



## PROGRESS REPORT

PROJECT TITLE: Hyperstable Enzyme to Control Plant Diseases – Phase VI

PROJECT NUMBER: 6099-23DD

REPORTING PERIOD: Sept, 2024 – April 2025

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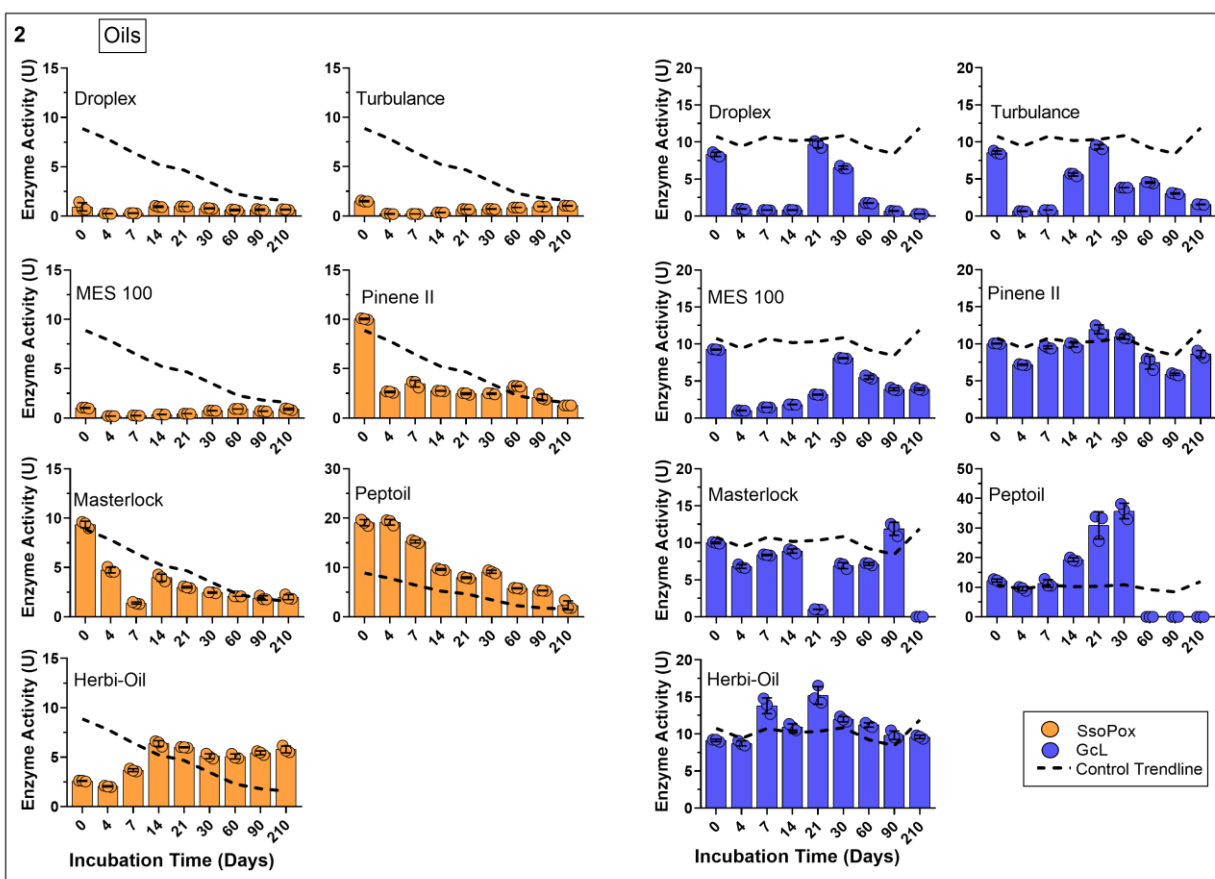
**\*Most Recent update is highlighted in CYAN\***

### Summary

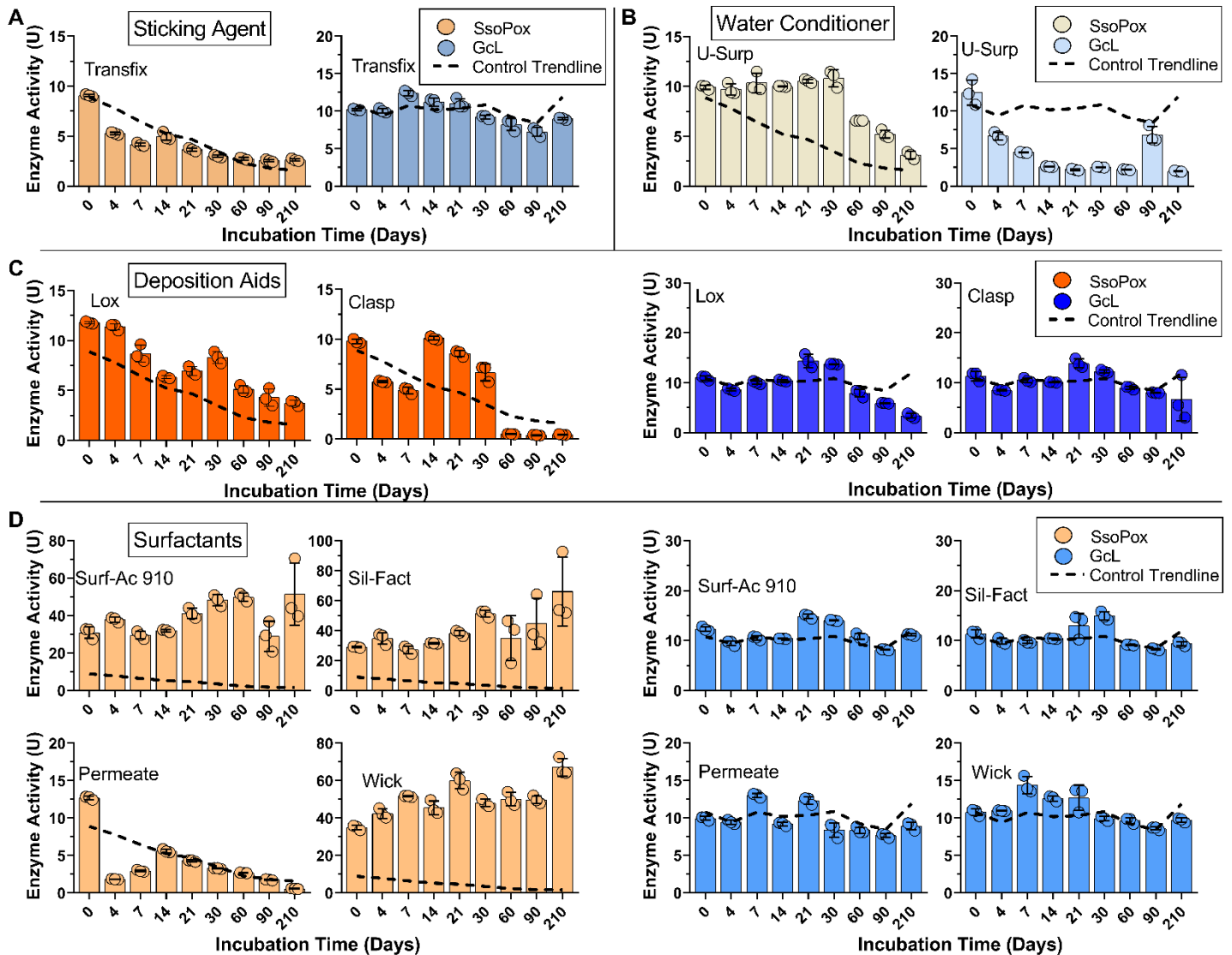
Many bacterial pathogens infect crop plants, representing major economic burdens. Current methods for controlling plant diseases due to bacterial infection have had limited success, in large part due to bacterial resistance, specificity, and environmental concerns associated with antibiotics. Novel strategies are therefore greatly needed to control microbes. Here, we take advantage of the ability of bacteria to communicate using small signaling molecules (e.g. AHLs). Bacteria communicate to regulate numerous behaviors, including pathogenicity. Our group has isolated and engineered enzymes that can degrade AHLs, and thereby interfere with microbial communication. By doing so, these enzymes do not kill bacteria but prevent their pathogenicity. In previous years of funding, we found that lactonase spray on infected corn leaves dramatically reduced Goss's Wilt in UMN fields, MN, during the 2021 season, and in 2022 we extended these field testing by performing a mid-scale study. In 2023, we confirmed these results in a mid-scale study and found that the lactonase enzyme is compatible with numerous commercially available surfactants and adjuvants. Interestingly, field results show a synergistic effect between the enzymatic treatment and the tested surfactant. Such an effect is also seen in corn yield measurements. These promising results prompt us to propose the logical next steps to accelerate the translation of the technology: **i) continue to evaluate the compatibility of the enzyme molecule with current market products; ii) investigate the possible synergies between enzyme treatments and surfactants and iii) evaluate the effect of treatment in the context of plant biostimulation.** The proposed work is expected to provide robust, replicated fieldwork data that will advance the usefulness of this technology to protect crop fields. The proposed experiments performed in combination with existing products and surfactants is expected to facilitate the technology translation, adoption, and use.

**1. Evaluate the compatibility of the enzyme molecule with current market products and surfactants and maximize treatment reproducibility and durability.**

One of the main objectives of this year’s project is to continue to evaluate the possibility of direct incorporation of the enzymes in commercial surfactants. After the yearly meeting at the UMN in 2023, and a discussion with the corn growers, we received the suggestion to also evaluate the compatibility of the enzymes in commercial adjuvants. To assess this possibility, we used purified, lyophilized enzyme and diluted it into 5 different commercial surfactants used for crops, at the recommended surfactant concentration. We previously reported very encouraging results with commercial adjuvants, mainly sourced from Drexel Very interestingly, we observe that the enzymes, including variants, remain active in the tested adjuvants (**Figure 1,2&3**). Yet, the enzymes show better performance in some surfactants, as opposed to others. Overall, these results are very encouraging and reinforce the potential of these enzymes for translation. These results are currently being prepared for publication as a scientific article.



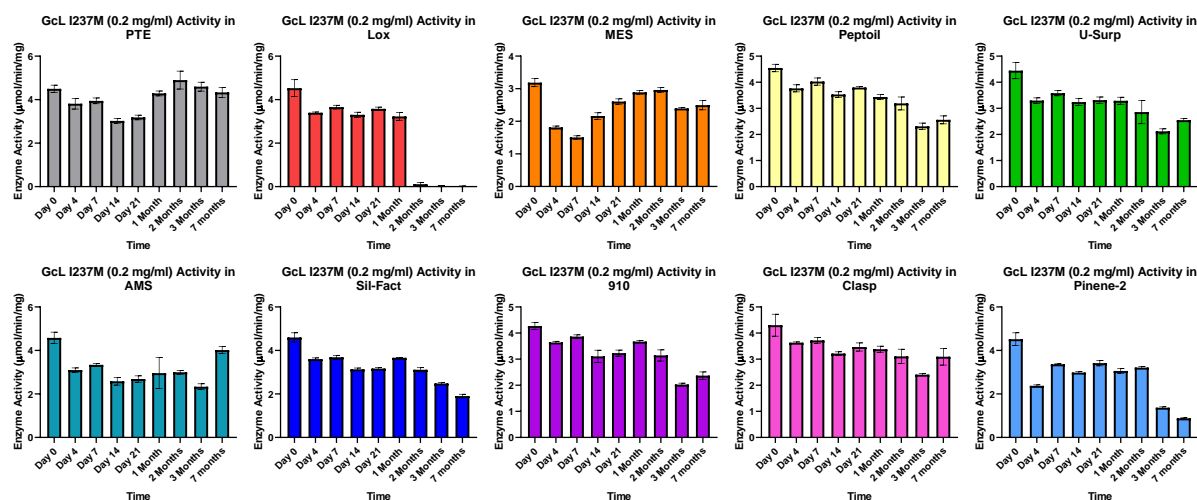
**Figure 1. Adjuvant Group 1.** Lactonase enzymes SsoPox (orange) and GcL (blue) remain active when added to many commercially used adjuvants **for over 6 months**). Dashed black line indicate a control where the enzyme is kept in aqueous buffer (control without adjuvant).



**Figure 2. Lactonase enzymes show different levels of activity over time in different crop adjuvant classes – Group 2.**

Enzyme activity in presence of (A) surfactants, (B) deposition aids, (C) water conditioner, and (D) sticking agent. The dotted trendline (black) is the enzyme activity in the control conditions (activity buffer) and serves as reference. The activity of lactonases SsoPox (orange) and GcL (blue) (0.2 mg/mL) were measured in the presence of manufacturer’s recommended concentrations of adjuvants (see “Enzyme tolerance to crop adjuvants” in methods). Activity over time was tested via a paraxononase assay. Enzyme activity is reported as U, which is 1 uM of substrate catalyzed per minute

## Attachment A: Workplan for Lactonase Biocontrol Project – Phase VI

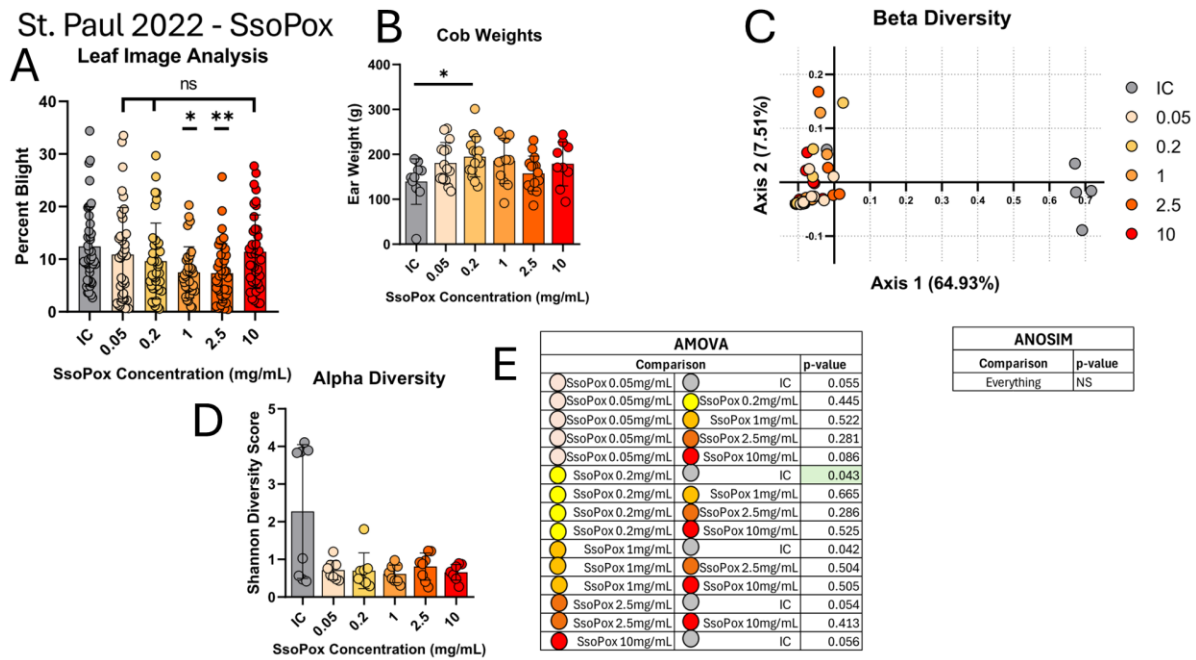


**Figure 3.** Lactonase improved mutant I237M activity over time in different crop adjuvant classes.

### 2. Evaluate the efficacy of surfactant-lactonase formulations in a mid-scale field trial.

We used the months of the winter where little field work is possible to continue to analyze our data, and particularly our sequencing data. In previous field season work, we collected surface leaf DNA samples for treated and untreated plants, and submitted these samples for sequencing of the 16s rRNA v4 region using the University of Minnesota Genomics Center.

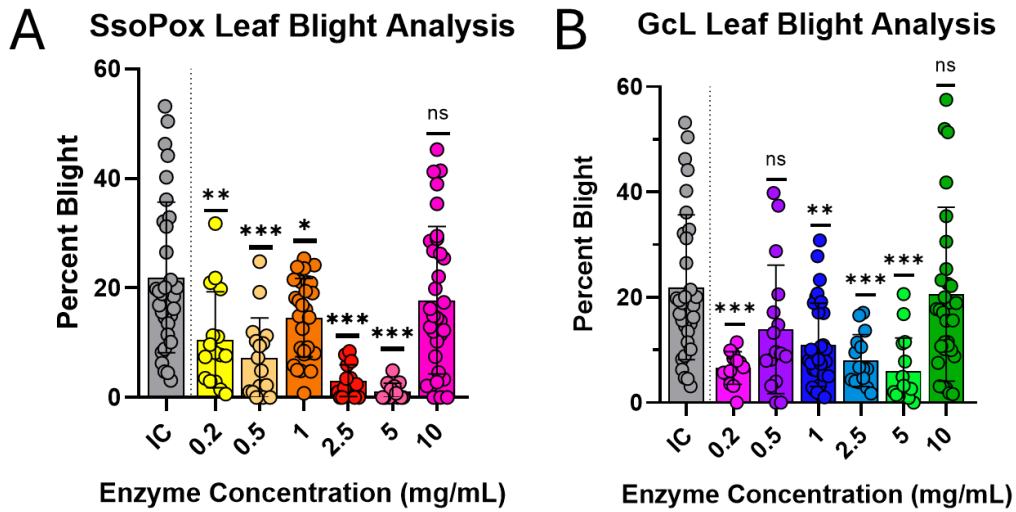
We examined the samples from the 2022 field season work (**Figure 3**). Then, we observed reduction of blight symptoms as a function of the enzyme dose (**Figure 3A**), an increase in cob weight with enzyme treatment (**Figure 3B**), and show that this corresponds to changes in alpha diversity in the microbial community population (**Figure 3D**), and one treatment concentration leads to a significantly different surface community (**Figure 3C and E**)



**Figure 3.** Effects of the lactonase SsoPox in the 2022 field season. **(A)** Dose response shows a reduction of leaf blight with higher enzyme dose. **(B)** enzyme treatment increased cob weight relative to control. **(C)** Beta diversity for surface leaf community. **(D)** Alpha diversity for surface leaf community. **(E)** Statistical analysis for surface leaf community.

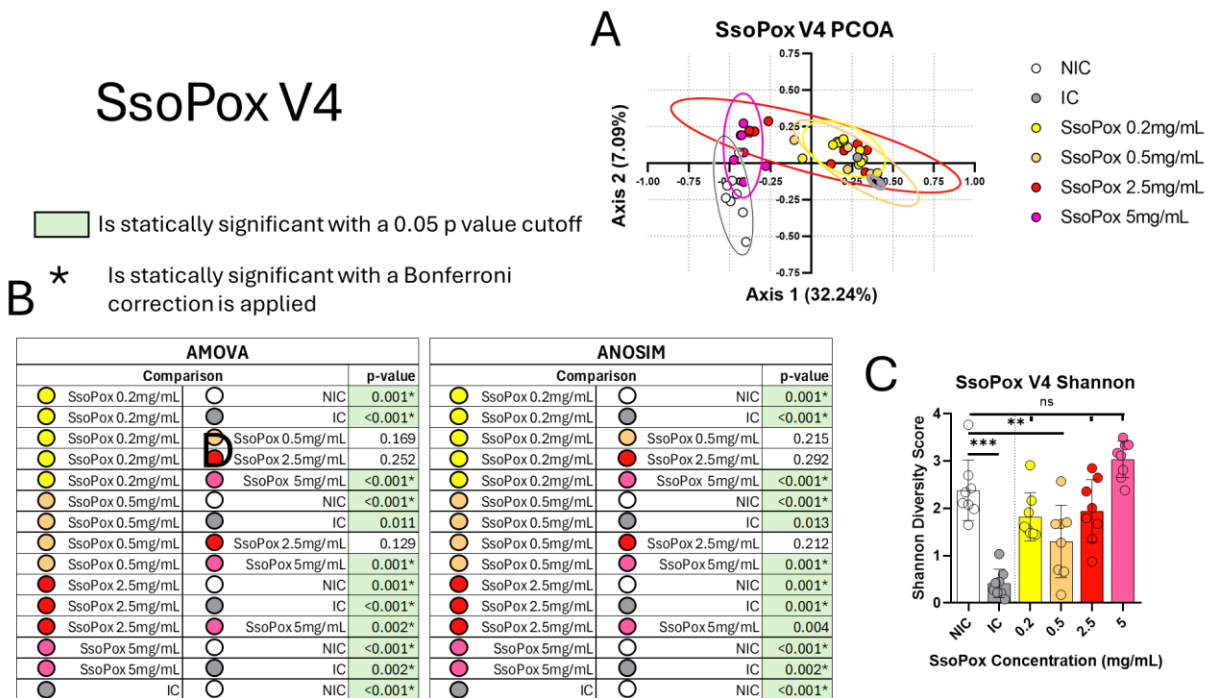
### Analysis of the 2023 field Dose-response experiment.

In the summer of 2023, we reported that the two different enzyme treatments reduced leaf blight caused by Goss' Wilt in a dose-dependent manner (**Figure 4**).

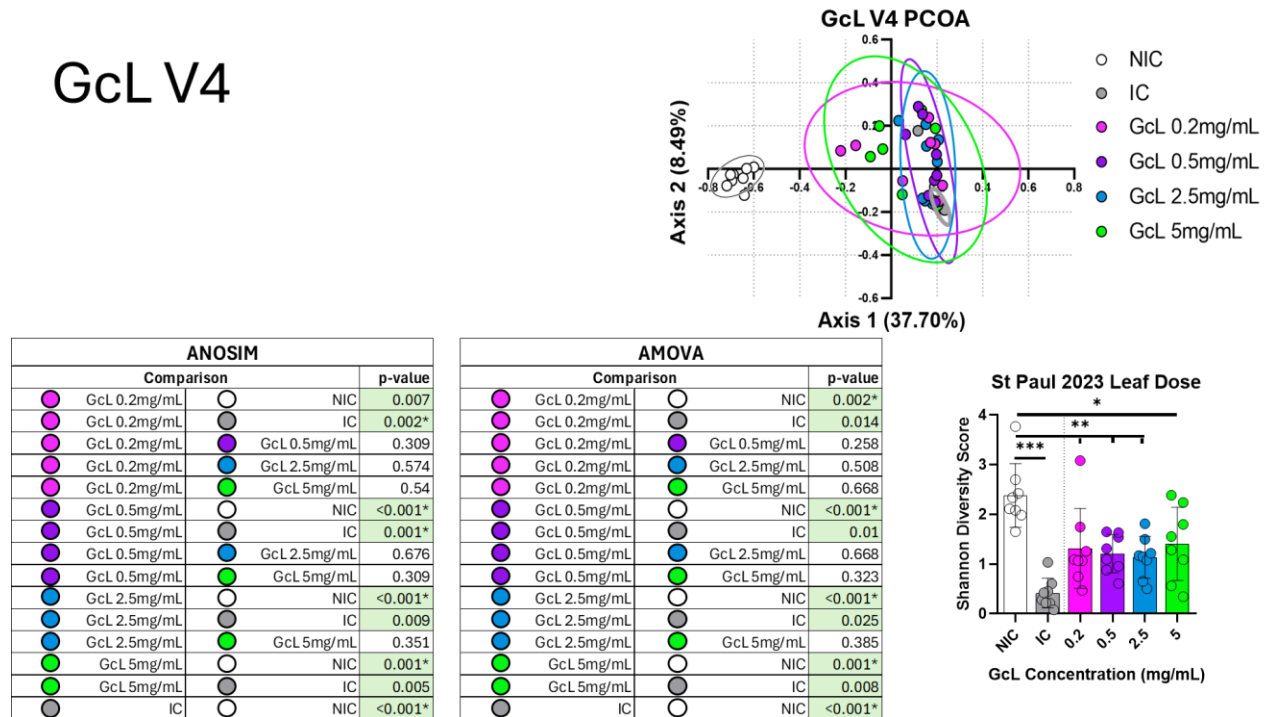


**Figure 4.** Enzyme treatments SsoPox (A) and GcL (B) reduce leaf blight caused by Goss’s Wilt in field testing in St Paul, MN, 2023.

Similarly to the 2022 field season, we analyzed DNA samples from leaves and submitted these samples for sequencing of the 16s rRNA v4 region using the University of Minnesota Genomics Center. Analysis reveals are being finalized, but highlights that **enzyme treatment significantly alters the surface microbial community** as illustrated by Principal Component analysis and statistical analysis (Figure 5 and 6). We are currently investigating the possible links between taxa variation and symptoms.

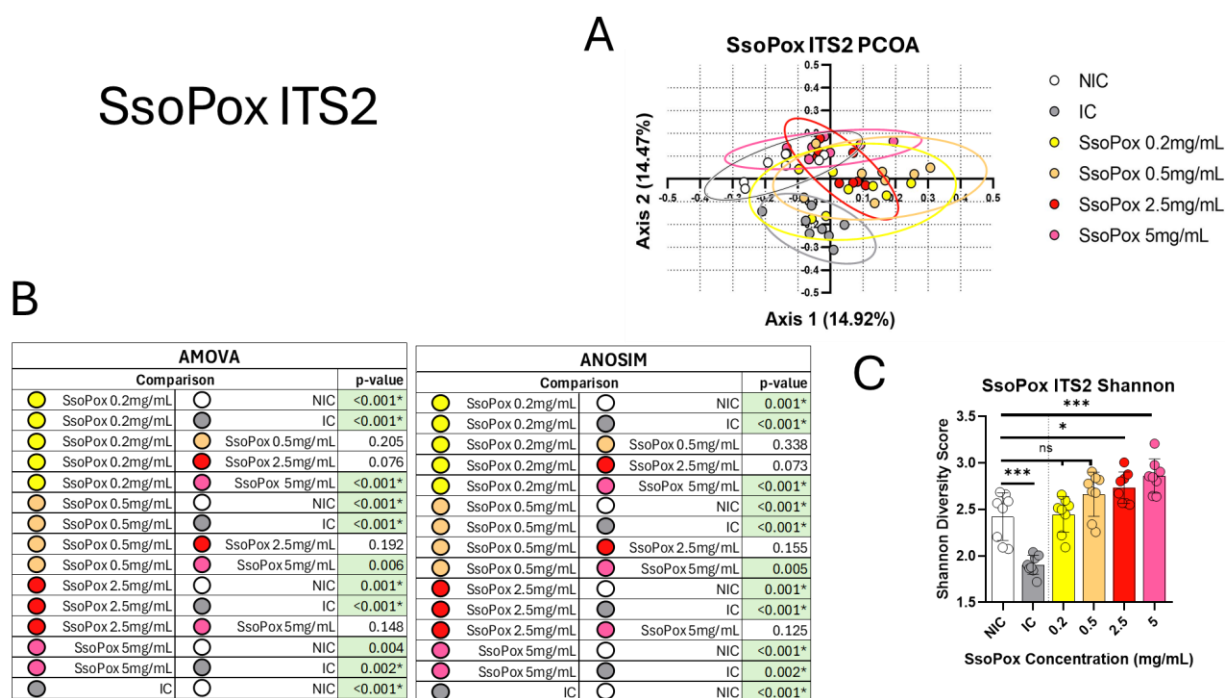


**Figure 5.** Effects of the lactonase SsoPox in the 2023 field season in St Paul on surface microbial community. (A) Principal component analysis of surface leaf communities. (B) Statistical analysis for surface leaf community. (C). Alpha diversity (Shannon index) for surface leaf community.



**Figure 6.** Effects of the lactonase GcL in the 2023 field season in St Paul on surface microbial community. (A) Principal component analysis of surface leaf communities. (B) Statistical analysis for surface leaf community. (C). Alpha diversity (Shannon index) for surface leaf community.

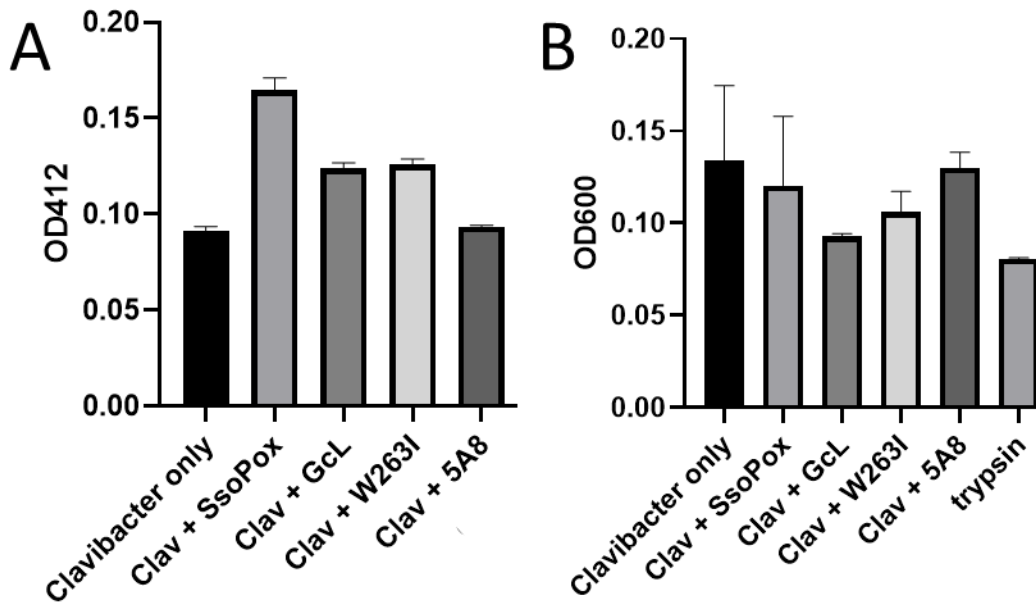
We took advantage of this sampling to investigate the potential influence of enzyme treatment on the fungal population. To do so, we sequenced the ITS2 region using the University of Minnesota Genomics Center. Analysis reveals are being finalized. However, results suggest that SsoPox enzyme treatment affects the fungal population in a dose-dependent manner (**Figure 7**). **This unexpected result is extremely interesting and may justify the evaluation of the efficacy of these biological molecules on fungal plant diseases.**



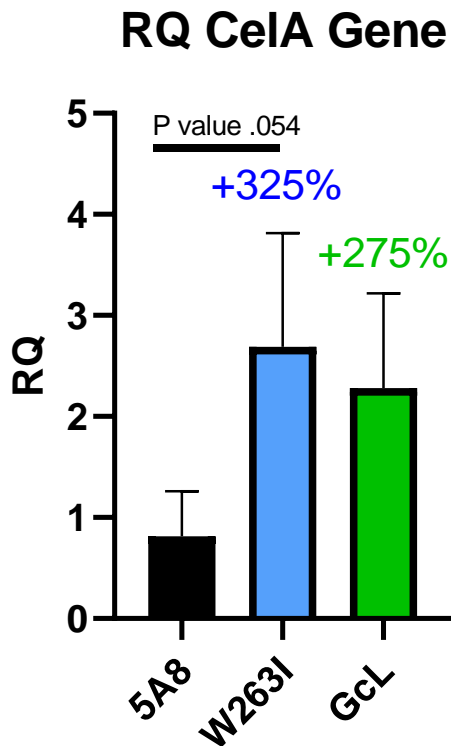
**Figure 7.** Effects of the lactonase SsoPox in the 2023 field season in St Paul on fungal community. (A) Principal component analysis of surface leaf communities. (B) Statistical analysis for surface leaf community. (C). Alpha diversity (Shannon index) for surface leaf community.

### Mechanistic understanding of the enzyme’s effect on the pathogen.

Understanding how enzymatic treatments can reduce pathogenicity is important to potentially improve the potency and specificity of treatments. To evaluate a potential direct effect, we tested whether the exogenous addition of enzyme in the culture media had any effects on the production of certain key gene products potentially involved in virulence. Specifically, we tested secreted protease activity using two different assays, azocasein and skil milk (Figure 8). Both assays suggest that the treatment of the culture with the enzyme stimulates the production of proteases. We attempted to confirm some of these results using RT-qPCR, and we find that enzyme treatment increases the production of the *CelA* gene that codes for a cellulase enzyme (Figure 9). Overall, these results suggest a possible direct effect of enzymes on the bacteria, and more investigations are currently ongoing, particularly using transcriptomics methods.



**Figure 8.** Secreted protease production (**A**: azocasein assay (higher value is more protease activity); **B**: skim milk assay (lower value is more protease activity)) by Clavibacter in presence or absence of lactonase treatments.



**Figure 9.** RT-qPCR of the cellulase C<sub>el</sub>A gene in presence (W263I and GcL) or absence (5A8) of active lactonase.

**Aim 2 & 3: 2024 season work:** We performed a new field experiment in 2024. We planned on evaluating some of the compatible combinations in the field (**Figure 4**). The goals for these tests are:

- Repeat our mid-scale field experiment of 2023
- Test the effectiveness of surfactant with lactonases as a treatment.
- Evaluate the effect of the enzyme on plants in absence of disease (**Aim 3**)

Samples are currently being collected and analysis of the samples is ongoing. We note that the plants did not grow as usual this year, possibly due to the weather (and the heavy rains). In particular, the Rosemount site that we planned to evaluate the effect of the enzymes in absence of disease (Aim 3 – biostimulation) barely grew, and some plants did not even grow at all (**Figure 10**). We also note that, also possibly due to weather, some plants in St Paul showed signs of disease before any intervention (**Figure 11**). We hope that meaningful results can still be collected from these experiments.

**Waseca – No Bacteria, Only Enzyme Treatment**



- By far the worst looking site
- Really small and sparse plants,

**Figure 10.** Plants did not grow well at the Waseca experimental site in 2024.

**St. Paul**



- Growth stage was V4
- There was already some kind of disease in the field

**Figure 11.** Some disease was present on some plants at the St Paul site in 2024.

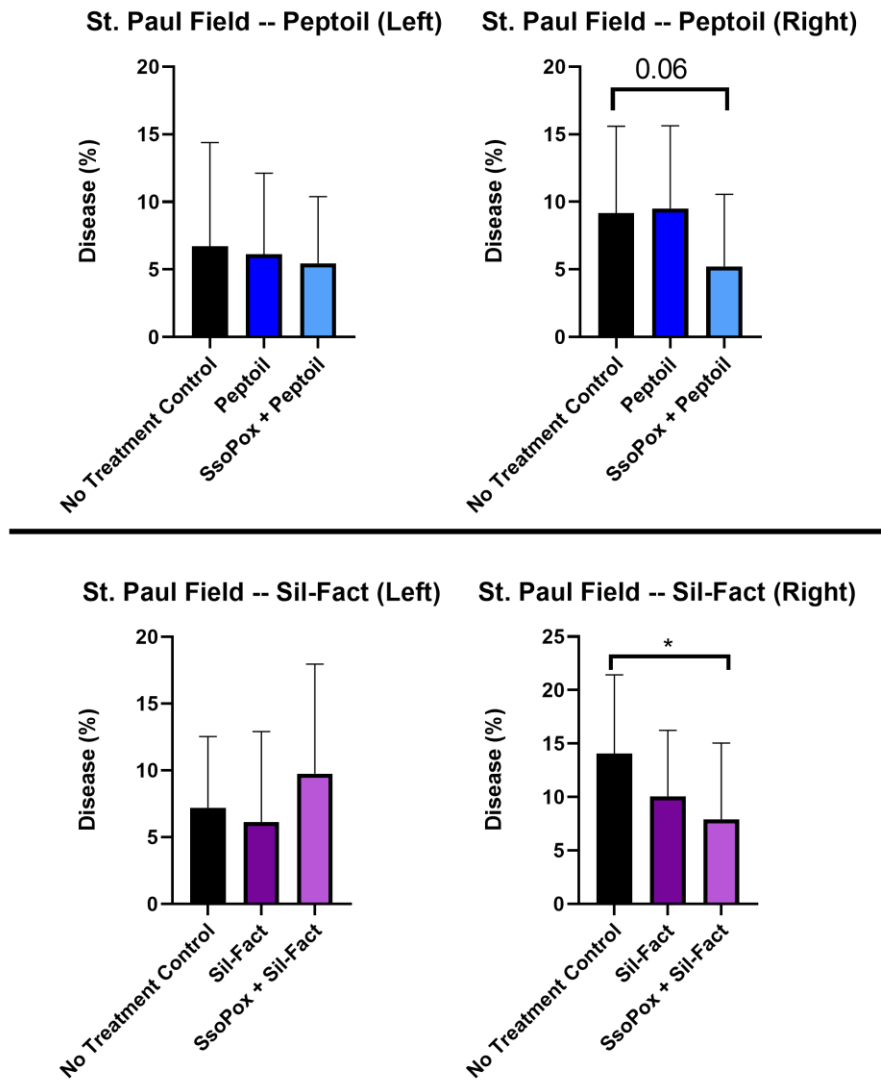
In Saint Paul, we performed testing with different surfactants and enzymes. We observe that despite low levels of disease, the combination of enzyme and surfactant reduces the disease level, significantly for the combination enzyme with Sil-fact (**Figure 12**).

Over the years, we have noticed that when low levels of diseases are recorded, lower levels of protection are observed. This is logical because there are fewer symptoms to reduce, and it squeezes the difference between healthy plants and sick plants, making it more difficult to observe statistical significance.

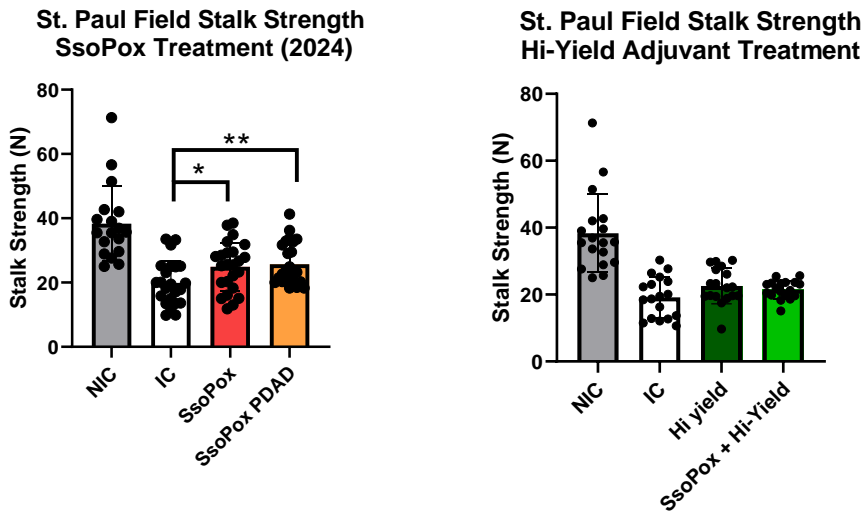
We continued our analysis of the 2024 season data and used measured stalk strength at the St Paul site (**Figure 13**). Compared to the infected control, we observe that the enzyme spray treatment significantly increases stalk strength, but not in the presence of an adjuvant. In Rosemount, the same analysis (**Figure 15**) reveals that our other lactonase treatment (GcL) also increases stalk strength, including when combined with surfactants. Overall, these results confirm previous results and are promising since stalk strength is generally associated with plant health.

Additionally, this season's new results confirm previous observations on cob weight (**Figure 14**). Treatment with enzyme alone increased cob weight from the infected control, although the weights remain lower than that of the non-infected control. Results also show that some surfactants (e.g. Hi-Yield, Silfac) also increase cob weight. However, the combination of enzyme and surfactant did not result in higher cob weight rescue than the treatments alone.

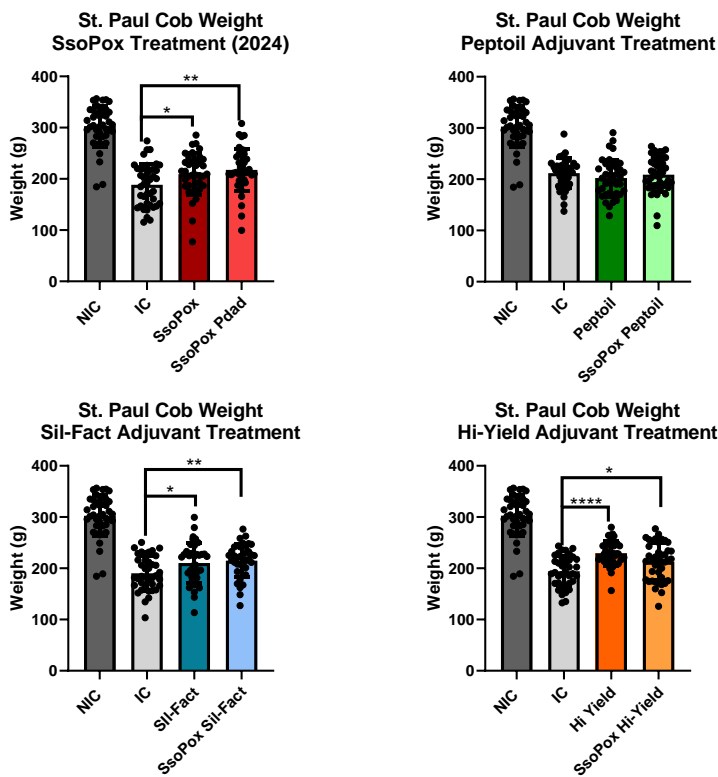
**Project summer season field work:** We are preparing for the upcoming field season. We are enthusiastic about reproducing the 2023 and 2024 mid-scale work. We are also preparing for evaluating the efficacy of the enzymes with identified, highly compatible commercial adjuvants and evaluate the effective treatment time window.



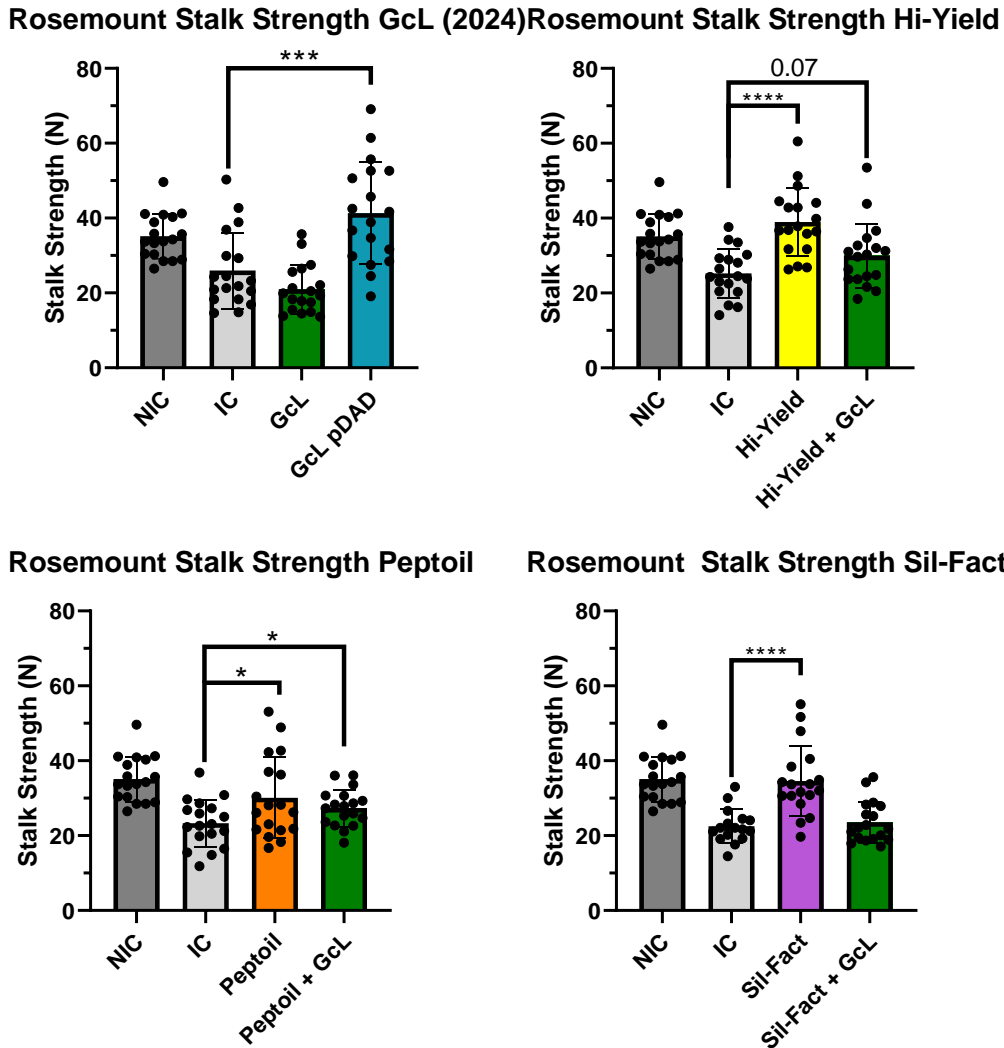
**Figure 12.** Disease level reduction was observed at the Saint Paul site (2024) with enzyme and adjuvant combinations.



**Figure 13.** Stalk strength at the St Paul site (2024) shows for (*left graph*) two different lactonase prep (SsoPox and SsoPox pDAD) as well as (*right graph*) enzyme and adjuvant combination. n=18. NIC= Non infected Control. IC= infected control.



**Figure 14.** Cob weight at the St Paul site (2024). The cobs of infected and healthy plants were collected and weighed. The enzyme SsoPox was used in this field varying only the adjuvant used. . n=40. NIC= Non infected Control. IC= infected control.



**Figure 15.** Cob weight at the Rosemount site (2024). The stalks of infected and healthy plants were measured using a force probe. Treatments with GcL pDAD greatly increased the stalk strength of infected plants, even surpassing that of healthy plants. Other treatments including adjuvant alone also showed an increase in the overall strength of the stalk. n=18. NIC= Non infected Control. IC= infected control.

## **Outreach and products**

Jacobson, Castonguay, Elias, manuscript under review at *Biotechnology and Applied Biochemistry*

Interview. Jan 16 2025. <https://www.mncorn.org/2025/01/16/speaking-the-language-of-bacteria/>  
Speaking the language of bacteria.

Ag Expo January 22 and 23 2025, Mankato, MN. The Use of Quorum Quenching Enzymes to Treat Corn Disease. Minnesota State Capitol.

Elias M. Feb 18 2025. Example of MN Biotechnology: The Use of Quorum Quenching Enzymes to Treat Corn Disease. Minnesota State Capitol.  
<https://bti.umn.edu/blog-posts/biotech-advocacy-capitol>

Castonguay Colton. The Use of Quorum Quenching Enzymes to Treat Corn Disease. Minnesota Corn Growers Association Reception. August 9 2023. [attendance: approx. 70].

Castonguay Colton. The Use of Quorum Quenching Enzymes to Treat Corn Disease. Rosemount ROC summer Research Tour. August 16 2023. [attendance: approx. 40].

Elias. Interference in quorum sensing, application in biotechnology. 11/2/2022. South Dakota State University seminar. [attendance: up to 25].

Elias. Molecular Determinants for Enzymatic Promiscuity and Exquisite Molecular Specificity. 3/9/2022. The Protein Society. Webinar. [attendance: up to 1,000].

Elias. Lactonases and biotechnological applications. 7/28/2022. Discussion with scientists at the german chemical company Evonik. [attendance: 3].

Jacobson. Outreach at the Davies High School in Fargo, North Dakota where Reed spoke to 7 different biology class periods, 2 of them were AP biology classes meaning they were taking college biology in high school, and 5 class periods were general bio. Each class period was about 45 minutes long, and there were about 20-25 students per class meaning about 150 students in total. His presentation covered, among other topics, his current thesis work looking at quorum sensing.

As part of our diversity and inclusion activities, our lab was involved in Grass-Roots Advancement in STEM Professions (GRASP) MN - Lab tour for 8th graders. April 24<sup>th</sup> 2023 and on November 17<sup>th</sup> 2023.