BETA GERMPLASM CONSERVATION AND REGENERATION
BSDF Project 280: Executive Summary

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The Western Regional Plant Introduction Station (WRPIS), part of the USDA-ARS National Plant Germplasm System, acquires, preserves, evaluates, documents and distributes *Beta* germplasm for use by the plant breeding community. Regeneration of *Beta* germplasm is an integral part of maintaining this valuable resource for the future. Currently the NPGS *Beta* collection contains 1,852 accessions of *Beta vulgaris* ssp. *vulgaris* (sugar beet, fodder beet, table beet and chard), 627 accessions of *Beta vulgaris* ssp. *maritima* (sea beet), and 271 accessions of 13 additional wild beet species. Our increase and regeneration activities are done in glasshouses, both WRPIS and rented space from Washington State University, and isolated field plots. We have 13-19 greenhouse spaces, 2 lathhouses, and 5 isolated field areas available for beet growing.

Greenhouse and field increase/regeneration protocol. Our target regeneration population is 100 plants per accession for both greenhouse and field regenerations. For greenhouse and field grown accessions seed is started in the greenhouse, once germinated it is transplanted to pots or Rootrainers^{TM} and grown for approximately 3 months. After 3 months, plants are placed at 4^{o}C for 4 months (wild beets) for vernalization. When plants are removed from vernalization, they are potted into 2-gallon pots and placed in greenhouse spaces isolated from other *Beta*. General maintenance is performed (daily watering, fertilizing, staking, and pruning), until seed harvest. For field grown accessions, plants are transplanted to the field in early to mid-May after vernalization or in September for overwintering in the field. Plots are spatially isolated by a minimum of 1.5 km. Seed harvest generally starts in mid- August and continues until October.

Increase/regeneration and collection management activities. From April 1, 2025 to March 31, 2026 we completed harvests from 28 accessions and have started harvest on 2 accessions and failed to meet our target number of 30 (15 accessions of *Beta vulgaris* ssp. *maritima*, 7 accessions of *B. vulgaris* ssp. *vulgaris*, 1 accession of *B. spp.*, 1 accession of *B. corolliflora*, 2 accessions of *B. trigyna*, 2 accessions of *B. macrorhiza* and 2 accessions of *B. lomatogona)*. This failure was due to the government shutdown, where several accessions were not managed correctly by the essential employees and the accessions failed to flower and are in the process of revernalizing them. For the accessions harvested, our plant populations ranged from 2 to 7 plants with our difficult species and 21 to 110 plants for *Beta vulgaris* accessions. The accessions with low seed production are revernalized and regrown for further harvest if they are not annuals. In addition to seed production activities, we took images and descriptor data on the above accessions grown for seed increase and the WRPIS seed germination technician tested 210 inventories for viability. All the above work was accomplished with the support of 2 student employees, hired with BSDF funding, who worked for us between April 2025 to March 2026.

One of the main missions of the NPGS is seed distribution for research and education. During the above time period, WRPIS sent 432 *Beta* seed packets in 20 orders to both domestic and foreign requestors. For all taxa managed by the WRPIS, 15,091 seed packets in 475 orders were distributed from April 1, 2025 to March 31, 2026.

POLLEN ISOLATION TENTS FOR *BETA* GERMPLASM REGENERATION IN THE FIELD
BSDF Project 281: Executive Summary

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Background. The U.S. Beta germplasm collection is maintained at the Western Regional Plant Introduction Station, part of the USDA-ARS National Plant Germplasm System (NPGS) that acquires, preserves, evaluates, documents and distributes plant germplasm for use by the plant breeding, research and education communities. Currently the NPGS *Beta* collection has 2,755 accessions of sugar, table, and fodder beet, chard, sea beet and other wild *Beta* relatives. Sixty-eight percent of the collection is available for distribution based on seed quantity and/or quality. The remaining 32% needs to be increased/regenerated before it can be distributed for use. At the current rate of regenerations (30 / year), it will take 30 years to regenerate and increase the current backlog. Seed increase and regeneration are integral parts of maintaining this valuable resource for the future, but due to the nature of Beta species being wind pollinated with a very fine pollen size in wild species (15- 20 µm) regenerations have been limited to physically isolated greenhouses and spatially isolated field plots.

Progress on this project was delayed due to difficulties in logistics in obtaining the isolation tunnel frames from Burley, Idaho. There were inconsistencies in the reported dimensions of the tunnels as well, so ordering materials was delayed until we could ensure the correct materials were obtained.

Isolation tunnel frames. In September 2025, we drove a flatbed truck from Pullman, WA to Burley, ID to pick up isolation tent frames from Magno Seed, formerly SESVanderhave, that were graciously donated to our project. We are currently in the process of ordering polyethene covers for the frames as well as 15 micron mesh fabric for use as end caps for each tunnel to prevent pollen from drifting to other plots.

Plant material preparation. Seeds of 15 accessions *Beta vulgaris* subsp *vulgaris* have been germinated and transplanted into root trainers. To ensure bolting this summer, all the accessions have been placed into a vernalization chamber set to 4°C for at least 4 weeks. Planting to the field is scheduled for the middle of May and irrigation manifolds will be assembled for each test plot. Sorghum Sudan grass will also be planted in 6 foot strips in front of the tunnels to act as a second layer of protection from pollen movement outside of the tunnels. Tent frames will be assembled over the plots after 4 weeks in the field and polyethylene covers will be secured over each tunnel. Fine mesh end caps will be secured at the same time to prevent pollen movement out of the tunnel.

GENOMIC CHARACTERIZATION AND DEVELOPMENT OF MOLECULAR MARKERS IN A NEW GENETIC SOURCE EXHIBITING STRONG RESISTANCE AGAINST BEET CURLY TOP VIRUS

BSDF Project 342: Final Executive Summary Report

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Sugar beet is highly susceptible to the beet curly top virus (BCTV) which significantly reduces sugar yield in semi-arid sugar beet growing regions. Insecticide seed treatments with neonicotinoids have been a primary management option to supplement host resistance but the use of neonicotinoids is gradually being restricted worldwide. Genetic resistance against BCTV in commercial cultivars is low to intermediate. This project aimed to generate a high-quality, contiguous genome assembly of the sugar beet line CTN_2, putatively a novel source of BCTV resistance. Chromosome-scale and haplotype-resolved assemblies were generated using PacBio HiFi long-read sequencing data along with OmniC chromatin conformation capture data. The combination of HiFi and OmniC enabled construction of highly contiguous assemblies with accurate phasing and scaffold ordering, ensuring a robust representation of both haplotypes (Table 1). A representative synteny analysis of chromosome 3 (possessing major QTL for BCTV resistance in a double haploid line KDH4-9; unpublished) is presented in Figure 1. Overall, the haplotype resolved assemblies are highly syntenic and have high concordance between each other, suggesting high homozygosity. This degree of homozygosity may suggest a singular; primary assembly may be sufficient to track the genome scale variation in CTN_2. Further analysis is being performed to identify any gene content variations or structural variations responsible for BCTV resistance in the CTN_2 line.

Metric	Haplotype 1	Haplotype 2
Assembly Size (Mb)	614.4	630.2
# Contigs	71	49
Contig N50 (Mb)	15	26.7
# Scaffolds	9	9
BUSCO (%)	98.2	98.50

Table 1. Genome assembly metrics for the resolved haplotypes of CTN_2.

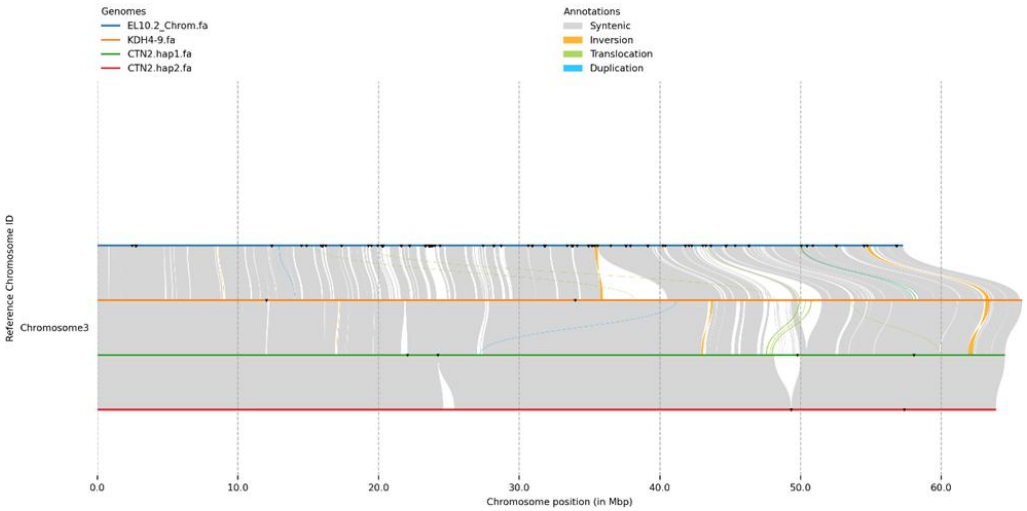

Figure 1. Synteny Plot of Chromosome 3. Genome-wide synteny displays collinearity with the reference genome (EL10.2) and another closely related BCTV resistant line KDH4-9.

SYSTEMATIC PREDICTION OF EMS-INDUSCED MUTATIONS IN A SUGAR BEET MUTANT POPULATION IN KIMBERLY, ID
BSDF Project 350: Annual Report

Eric Vincill[1], Imad Eujayl[1], Carl Strausbaugh[1], Rajtilak Majumdar[1], and Kevin Dorn[2]
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Identifying and tracking new and novel crop enhancing traits in elite germplasm is an important strategy for agricultural breeding programs to deliver superior seed to the farmers. It is frequently done by using naturally occurring mutations (wild accessions) or inducing mutations with radiation or chemicals. Subsequent breeding then takes place, mutant individuals are isolated, and the causal gene/variants are mapped to specific loci in the genome. The utility of this *forward genetics approach* (or classical genetics approach) used to identify and genetically track agronomically important traits in crops has historically been hampered by the inefficiency in linking loci changes in the genome to the desired phenotypic changes. However, once crop enhancing traits have been *identified and directly linked* to specific loci in the genome, a tractable system is established which allows a crop breeding program to efficiently and predictably introgress these crop enhancing traits into appropriate product releases.

Objectives. The objectives are to 1) deliver to the sugar beet crop breeding community a predictive database of all the genes that were impacted (changed/disrupted) in a Kimberly EMS mutagenesis sugar beet population and 2) group these allelic changes based on how impactful these changes are inside active/critical genes in the plant: a) *Severe* (premature stop codon/gene disruption), b) *Moderate* (amino acid change of the encoded protein), and c) *Mild* (no amino acid change)

Materials and methods. The EMS mutagenized population that will be used in this study has already been generated in Kimberly (22 lines including the parental non-mutagenized reference line) and selfed-pollinated for at least 4 generations. Twenty-five plants from each of the 22 individual lines will be grown under greenhouse conditions. Leaf material from the first set of true leaves will be isolated and subjected to standard plant genomic isolation methods. Genomic DNA will be quantitated and standardized across all samples. In collaboration with Dr. Rachel Naegele (USDA-ARS, East Lansing, MI), these 22 individual lines will be DNA sampled and analyzed for unique SNPs on the sugar beet 4K SNP chip platform that Naegele has designed.

Results (2025-2026 year). In collaboration with Dr. Rachel Naegele (USDA-ARS, East Lansing, MI) who is developing a comprehensive next generation sugar beet 4K SNP Chip platform in order to systematically 'fingerprint' USDA sugar beet accessions with observable phenotypes (traits). The updates for this year are that the 22 Kimberly EMS lines will be analyzed on the 4K SNP Chip platform and tested in the field (2026 field season) for Rhizoctonia resistance with the objective to identify unique SNPs that are closely linked to Rhizoctonia resistance.

While the original goal of this project was to identify all the EMS-induced mutations in a sugar beet mutant population generated in Kimberly, ID. This goal did not have the capacity to directly link SNPs to desirable traits. By switching to the comprehensive next generation sugar beet 4k SNP Chip platform to Dr. Rachel Naegele is developing, linking phenotype to genotype will be far more attenable and cost affective for the sugar beet community.

DEVELOPMENT OF NOVEL BIOBASED ANTIMICROBIALS FOR SUGAR BEET BACTERIAL CONTAMINATION CONTROL

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The United States is the world largest sugar producer and nearly all sugar beets grown are used for sugar production accounting for 55% of the sugar produced and 1.1 million acres planted. Post-harvest bacterial rot and slime formation of sugar beets attributable to *Leuconostoc* species can result in yield losses exceeding 50% in sugar beet production. Furthermore, *Leuconostoc* spp. infection can substantially reduce root sucrose level and overall crop tonnage which further diminishes the quality and market value of harvested sugar beets. Current post-harvest bacterial mitigation methods remain minimal with heavy reliance on physical intervention for controlling bacterial contamination of sugar beets.

Endolysins offer a promising natural approach for antimicrobial control with high specificity for *Leuconostoc* spp. and minimal off-target effects unlike traditional antibiotics. Endolysins are a type of enzyme that attack and degrade the cell walls of specific bacteria. The novel approach of using endolysin technology can easily be used for sugar processing. It also has the potential to be used for pre- and post-harvest contamination. Endolysins have not shown development of bacterial resistance and thus provides an attractive solution for long-term sustainable control against sugar beet bacterial pathogens.

The objectives of this research are to 1) identify new endolysins by searching available genome sequences of sugar beet bacterial pathogens, and 2) develop the newly identified endolysins into a treatment against sugar beet bacterial pathogens to prevent root rot, slime formation, and sucrose loss in sugar beets, thereby improving yield and quality during storage and processing.

This research identified 18 potential endolysin sequences found from the genomes of sugar beet bacterial pathogens. Fourteen of which were synthesized for preliminary testing. We successfully produced 6 endolysins and of these, 4 endolysins were effective against *Leuconostoc* species such as *L. mesenteroides* and *L. suionicum*. Future research will expand testing of these endolysins to include additional *Leuconostoc* species isolated from diseased sugar beet root tissues.

OPTIMIZATION OF DNA-GUIDED ARGONAUTE NUCLEASES FOR REAL-TIME DETECTION OF *CERCOSPORA BETICOLA* VIRULENCE IN A FIELD SETTING

Joshua Chappie
USDA-ARS, Fort Collins, CO

Cercospora Leaf Spot (CLS), caused by the fungal pathogen *Cercospora beticola*, is an economically damaging pathogen in all sugar beet production regions. Successful management of CLS relies heavily on timely and consistent fungicide applications throughout the growing season, which is complicated by the emergence of *C. beticola* strains that are resistant to most fungicide chemistries in use. The ability to detect *C. beticola* and quickly assess its virulence and resistance properties is therefore crucial for managing disease outbreaks and minimizing economic loss. Current diagnostic approaches suffer from issues with sensitivity and/or require expensive instrumentation and technical expertise, which makes them largely inaccessible to growers and agriculturists. Although laboratory analysis of samples off site can provide a detailed description of the pathogen population in a specific area, the time required for this processing can delay fungicide applications and negatively impact the overall efficacy of the treatment over the course of the growing season. This project to develop a cost-effective diagnostic kit that can detect *Cercospora* in the field and provide accurate information about strain virulence and fungicide resistance to inform management strategies in real-time. Our approach employs programmable DNA-guided Argonaute (Ago) nucleases and fluorescent reporters to provide genotypic analysis of field samples using a minimal number of thermostable reagents. By combining different DNA guides and probe sets, our assay can be readily adapted to address other region-specific diseases and can be multiplexed to detect other sugar beet pathogens (e.g., beet curly top virus, beet western yellows virus, *Alternaria*, etc.) simultaneously. The programmable nature of Ago enzymes also affords the opportunity to regularly update DNA guide sequences to allow detection of new variants/mutations as soon as genomic and epidemiological data become available. We have thus far purified the thermostable Ago enzyme from *Pyrococcus furiosus* (PfAgo) and confirmed its activity in the laboratory. We have additionally optimized primary DNA guide sequences that target the PfAgo nuclease to unique regions of the *C. beticola* genome and are optimizing fluorescent reporters to enable real-time analysis of samples using a portable, low cost/high sensitivity fluorometer. Successful completion of our objectives will provide growers, agronomists, and pathologists a novel tool to obtain a real-time read out describing the presence, virulence, and fungicide resistance of *Cercospora* in any field, thereby improving management decisions and reducing CLS disease spread.

Pre-breeding for tolerance to the sugar beet cyst nematode and understanding the diversity of tolerant sources

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Executive Summary

We are continuing to develop genotyping approaches that lay the groundwork for mining useful resistance from wild sea beet including cyst nematode resistance In 2026, we demonstrated that whole-genome pooled sequencing provides a scalable, cost-effective framework for characterizing heterogeneous germplasm. Using 5,000 individuals across the primary *Beta vulgaris* gene pool, we established analytical pipelines that directly connect digital sequence data to physical gene bank accessions.

Major Outcomes

Wild germplasm diversity quantified. A geographically representative *B. vulgaris ssp. maritima* panel captured the highest allelic diversity within the primary gene pool and contained numerous private alleles absent from modern U.S. sugar beet breeding materials.

Improved genomic resources for wild beet. We developed and released a high-quality reference genome for *ssp. maritima*, comparable to the EL10 sugar beet reference.

Resistance allele discovery. Allele mining at the Rz2 rhizomania resistance locus identified multiple putatively functional alleles. Functional variants were common in wild and table beet germplasm but comparatively rare in U.S. sugar beet breeding materials, highlighting immediate opportunities for strategic introgression and resistance diversification.

Genome-wide selection scans using gene bank data. By integrating pooled sequence data with curated phenotypes, we detected strong differentiation signals consistent with known loci controlling monogerm seed development.

Strategic Significance.

Whole-genome pooled sequencing establishes durable genomic infrastructure for heterogeneous germplasm collections. The approach:

- Reduces sequencing costs relative to individual resequencing
- Supports allele mining, core set design, and genome-wide scans
- Directly informs resistance introgression strategies

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BEETBASE: A TOOLBOX FOR SUGAR BEET GENETICS, GENOMICS, AND GERMPLASM RESEARCH

Kevin Dorn, Rachel Naegele, Eric Vincill, Evan Long, Chenggen Chu, Carl Strausbaugh, Linda Hanson, Alex Cornwall, Olivia Todd, and Samuel McNeill

This project aims to establish BeetBase as a comprehensive, centralized "one-stop -omics shop" for the public sugar beet research community. The platform is designed to be a unified, web-based tool for genetics, genomics, and germplasm research, with its development directly addressing key gaps in current information infrastructure identified by BSDF member organizations. BeetBase is intended to serve both public and private researchers engaged in research spanning the diverse disciplines of breeding, genetics, genomics, and phenomics. Collectively, this project represents a grassroots initiative to preserve raw publicly produced datasets and enable new insights to be extracted from ‘legacy’ large scale phenotyping efforts.

In the 2025-2026 proposal cycle, a proposal was submitted to BSDF seeking first time support to continue the development of BeetBase tools and datasets. Two objectives were proposed, 1.) Curly Top, Rhizomania, and Cercospora phenotypic dataset curation, tool development, and deployment and 2.) Deploy a genetic variant mining toolkit enabling researchers to find germplasm lines with specific alleles of interest using publicly available genome sequence datasets. Due to partial funding of the 2025-2026 proposal, the project's scope was necessarily adjusted, and Objective 2 was put on hold.

To date, all components of Objective 1 have now been completed. The team has also navigated unanticipated administrative reviews that have impacted some of BeetBase's online functionality. As an interim solution to ensure continued community access, the R/Shiny code and raw data required to run the tools have been made available on the BeetBase website (beetbase.scinet.usda.gov) for users to download and operate on their local systems. As these administrative reviews are addressed and resolved, we anticipate full functionality will be restored to original capabilities and include all new datasets and tools developed over the past year. The project remains committed to its goal of publishing a manuscript to officially unveil the first complete version of BeetBase upon the completion of its foundational objectives.

PART III, EVALUATING THE EFFICACY OF MINIMUM INHIBIORY CONCENTRATION (MIC) VALUE ANTIMICROBIAL AGENT APPLICATION IN POSSIBLE COMBINATION WITH DEXTRANASE TO MICROBIAL ISOLATES TO MITIGATE SUCROSE LOSSES AND INCREASED VISCOSITY
BSDF Project 501: Executive Summary

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USDA-ARS, New Orleans, LA BSDF Project 501
Project Team: Gillian Bruni, Zianab Yassin, Evan Terrell
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Microorganisms are carried into sugar beet factories by infected roots and associated soil. Microbial growth in the sucrose-rich factory processing streams cause sucrose losses and downstream operational challenges during raw sugar extraction. This results in economic losses and operational inefficiency. Since the number and identity of microorganisms are like a moving target, constantly fluctuating, we took the approach of designing our research objectives using factory-derived bacterial strains to answer questions about the benefits and conditions of dextranase addition compared to optimized antimicrobial dosing.

We found that dextranase effectiveness against bacterial culture viscosity was largely temperature-dependent. Dextranase was most effective at 60ºC, followed by 50ºC and to a much lesser extent 28ºC. This temperature-dependence means that less dextranase is required at higher temperatures such as 50-60ºC while treatment at lower temperature was much less effective even at the highest concentrations. This may help to inform operators on optimal temperatures and points of addition during processing to add dextranase if indicated. Next, dextranase activity was also concentration-dependent and somewhat bacterial strain dependent. The lowest concentrations measured for *Leuconostoc* and *Weissella* strains treated at 60ºC were 2.5 to 10 ppm.

Further, with a few exceptions, the addition of dextranase did not improve the effectiveness of antimicrobials, meaning that dextranase and antimicrobial administration together would cost more without additional benefit. Next, we also determined that addition of dextranase (even in excess) together with optimal antimicrobial concentration did not provide additional benefit in terms of sucrose savings. Moreover, during comparisons of dextranase treatment vs. optimal antimicrobial treatment, the latter provided dramatic sucrose savings.

In conclusion, it should be noted that optimized dextranase treatment can be very effective at reducing viscosity caused by dextran-forming bacteria at elevated temperature at 60ºC. However, it has no effect on non-dextran bacterial exopolysaccharide products. In contrast, optimal antimicrobial dosing used prophylactically was effective at inhibiting sucrose losses caused by all bacterial strains tested.

INNOVATIVE NANOFIBER-BASED INSECTICIDE FOR SUSTAINABLE SUGARBEET ROOT MAGGOT CONTROL

Long Jiang[1], Mark Boetel[2], Chenggen Chu[3], Raj Hazra[1], Patric Beauuzay[4], Reed Thoma[2]

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This project developed a new, more sustainable pesticide to control the Sugarbeet Root Maggot, a major agricultural pest. By attaching a common insecticide (cypermethrin) to natural cellulose nanofibers, the team created a slow-release system that keeps the pesticide in place longer, improving effectiveness while reducing environmental impact.

Laboratory testing showed that the new material successfully kills maggots, with higher concentrations and longer exposure leading to stronger results. In some tests, nearly all larvae were eliminated within 48 hours, demonstrating both potency and sustained activity over time.

These findings suggest the material could outperform traditional pesticides, which often degrade quickly or wash away in soil. The fiber-based system acts like a “nanonet,” allowing for longer-lasting protection and potentially lower environmental contamination.

Next steps include refining soil-based testing, scaling production, and conducting field trials. If successful, this approach offers farmers a more effective and environmentally responsible way to protect crops.

FIELD VALIDATION OF A COMPREHENSIVE RISK MODEL FOR CERCOSPORA LEAF SPOT OF SUGARBEET

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EXECUTIVE SUMMARY

The purpose of this project is to collect data tied to in-field trial results in order to update and improve the Cercospora leaf spot risk model currently used in North Dakota, Minnesota, and other regions of the United States. Cercospora leaf spot (CLS) caused by the fungal pathogen, *Cercospora beticola*, continues to limit economic returns for growers and cooperatives warm and humid areas of sugarbeet production regions. Due to the need for robust data analysis and sample sizes, this project will continue in the 2026 crop year thanks to support from the Beet Sugar Development Foundation.

Objectives:

1) Conduct field-scale validation of an updated Cercospora leaf spot risk model incorporating free water (dew formation and rainfall) in addition to current parameters of temperature and relative humidity.
2) Compare the efficacy of fungicide programs utilizing the updated CLS model to the currently available risk- and calendar-based fungicide application timings.

Summary of results:
Two field trials were evaluated in 2025, at Kragnes, MN and Prosper, ND. Overall, CLS disease pressure was low in these non-inoculated trials at these two locations due to relatively low rainfall. Fungicide programs initiated at row closure performed well to the nontreated plots, but were not significantly different from daily infection value (DIV)-based models incorporating environmental variables. Research and analysis continues on 1) whether DIV-based models offer improvement of calendar-based fungicide programs and 2) how updates to the DIV-based model can better reflect CLS epidemiology including latent CLS infections, free water (dew or rainfall) and additional data supporting a trend of earlier fungicide applications for optimal recoverable sugar.

THERMAL WEEDING FOR HERBICIDE-RESISTANT WEED CONTROL

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Executive Summary:
On-the-go weed detection and hot water treatment was proposed and evaluated on a thermal spot weeding robot for herbicide-resistant weed control. Initial efforts were focused on three main developments: robotic platform design, weed detection, and spray control. The robot was designed to integrate intelligent sensors and spray controller with a thermal device, carrying two cameras, a global positioning system (GPS), and artificial intelligence (AI)-capable brain for sensing and navigation, an on-demand water heater, and a sprayer. The robot was operated in auto track mode where path planning is implemented through global positioning with a mission to perform weed identification and thermal treatment.

AI-enhanced image processing algorithm was developed to detect weed pixels from background soil and crop canopy in images captured by the robot. Weed detection was evaluated by using a machine learning model and computer vision algorithms. Images were first processed to detect target weeds by fine-tuned conditions and refined through noise filtering, resulting in each weed pixels in a bounding box (bbox) in three columns matching to nozzle locations. The bbox was further grouped with near neighboring bboxes and stretched to the column width. The detected weed bboxes were converted to GPS coordinates so that spray nozzles can be activated only where the weeds are present. The nozzle activation was conditioned by geofencing of the bbox, i.e., turning a nozzle on if the nozzle is inside the bbox. The algorithm was evaluated with green flags in the lab and successfully validated for timely nozzle responses. Evaluation continues to quantify the accuracy of weed detection and nozzle spray in crop fields with natural weeds.

For thermal spray weeding, mechanism of heat energy supply was designed and implemented. Water in a water tank is fed by a water pump to the on-demand water heater that instantaneously heats up the water to the boiling temperature. Solenoid valves are activated through a relay to individually turn the nozzles on and off corresponding to the weed detected by the front camera. The 25-gal water tank and three nozzles are for the prototype and can be upscaled to a larger tank and more nozzles after the initial validation. AI-driven thermal spot weeding robot integrated with safeguarded navigation will create new opportunities with data-driven management for digital agriculture and help reduce chemicals use by selectively applying hot water to only when and where they're needed.

This study demonstrated the potential of computer vision models for weed detection through methodical experimentation and preprocessing innovations. By refining techniques and adopting scalable solutions, the project is well-positioned to advance weed detection and spray control capabilities in future phases.

GENETIC CHARACTERIZATION OF GENES CONFERRING RESISTANCE TO SUGARBEET ROOT MAGGOT THROUGH NESTED ASSOCIATION MAPPING

Chenggen Chu
USDA-ARS, Fargo, ND

Sugarbeet root maggot (SBRM) is a major pest of sugarbeet in the United States and Canada that causes significant yield reduction. Growing varieties of tolerance to SBRM would be the most economical and environmentally friendly way for SBRM management. However, genetic control of SBRM resistance in sugarbeet remains largely unknown. Methods for selecting SBRM resistance in breeding mainly depends on the assessment of insect damage to roots under natural infestation in field nurseries. Such evaluation is time-consuming and not always reliable due to the non-uniform distribution of the insects in the field and varied insect pressure across years. Therefore, this research aims to conduct genetic studies about SBRM resistance and then develop markers linked to resistance genes to enable marker-assisted breeding. Currently, three sugarbeet lines F1024, F1016 and F1015 and one red beet line PI 179180 are only publicly available SBRM resistance sources for breeding. Resistance in F1024, F1016, and F1015 could be the same due to their same origin. PI 179180, however, may carry different resistance genes since it belongs to a different beet type. Therefore, this research focuses on using the nest-association method to detect resistant genes in lines F1024 and PI 179180. Previous work has developed two recombinant inbred lines (RILs) populations each initially included 300 RILs that used F1024 and PI 179180 as resistant parent, respectively. In 2025, we planted 223 RILs from population derived from PI 179180 and 200 RILs from population derived from F1024 in both locations near St. Thomas and Reynolds, ND. However, germination rate in St. Thomas is very low and the experiment was unfortunately abandoned. Germination in Reynolds is better, but insect pressure is too low in our plots, and many plants have no feeding injuries. Data collected from Reynolds thus is not suitable for gene identification. We currently have seeds from 185 RILs of population derived from PI 179180 and 178 RILs of population derived from F1024 that have sufficient seeds for growing in one field location in this year. For safe, we will grow one population in Reynolds and the other in St Thomas in 2026 and will evaluate and harvest roots in fall of 2026 for conducting genome-wide association study and amplify seeds for SBRM evaluation in 2027. Meanwhile, we will use greenhouse to increase seeds of RILs that each have low seed amount, and those RILs will also be included in the evaluation and genetic study in 2027.

IDENTIFY SUGARBEET ROOT MAGGOT RESISTANCE GENES IN WILD SEA BEET THROUGH GWAS

Chenggen Chu
USDA-ARS, Fargo, ND

Sugarbeet root maggot (SBRM) is a major pest of sugarbeet in the United States and Canada that causes significant yield reduction. Wild sea beet (*Beta vulgaris* ssp. *maritima*), the progenitor of cultivated beets, is an important gene resource for sugarbeet sustainable improvement. Through evaluating 300 sea beet accessions for tolerance to SBRM, a set of 32 accessions were considered tolerant to the insect, which proved the potential of using sea beet to improve sugarbeet SBRM tolerance. However, substantial phenotypic variation presents in most sea beet accessions indicate that significant genetic variation exists within the accession, which means current sea beet accessions in stock are mostly the mixture of multiple genotypes and are not suitable for genome wide association studies (GWAS) to identify resistance genes. This project was designed to purify sea beet accessions according to phenotypic observations by removing off type plants having appearances that differ from the majority of the plants in the accession or dividing the accession to subgroups if substantial portions of individuals showed different appearance, and then the purified accessions/sub-groups will be used for conducting GWAS. Purification in sea beet accessions is very difficult due to its open-pollination and self-incompatibility nature that requires keeping more than two plants for seed production, which unfortunately maintained a fair amount of genetic variation in each accession/sub-group even after a one-time purification. Therefore, adding additional rounds of purification is preferred and will improve phenotype accuracy in each accession/sub-group, which further improves accuracy of gene identification. In year 2025, our purification in sea beet regrouped 300 original accessions into 388 subgroups. After growing the subgroups in greenhouse, seeds were obtained from 356 subgroups with 314 having annual growth habit and 42 are biennial. To increase the number of biennial subgroups, we grew 300 original accessions in a field at Fargo, ND in 2025, and selected biennial plants from over 220 accessions followed by producing seeds in greenhouse. Our target is to obtain a total of about 500 subgroups that will give us enough flexibility to select 300 subgroups with about equal amount of annuals and biennials to form a diverse panel for conducting GWAS. Meanwhile, we started using bulk-segregation-analysis (BSA) to quickly identify the indicative genomic regions for SBRM tolerance. We used equally mixed seeds from 300 sea beet accessions and planted them at St. Thomas and Reynolds, ND in 2025, to select heavily and slightly damaged roots to form pools of a susceptible and a resistant, respectively, and all roots in each pool will be genotyped to identify resistant loci through BSA. However, experiment in St. Thomas was abandoned due to severe drought condition. Experiments in Reynolds obtained 108 plants in the susceptible pool and 84 in the resistant pool. Marker genotyping is ongoing, and BSA will be done in early May 2026. Overall, the project was conducted as planned, and we added bulk-segregation-analysis that will enable us to quickly obtain useful information and provide an additional way for verifying resistance genes. In 2026, we will do the second round of purification in sea beet subgroups and will repeat BSA experiments in St. Thomas and Reynolds, ND. This project will provide critical information for introgression of SBRM resistance from sea beet into sugarbeet and develop new germplasm for sugarbeet breeding.

Identification and characterization of pathogen effectors

Melvin D. Bolton
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Cercospora leaf spot (CLS), caused by *Cercospora beticola*, poses a severe economic threat to global sugarbeet production. This research investigated how *C. beticola* overcomes *BvCr4*-mediated resistance (CR+), demonstrating through a controlled passage study that the pathogen rapidly adapts under selection pressure. Specifically, isolates quickly lost the *CbAvrCr4* gene, which is critical for CR+-mediated resistance, by deleting a large (~45Kb) GC-rich genomic region where this gene resides. This finding reveals a mechanism by which *C. beticola* can swiftly circumvent CR+ resistance, presenting a significant challenge for sugarbeet breeding programs relying on *BvCR4*.

MOLECULAR BASIS OF FUNGICIDE RESISTANCE
BSDF Project 643: Executive Summary

Melvin D. Bolton
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Cercospora leaf spot (CLS), caused by *Cercospora beticola*, is the most damaging foliar disease of sugar beet globally. The disease is managed through a combination of resistant varieties, cultural practices, and timely fungicide applications, including systemic benzimidazoles, strobilurins, and triazoles. However, *C. beticola* isolates are increasingly developing resistance to these fungicides. Previous research identified mutations in the *CbCyp51* gene linked to resistance against the triazole tetraconazole, with specific codon usages at amino acid positions 144 and 170 influencing the level of resistance. Additionally, a polyketide synthase (PKS) gene involved in fulvic acid production was found to be induced by tetraconazole.

Expanding on this, recent research investigated genetic resistance to triphenyltin hydroxide (TPTH), an area previously unexplored. A preliminary study identified the fulvic acid PKS gene as significantly associated with elevated TPTH resistance. Subsequent work focused on characterizing the fulvic acid biosynthesis pathway. Mutants lacking the *flv1* gene, which encodes the PKS responsible for fulvic acid production, exhibited a remarkable increase in sensitivity to fungicides, showing 10-fold greater sensitivity to TPTH and nearly 1000-fold greater sensitivity to tetraconazole. This suggests that fulvic acid plays a crucial role in fungicide resistance, potentially by directly binding to fungicides or by acting as a protective shield for the fungal hyphae.

PROFILING MICROBIAL DIVERSITY IN THE SUGARBEET PROCESSING
BSDF Project 650: Executive Summary

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The U.S. sugarbeet industry is a vital economic pillar, contributing approximately $23.3 billion annually and accounting for nearly 55% of domestic sugar production. Pre- and postharvest farm operations and sugar extraction process in the factory face continuous challenges from pathogens, pests and microbial contaminants. Some of the big financial losses for the U.S. sugarbeet industry occur during the sucrose extraction process in the factory. A major driver of these financial losses is microbial contamination within the factory, where bacteria like *Leuconostoc* consume sucrose and produce impurities that disrupt juice purification and crystallization. Despite the clear financial and operational damage caused by these contaminants, there is a critical knowledge gap in sugarbeet processing microbiology, particularly regarding fungal contaminants. In this study, we aimed to profile fungal contaminants in raw sugarbeet juice at sugarbeet processing factories across the U.S. and a factory from Canada. By deep sequencing the fungal ITS rRNA gene, we characterized full spectrum of fungal contaminants in the raw sugarbeet juice which are primarily yeast species. Major yeast contaminants included *Tausonia, Mrakia, Candida, Pichia, Saccharomyces,* etc. which are known to metabolize sucrose and other carbohydrates. The knowledge of fungal contaminants present in the factory processing streams aids in developing management strategies that limit sucrose loss and technical challenges during sugarbeet roots processing.

DEVELOP ARTIFICIAL MICRORNA-BASED TECHNOLOGY, A NEW METHOD TO CONTROL BEET NECROTIC YELLOW VEIN VIRUS CAUSING RHIZOMANIA DISEASE IN SUGARBEET

Vanitharani Ramachandran
USDA – ARS, Fargo, ND

Rhizomania, caused by beet necrotic yellow vein virus (BNYVV) and transmitted by the soil-borne vector *Polymyxa betae*, continues to pose a threat to sugarbeet production. Although the disease is currently managed through resistance genes incorporated into commercial cultivars, the emergence of viral strains capable of overcoming host resistance has become an increasing concern. RNA silencing is a major antiviral defense mechanism in plants, and artificial microRNA (amiRNA) technology offers a highly specific and stable approach for engineering durable virus resistance. This project focuses on developing amiRNA-based approaches targeting viral genomic regions most susceptible to host-induced silencing.

We established a robust system for generating BNYVV-infected sugarbeet roots and constructed small RNA libraries from ELISA-confirmed infected plants. High-throughput sequencing and mapping of virus-derived small RNAs (vsiRNAs) across RNA1-RNA4 revealed consistent silencing "hotspots" over multiple timepoints. These hotspots reflect stable, targeted host RNA silencing responses to BNYVV infection. Viral sequences corresponding to these regions were selected for amiRNA construction. We have established sugarbeet transformation system now in place, we are advancing to generating stable-plant testing of the amiRNA constructs. To evaluate their antiviral efficacy, we completed the development of agrobacterium-compatible BNYVV infectious clones. Earlier versions lacked robustness, leading to reconstruction of the viral genome in a binary vector system, which is currently being assessed for infectivity. Given the increasing prevalence of resistance-breaking BNYVV isolates, we also generated infectious clones with resistance breaking potential to ensure that amiRNA constructs are evaluated against emerging viral strains.

This project has achieved with several key milestones, including detailed characterization of BNYVV-derived vsiRNAs and the host silencing response during infection. These foundational insights directly support the development of amiRNA-mediated viral targeting as an alternative for managing rhizomania. The anticipated outcomes include more durable virus control, reduced dependance on conventional resistance genes, and long-term improvements for enhanced and sustainable sugarbeet productivity.

IDENTIFICATION OF N^6-METHYLADENOSINE MODIFICATION ON BNYVV AND ITS EFFECT ON RHIZOMANIA DISEASE DEVELOPMENT

Vanitharani Ramachandran
USDA–ARS, Fargo, ND

Rhizomania caused by beet necrotic yellow vein virus (BNYVV), remains a major threat to sugarbeet production. This project investigates whether BNYVV genomic RNAs carry N6-adenosine modifications and how these marks may influence viral infection and disease development in sugarbeet.

We successfully established BNYVV infection in sugarbeet roots using infectious clones and virus infection confirmed by using ELISA. Immunoprecipitation of BNYVV RNA to capture N6-adenosine modifications was challenging due to the excessive presence of host RNA, that prompted to shift toward purifying intact virus particles to obtain enriched viral RNA.

Current efforts focus on optimizing protocol for BNYVV purification from infected sugarbeet roots. This will enable efficient recovery of viral RNA with reduced host RNA contamination. In parallel, we are developing N6-adenosine immunoprecipitation and high-throughput sequencing workflow to map N6-adenosine modification-sites across BNYVV RNA1-RNA4 by comparing with mock-inoculated roots as controls.

This workflow will clarify the role of RNA modifications in BNYVV infection and may inform future management strategies for rhizomania. The project is ongoing and scheduled for completion in 2026 with no-cost extension.

CERCOSPORA BETICOLA IN-SEASON ADAPTIVE POPULATION DYNAMICS IN RESPONSE TO MANAGEMENT PRACTICES
BSDF Project 693: Executive Summary (Final)

Nathan Wyatt
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Cercospora leaf spot (CLS), caused by *Cercospora beticola*, is an economically damaging pathogen hindering sugarbeet production. Management of CLS is reliant on fungicide applications with the timing and order of applied chemistries being critically important for effective management. During the 2023 Red River Valley growing season, 14 fields were sampled throughout the growing season by collecting leaf samples prior to fungicide application and subsequently sampled before subsequent fungicide applications. For all samples collected, molecular assays were conducted for strobilurin and triazole resistance to observe fluctuations in the prevalence of resistance surrounding fungicide applications. The mutations G143A (G143 = sensitive, A143 = resistant) in the strobilurin (QoI) target CytB gene and the E170 (resistant) and L144 (sensitive) mutations in the triazole (DMI) target CYP51 were used for this study.

Results of this survey showed that *C. beticola* field populations dynamically changed throughout the growing season and surrounding fungicide applications. The number of fungicide applications ranged from two to five applications per field with fields planted with CR+ varieties receiving one fewer spray on average than non-CR+ varieties. Due to the variety and timing of fungicide applications across the fields sampled, strict statistical analysis is not possible on this data. However, common features of each fungicide application program may be examined for potential trends that will be validated with data from additional years. The change in prevalence of each monitored mutation following specific fungicide chemistries is potentially a useful tool for assessing the selection pressures of fungicides and the impact of contrasting chemistries on the *C. beticola* populations.

Most notably, this data demonstrates that the population of *C. beticola* is far from static and management practices such as fungicide applications induce selection on the pathogen population. This highlights the complexity of CLS management as the *C. beticola* population is ever changing in response to management practices.

These preliminary results have been used to apply for funding through the Sustainable Sugar Beet Research Initiative to further examine the in-season dynamics associated with management of this complex pathosystem.

RESISTANCE GENE DIVERSITY IN PUBLICLY AVAILABLE GERMPLASM
BSDF Project 695: Executive Summary (Final)

Nathan Wyatt
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The objectives of this research project were to screen publicly available sugar beet and sea beet germplasm for close homologs to *BvCR4*. We successfully screened a total of 71 sugar beet and 514 sea beet accessions and identified homologs of *BvCR4* in 32 accessions. Eight homologs were identified in sugar beet accessions with five encoding functional proteins. In these sugar beet accessions, the percent identities to the reference BvCR4 amino acid sequence were 76% and 90%. In sea beat accessions we identified 25 *BvCR4* homologs having a range of 78 to 92% identity to the published BvCR4 amino acid sequence. By comparing mutations between the different countries of origin we can estimate a theoretical center of diversity for the *BvCR4* gene. The center of origin should be the population contributing the most identified homologs and would have the highest genetic diversity. Most of the accessions containing a *BvCR4* homolog were originally collected in Italy and Greece with a total of 16 accessions contributing to this population and ranged from 75 to 93% identity to the published *BvCR4* genetic sequence, the largest and most diverse population in this study. The next notable population was comprised of homologs contributed by Spain and Portugal with five accession having homologs. The genetic variability of this population was slightly narrower than the upper Mediterranean population with genetic identity to the published BvCR4 sequence ranging from 82 to 90% similar. The results of this work provide information that can be used to evaluate accessions for their utility in developing CLS resistant sugar beet varieties. The presence of a *BvCR4* homolog in sugar beet accessions provides a good starting point for these evaluations. Future investigations should examine whether these sugar beet varieties have been incorporated into the breeding structure for any commercial varieties available as this would indicate that the BvCR4 resistance may have been inadvertently deployed though with slight differences in the resistance gene. These results also indicate that the origin of the *BvCR4* resistance may be in the upper Mediterranean region and future efforts to collect new accessions of sea beet may focus on sampling these regions to obtain the most diverse individuals for resistance evaluation. Finally, the range of genetic and amino acid identity to the original *BvCR4* reference indicates that *BvCR4* diversity may be a useful route of inquiry to identify CLS resistance traits that could be stacked with existing *BvCR4* genes to provide more durable resistance. The results of this work were recently accepted for publication in the journal Plant Disease under the manuscript title “High natural variation of *BvCR4* homologs in sea beet contrasts with narrow genetic base of sugar beet: Implications for Cercospora leaf spot resistance”. Additional results of this project can be found in the publication.

COMPETITIVE FITNESS ASSAYS: DIRECTLY MEASURING FITNESS PENALTIES ASSOCIATED WITH *CERCOSPORA BETICOLA* ADAPTATION TO MANAGEMENT PRACTICES

Nathan Wyatt
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Cercospora Leaf Spot (CLS), caused by the fungal pathogen *Cercospora beticola* (*Cb*), is an economically damaging pathogen in all sugar beet production. CLS is typically managed with a combination of cultural practices, timely fungicide applications, and resistant sugar beet varieties (Rangel et al. 2020). Each management practice represents a selection pressure exerted on the *Cb* population and *Cb* must adapt to succeed in the agricultural environment. Adaptive mutations, such as mutations conferring fungicide resistance, are often accompanied by tradeoffs in relative fitness. Fitness penalties may reduce the selective advantage of resistance without fungicide treatment, potentially leading to reversals of resistance evolution given sufficient time (Hawkins and Fraaije 2018).

We proposed establishing and validating head-to-head competition assays to obtain direct measurements of fitness and fitness penalties for *Cb* isolates *in-planta*. By co-inoculating *Cb* isolates at equal spore volumes and quantifying the amount of CLS disease attributed to each isolate after symptom development, we aim to provide direct quantitative measurements of relative fitness for *Cb* isolates with adaptive mutations. Furthermore, by combining competition assays with serial passage experiments, we can estimate the number of generations necessary to shift the pathogen population towards sensitivity in the absence of the selection pressure and/or towards resistance with exposure to the selection pressure (fungicide or sugar beet variety).

In this first year of the project, we successfully verified that we can accurately measure differential proportions of *Cb* isolate DNA as a proxy for *in-planta* fitness. By co-inoculating strobilurin sensitive and resistant isolates at equal spore ratios, we observed that strobilurin sensitive isolates appear to have an *in-planta* advantage over their strobilurin resistant counterparts. Another important finding is that background genetics play an important role in *Cb* isolates relative fitness as we observed with one sensitive isolate that was less competitive compared to all other isolates tested against it. We were further able to confirm that the competition assay can measure competitive fitness on different host varieties. By co-inoculating a CR+ adapted and non-adapted isolate on susceptible and CR+ sugar beet varieties we observed that the CR+ adapted isolate dominated the recovered *Cb* DNA on the CR+ variety. However, on the susceptible sugar beet variety, the non-adapted isolate was more competitive than the CR+ adapted isolate, potentially indicating a fitness penalty associated with CR+ adaptation.

Moving forward we intend to evaluate a wider variety of strobilurin resistant and sensitive isolates to further resolve the fitness penalty associated with resistance. We also are moving forward with evaluating fitness penalties associated with resistance to benzimidazole and triazole compounds. Due to the genomic location of resistance mutations for benzimidazole and triazole resistance, we can generate lab strains with resistance mutations to compare to the wild type counterparts, thus eliminating variation associated with background genetic variation. We also intend to evaluate competitive fitness under fungicide selection moving forward.

SEEDLING DISEASES OF SUGAR BEET
BSDF Project 728: Executive Summary

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Project also funded with support from Michigan Sugar.

To better understand the current pathogens causing seedling damage, a renewed survey of Michigan sugar beet fields was started in 2023. Seedlings showing damping-off or symptoms such as yellowing and stunting were collected from both commercial fields and research plots throughout the state. In the current survey, *Fusarium* spp. were the most frequently isolated pathogens. They were found in a similar proportion of fields as in a survey from 2008 to 2014, averaging isolation from 60 to 70% of fields. In addition to *Fusarium* spp., two other pathogen genera were consistently detected from multiple fields including *Rhizoctonia solani* (35 to 45% of fields) and *Aphanomyces cochlioids* (25 to 35% of fields). In this survey, *Alternaria alternata* has been isolated at a much higher level than either in the prior survey or other published surveys, where it was found in 1-2 fields, 1-2 plants. The first two years it was found from approximately 45% of fields, but in 2025 was identified from 70% of fields sampled, with more than one plant having the fungus from the majority of these fields. The *A. alternata* isolates tested caused stunting, yellowing, and delayed growth but did not kill any beets in greenhouse tests. This needs some further confirmation to determine how widespread this might be. Isolates of all three other commonly isolated genera killed some seedlings. Based on the prevalence of *Fusarium* spp., the current focus is on response to this group. As previously reported, a larger number of species were found than are reported on adult beets in the field. Tested isolates of *F. oxysporum, F. acuminatum*, and *F. avenaceum* all caused seedling death on two beet varieties.

Project 751

SUGAR BEET HOST RESISTANCE TO *BOTRYTIS CINEREA* DURING POSTHARVEST STORAGE

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USDA ARS, East Lansing, MI

Summary: Sugar beet germplasm showed little variation in susceptibility to *Botrytis* during postharvest storage tests after 16 weeks of cold storage. Of the *Botrytis* isolates evaluated, little variation in disease severity was observed across genotypes

Postharvest storage is the major source of loss in sugarbeet production. Fungi collected from beet piles consistently show *Botrytis cinerea* as a major postharvest storage pathogen. Little work on the diversity of *Botrytis* in storage piles or on sugar beet resistance to the pathogen has been don since that time. Evaluating diversity of the pathogen can provide important information on pathogen variability and inform screening methods for host resistance. This information is critical for improving management strategies and breeding *Botrytis*-resistant beets.

Botrytis diversity by sampling. Only 15 isolates of *Botrytis* were collected from piles during the 2023-2024 processing campaign. Additional isolates (6) collected from beet by Dr. Hanson prior to 2023 as well as isolations of *Botrytis* from stored beets from the 2023-2024 field season will also be included. Diseased beets collected during the 2024 – 2025 processing campaign are also being sampled to isolate *Botrytis*. Collected isolates are being cleaned to ensure each culture represents a single genetically pure isolate. DNA has been extracted from all genetically pure isolates collected to date. Genes related to genetic diversity testing, fungicide resistance, and virulence have been amplified.

Postharvest evaluation of beets. A single isolate of *Botrytis* was used to evaluate disease severity across 10 genotypes of beet. These genotypes were evaluated after 0, 4, 8, 13 and 17 weeks of cold storage to detect potential changes in disease susceptibility during storage. Three of the genotypes were further evaluated for transcriptional changes during cold storage. None of the sugar beet genotypes evaluated were resistant to *Botrytis* at any time point evaluated. This included genotypes that had previously been classified as having “good storage”. A slight increase in disease susceptibility was observed over time in 2023 and 2024.

An additional nine isolates of *Botrytis* were used to evaluate disease symptoms across 11 sugar beet genotypes. Due to limited root availability, not all genotypes were evaluated against each isolate. Only one isolate, B012-6, caused little to no disease on sugar beet roots. All remaining isolates caused visible disease on all sugar beet genotypes. Images of each root were collected and are currently being analyzed for disease severity (lesion area and lesion depth). Visible differences in disease severity were evident across hosts and isolates.

Project 752

CHANGES IN METABOLITES ASSOCIATED WITH SEEDLING RESISTANCE TO *RHIZOCTONIA SOLANI* IN SUGAR BEET ROOTS DURING GROWTH AND ESTABLISHMENT

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Summary: Field studies were conducted in 2025 at the Saginaw Valley Research and Extension Center to evaluate seedling Rhizoctonia disease severity using 11 breeding lines including five new populations based on crosses made from field selections in 2023. Disease scores varied within each population, indicating that the trait was still segregating. Root samples were collected from four of the beet lines for identification of semi-targeted metabolites.

Seedling diseases continue to be a problem in major beet production regions in the US. *Aphanomyces*, *Pythium*, and *Rhizoctonia* have historically been the primary seedling pathogens, but more recently *Fusarium* has been growing in frequency as a seedling pathogen. Root development in sugarbeet is known to undergo large organizational and transcriptional changes between 4 and 6 weeks post planting and this coincides with a decrease in disease sensitivity in sugar beet lines with adult plant resistance or tolerance. Phytoalexins are antimicrobial metabolic compounds produced by the host plant's defense system in response to a stress. In adult beets, metabolites such as the phytoalexin betavulgarin were present after infection with *Rhizoctonia* in resistant sugar beets. In seedlings with adult RRCR resistance, this phytoalexin was not detected when challenged with a fungal elicitor. It has been suggested that seedling sensitivity to *Rhizoctonia* may be associated with a lack of phytoalexin response.

Seedling rhizoctonia resistance. Eleven breeding lines including SR98/2 (with seedling and adult resistance) two seedling susceptible but adult resistant (FC709/2 and FC724), and two seedling and adult Rhizoctonia susceptible accessions (EL-A009983 and 95HS3) were planted in the field for seedling Rhizoctonia assays. An additional five breeding lines derived from seedling Rhizoctonia selections made in 2023 were included. Plots were inoculated with *R. solani* isolate R1. For four genotypes with good germination, seedlings were collected weekly from 0 to 6 weeks post inoculation, flash frozen in liquid nitrogen and stored in the -80 °C. Each sample was evaluated for disease based on a 0 to 5 scale (0 = no visible symptoms; 5 = dead root). For inoculated samples, five seedlings were collected. For control plants, three roots were collected.

For the five new populations, roots with a disease score of 3 or less at harvest were collected and stored in a cold room before planting in the greenhouse. Plants are currently growing in the greenhouse at Michigan State University for seed production.

Metabolite selection. Dr. Ilce Medina Meza has left MSU, pursuing an administrative role elsewhere. Per discussion with Dr. Anthony Schilmiller at the MSU Mass Spec facility, we are in the process of growing and inoculating beet seedlings in the greenhouse for an untargeted Q-TOF test run to identify potential differentially present metabolites beyond the subset listed in the previously recommended targeted approach. Root samples collected from the 2025 field season are currently stored in the -80 °C and awaiting processing pending test run results.

STORAGE ROT OF SUGAR BEET AND INFLUENCE OF GROWING CONDITIONS ON DISEASE

Jaime Willbur[1] and Linda Hanson[2]
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In Michigan, sugarbeets are stored for up to 200 days post-harvest, resulting in lower sugar yield due regular energy use from respiration, with additional losses if rot develops. Three major fungal rot pathogens have been identified in this region. Previous work has demonstrated variability among commercial varieties and experimental germplasm in their responses to these pathogens, indicating potential for genetic improvement; however, additional evaluation is needed to better inform breeding and storage management strategies. To address this, experiments using inoculated sugar beet root slices were conducted over two years to assess varietal responses to key storage pathogens. No single variety consistently performed best or worst; however, among eight varieties evaluated across both years, two repeatedly exhibited lower-than-average rot severity, consistent with reduced rot observed in commercial whole-root storage assessments by Michigan Sugar Company. Complementary research evaluating the impact of pre- and post-harvest factors on storage rot susceptibility found that mechanical damage at harvest significantly ($P < 0.05$) increased rot length in two of three years and influenced symptom development at multiple timepoints, underscoring its importance as a key driver of post-harvest losses. The combined effect of Cercospora leaf spot and mechanical damage showed a marginally significant ($P = 0.05$-0.06) increase in rot severity in one variety and trial year, suggesting potential interaction effects under certain conditions. Ongoing evaluations continue to highlight the importance of environmental conditions and in-season management. Current efforts, including microscopy-based studies, are focused on identifying physical and physiological traits associated with varietal differences in damage susceptibility and increased vulnerability to storage pathogens under severe in-season stress.

MONITORING AND CHARACTERIZATION OF NEW AND EMERGING FOLIAR PATHOGENS OF SUGAR BEET

Jaime Willbur[1] and Linda Hanson[2]
[1]Michigan State University and [2]USDA-ARS, East Lansing, MI

Domestic sugar beet production is challenged by a variety of diseases and pests impacting yield, quality, and storability. Reports of increased prevalence and fungicide resistance for the historically minor disease, Alternaria leaf spot, has indicated a need for improved management. This study aims to characterize current *Alternaria* species and other new and emerging fungal pathogens impacting Michigan sugar beet crops. Isolates were collected from symptomatic sugar beet leaf samples and were characterized using a leaf assay to measure virulence. A spiral gradient screen was also conducted to assess responses to several common fungicide classes in artificial media. In 2025, *Alternaria alternata* (n = 50) and *Cercospora beticola* (n = 80) isolates were tested for sensitivity to difenoconazole, mefentrifluconazole, prothioconazole, tetraconazole, pyraclostrobin, triphenyltin-hydroxide, and thiophanate methyl. The majority of *in vitro* screened sensitivities to difenoconazole and triphenyltin hydroxide were below the respective sensitivity thresholds for both *C. beticola* and *Alternaria* spp., supporting continued use for leaf spot management. However, the high prevalence of thiophanate-methyl resistance in *Alternaria* spp. (98%) and *C. beticola* (76%) isolates indicates this product may be ineffective in foliar programs. Based on the sensitives monitored from 2021-2025, recent isolates of *A. alternata* and *C. beticola* were significantly more resistant to several of the DMI active ingredients including mefentrifluconazole, tetraconazole, and prothioconazole. This observation followed the regional loss of CR+ resistance in 2024, where severe epidemics occurred amid extended fungicide intervals of 3-4 weeks. Though foliar observations remain limited, a small collection of Michigan *Colletotrichum* spp. isolates has also been assembled, and fungicide screening is in progress. These studies will help to inform in-season management considerations for important sugarbeet leaf spot pathogens.

Genome editing elite sugar beet germplasm for enhanced fungal and pest resistance with sugar beet genes BvPGIP and BvSTI
BSDF Project 811: Executive Summary

Vincent Klink
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The sugar beet root maggot is native to the U.S. It is capable of destroying an entire crop. The problem is made worse by earlier spring-like temperatures that make replanting non-feasible. The availability of specific types of resistant germplasm makes understanding its nature possible and important, especially with a U.S. value of greater than $1 B.

Prior experiments have identified two sugar beet genes that are expressed in resistant lines. The sugar beet genes were described as a polygalacturonase inhibiting protein (PGIP) and a serine protease inhibitor (SPI) that belongs to a class of Kunitz trypsin inhibitor (KTI) proteins. PGIP and KTI belong to classes of proteins that are known to inhibit polygalacturaonase (PG) and trypsin (Trp), respectively, proteins that pathogens use to infect plants. This fact made sequencing the SBRM genome of utmost importance since it would provide gene sequence information that could relate the 3-dimensional structures of the sugar beet and SBRM proteins. The SBRM genome, consisting of 28,276 genes, was identified as having both PGs and Trps. Complementary analyses were performed that identified the entire sugar beet PGIP and KTI gene families. This analysis allowed for the design of computer-assisted experiments that depict the lock-and-key interaction occurring between the sugar beet resistance protein and the SBRM pathogenicity protein. By doing so, experiments could be designed to express the sugar beet resistance gene to high levels in order to accomplish washing out the effect of the SBRM pathogen protein.

Experiments on a related experimental crop that also examined a root pathogen as a model identified its own PGIP and KTI gene families. Unlike the original experiments on sugar beet, the experiments were more precise, demonstrating that the genes were expressed in specific root cells undergoing a resistant reaction to the pathogen. The results provided experimental support to the similarity of the resistance process to that found in sugar beet-SBRM pathosystem. Experiments then transgenically-expressed the PGIP11 gene in the experimental model, in a line that is normally susceptible to the root pathogen, a parasitic nematode. The result was roots that suppressed infection by 75-77%. In contrast, inhibiting PGIP11 gene function increased infection by 4.1 to 4.3-fold. Related experiments were also done for KTI in the experimental model system. In those sets of experiments, each of the targeted genes, KTI20 and KTI30 were expressed. The roots suppressed infection by greater than 80%. In contrast, inhibiting KTI20 or KTI30 gene function increased infection by 7.5 to 7.7-fold.

The experiments reveal the importance of having plant and pathogen genomes to aid in resistance gene identification and pathogen proteins involved in their pathogenesis. The results add to the database being generated to managing SBRM as a threat while providing solutions to control other devastating sugar beet pathogens.