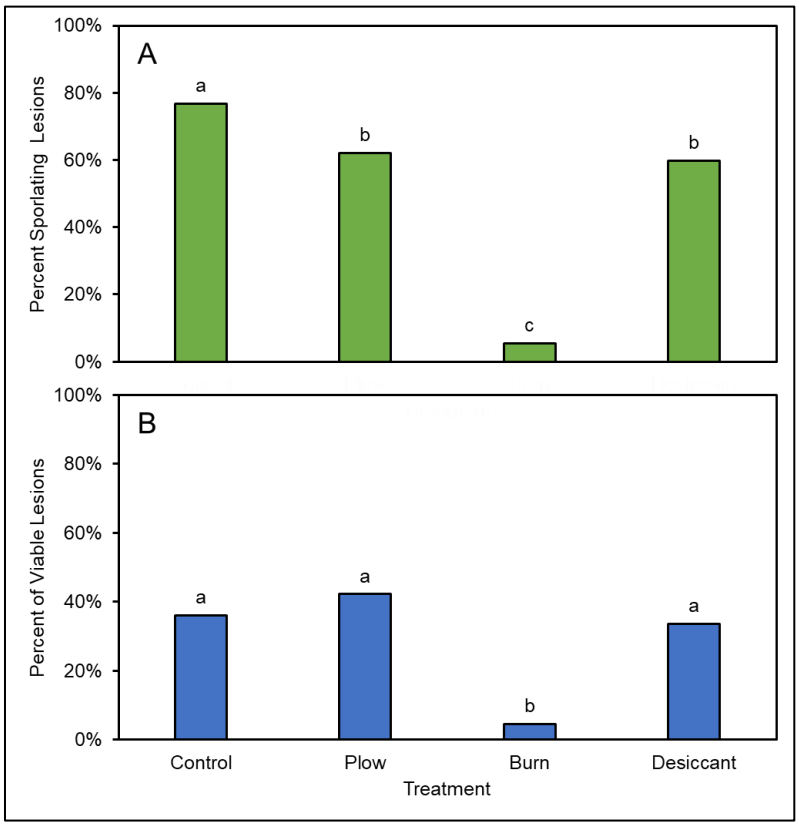


**Figure 1.** Significant treatment differences were detected in percentages of **A,** lesion sporulation ($P < 0.001$) and **B,** lesion viability ($P < 0.05$) from samples collected at-harvest (N=133 leaves or 240 lesions). The desiccant, burn, and plow treatments were applied seven days pre-harvest, prior to defoliation at harvest, and immediately post-harvest, respectively. Means comparisons were determined using Fisher's protected Least Significance Difference; bars with the same letter are not significantly different α=0.05.

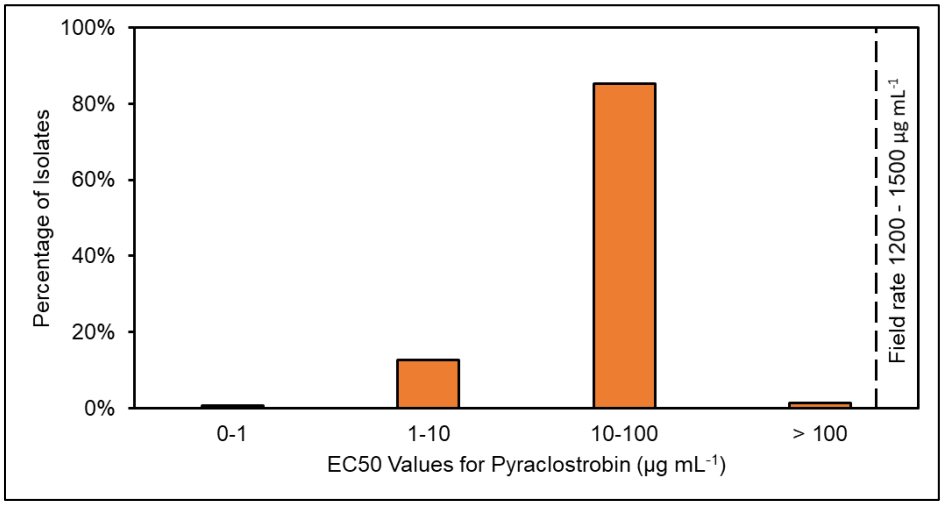


**Figure 2.** Frequency distribution of mean values of the effective concentration of pyraclostrobin inhibiting *Cercospora beticola* isolate growth by 50% ($EC_{50}$) from mid-season samples collected in July. Mid-season samples were subjected to three of six total fungicide applications for each treatment [nontreated control, mixed low-risk (mancozeb) and high-risk (pyraclostrobin) products, alternated low-high, and alternated high-low].