Bacterial
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Streak
Research Initiative

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2025 Bacterial Leaf Streak Meeting Abstracts

Session 1: Pathogen Biology

Loss of ice nucleation alters *Xanthomonas translucens* transmission

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Environmental transmission to hosts is an important ecological step for many pathogens. The underlying genetic mechanisms of transmission are poorly defined. Some bacterial pathogen including *Xanthomonas translucens* perform biological ice nucleation, a biophysical process where organisms facilitate water freezing near subzero temperatures. Ice nucleating bacteria like *X. translucens* uses InaZ for the formation of and movement in precipitation. Here we investigated the link between *inaZ* evolution and *X. translucens* transmission strategies. Our phylogenomic analysis found that *inaZ* is ancestral to Gammaproteobacteria and convergently lost from multiple species that are now ice nucleation inactive. Loss of *inaZ* frequently occurred across the Gammaproteobacteria, and most lineages that lost *inaZ* now rely on plant- or insect-mediated transmission. We determined that *inaZ* loss correlated with significant genome-wide gene loss and reduced gene flow within the *X. translucens* pv. translucens subspecies. Overall these results highlight gene loss as a key driver of transmission strategy evolution.

Rapid whole-genome sequencing and TALE identification of *Xanthomonas* strains isolated in two growing seasons across Minnesota

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Bacterial leaf streak of wheat, caused by *Xanthomonas translucens* pv. *undulosa* (Xtu), is an emerging threat to US wheat growers. Bacterial pathogens, like Xtu, have short generation times and evolve quickly in response to selective pressures to maintain pathogenicity. One class of rapidly evolving virulence proteins are the transcription activator-like effectors (TALEs). TALEs are required for full virulence in several Xanthomonas pathosystems, including Xtu on wheat. We leveraged a collection of Xtu strains isolated by the Dill-Macky lab at UMN to determine the diversity of Xtu effector content across two seasons at sites across Minnesota. In doing so, we hope to resolve questions surrounding how Xtu is changing over time and whether geographic origin is correlated to TALE and non-TALE effector content. We submitted genomic DNA to a nanopore sequencing provider to assemble the genomes of ten strains isolated from across Minnesota, five each from the 2022 and 2024 seasons. TALEs were detected and classified using AnnoTALE, then TALE and non-TALE effector content was cataloged. Assays of the strains on wheat cultivars will allow us to evaluate if specific effectors are associated with virulence. Our findings demonstrate: the utility of modern rapid sequencing services for assembling complex bacterial genomes, the diversity of effector content across geography and growing season, and can inform the selection of relevant Xtu strains for bacterial leaf streak nurseries.

Unraveling *Xanthomonas translucens* host range evolution

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Understanding the evolution of pathogen niche-shifting is key for predicting and mitigating emergent plant diseases. However, the mechanisms leading to pathogen ecological changes remain unknown for most plant diseases. Plant pathogenic bacteria colonize and infect host plants by secreting virulence factors, including effector proteins, that modulate plant cellular

pathways. Nonetheless, these same factors may trigger plant defense responses leading to resistance. Here, we determined how the evolutionary loss of a single effector gene contributed to the emergence of a broad host range subgroup in the bacterial plant pathogen *Xanthomonas translucens*. Comparative genomics analysis between the barley-infecting *X. translucens* pv. translucens (Xtt) and the wheat-infecting *X. translucens* pv. undulosa (Xtu) revealed the effector XopAL1 as a key factor in determining host range. Curiously, targeted deletion of *xopAL1* in Xtt allowed for host jump to wheat but with an inherent decreased fitness in the original host barley, while introduction of this effector into Xtu severely decreased its ability to infect wheat. RNA-seq analysis determined a XopAL1-mediated global transcriptional reprogramming in wheat, with an enriched upregulation of defense response genes. Artificially inducing specific XopAL1-induced defense genes restored the non-host response, highlighting their importance in the downstream effector-triggered defense response. Overall, our study demonstrates that evolutionary effector loss modulates host range across plant genera by altering the profile of plant immune activation.

Three can play that game: Exploring the dynamics between the three clades of *Xanthomonas translucens* pv. *translucens*

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The bacterial leaf streak disease, caused by *Xanthomonas translucens* pv. *translucens* (Xtt), has been an increasingly important disease of barley. Given its persistence across cropping years, and the lack of effective management strategies to mitigate infestations and losses, research on this pathosystem is more critical now than ever. Building on recent comparative genomic studies on Xtt diversity, our surveys confirmed that three distinct clades with varying sets of transcription activator-like effectors are present in major barley-growing states. Field surveys indicate that the prevalence and abundance of these clades vary between infested fields. In addition, virulence tests using representative strains from each clade on ten barley lines revealed differential disease severity responses. Both highlight potential interclade interactions within Xtt that may affect disease severity. To investigate microbial dynamics between Xtt strains, selected representative strains from each clade were transformed with internal DNA barcodes, and fluorescence genes. The internal barcode enables strain detection and quantification through qPCR or NGS. The fluorescence protein expressed by each strain will allow the exploration of their localization and assess their colonization behavior when co-inoculated. Through this study, we aim to explore potential interclade competition and colonization dynamics in Xtt to better inform our approach in developing effective disease management strategies.

Gaining molecular insights into wheat and *Xanthomonas translucens* interactions, plant immunity, and pathogen virulence

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Xanthomonas translucens (Xt) is the causal agent of a re-emerging bacterial disease of cereals (bacterial leaf streak, BLS). While the disease has been recognized for a century, there are still many unknowns about the mechanisms of infection and pathogen recognition in wheat and barley. First, to investigate the factors involved in host immune system activation, we heat-killed cultures of Colorado *Xt* pv. translucens (Xtt) and *Xt* pv. undulosa (Xtu) isolates and syringe-infiltrated them into wheat and barley seedlings. After 24 hrs, live Xtu or Xtt was infiltrated into the same leaf area as the previously infiltrated heat-killed bacteria. After 7 days, we observed that the pre-treatment with the heat-killed cultures reduced the development of water-soaking symptoms in both wheat and barley. This observation is consistent with 'pathogen associated molecular pattern activation', a mechanism in which the plant's immune system is primed for a subsequent bacterial infection after recognizing a conserved PAMP present in the pathogen. Second, to investigate how Xt may be directly suppressing the plant immune system, we investigated the role of xanthan. We hypothesized that xanthan, a polysaccharide known to be involved in bacterial quorum sensing and plant immune suppression plays a role in pathogen virulence and symptom development. We inhibited xanthan production in a Colorado Xtu isolate by deleting a core gene required for its production (*gumD*). We observed that the XtuΔ*gumD* mutant developed reduced water soaking symptoms compared to the wildtype, which could be recovered by adding exogenous xanthan. This suggests that xanthan plays an essential role in Xtu virulence. Together, our data begins to reveal insights into the molecular mechanisms of Xt recognition and host immune suppression.

Session 2: Genetics and Breeding

Phenotypic evaluation and genome-wide association mapping for bacterial leaf streak resistance in a worldwide cultivated emmer wheat collection

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Bacterial leaf streak (BLS), caused by the bacterial pathogen *Xanthomonas translucens* pv. *undulosa* (Xtu), is an important disease affecting wheat production worldwide. Recently, the Northern Great Plains of the USA—a key region for durum wheat and hard red spring wheat—has reported an increased prevalence and severity of BLS. Growing resistant wheat varieties remains the most effective and environmentally friendly strategy to mitigate yield losses caused by this disease. The extensive virulence and genetic diversity of Xtu strains, coupled with the quantitative genetic architecture of wheat-pathogen interactions, highlight the critical need to identify diverse sources of resistance. Cultivated emmer wheat, a progenitor of both durum and bread wheat, offers a broad genetic diversity that could be leveraged for resistance breeding. In this study, we phenotypically evaluated BLS disease severity in 508 cultivated emmer wheat lines at the seedling stage under controlled environmental conditions. Several emmer wheat lines demonstrated high levels of resistance to BLS. Genome-wide association mapping identified two quantitative trait loci (QTL) located on chromosomes 1A and 7B, accounting for 6.1% and 4.8% of the total phenotypic variation, respectively. The most significant marker on chromosome 1A, S1A_305713424, was notably absent in both durum wheat landraces and breeding lines, suggesting that this favorable allele may have been lost during domestication from cultivated emmer wheat. These findings provide a valuable germplasm resource for breeding BLS-resistant durum and bread wheat varieties. Further investigation is needed to identify the candidate gene and elucidate the underlying resistance mechanism associated with the identified QTL.

Efforts towards understanding and integrating key QTLs for bacterial leaf streak resistance in wheat

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Bacterial leaf streak (BLS) caused by *Xanthomonas translucens* pv. *undulosa* (Xtu), poses a considerable challenge to wheat production on a global scale. In North America, strong genetic resistance in hexaploid wheat is rare, and previous research failed to identify major genes or QTLs useful for resistance breeding. Consequently, it is imperative to develop spring wheat lines that exhibit durable resistance to BLS by utilizing both adapted and un-adapted genetic resources. A recent study from our group has pinpointed three robust QTLs associated with BLS resistance located on chromosomes 3B, 5A, and 7D, utilizing two distinct mapping populations, namely BoostND and ITMI, and SNP markers linked to the QTL peaks have been converted into Kompetitive Allele Specific PCR (KASP) markers. This study aims to develop near-isogenic lines for each of the three QTLs through marker-assisted backcrossing to assess their independent effects on BLS resistance across various environments and help in fine-mapping and developing diagnostic markers. Furthermore, we plan to integrate these three QTLs into elite hard red spring wheat varieties, with anticipation that developed lines will provide superior performance and robust BLS resistance and making them suitable for varietal development initiatives. This study will enhance the understanding of BLS resistance and aid in the development of diagnostic markers for the efficient selection of resistance alleles in wheat breeding programs. It will support in the management of BLS and help to reduce its social and economic impact on wheat production.

Mapping for Resistance to Bacterial leaf streak disease in Global Durum Wheat panel

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Bacterial leaf streak (BLS) caused by *Xanthomonas translucens,* is a major disease affecting small grains, including durum (*Triticum turgidum* subsps. *durum*; 2n=4X=AABB) and wheat (*Triticum aestivum*; 2n=6X=AABBDD). Although not new to North America, BLS has reemerged as a significant threat in the Northern Great Plains, leading to substantial yield and quality losses in wheat and barley. The disease begins with water-soaked lesions that expand into large necrotic streaks, reducing photosynthetic activity and grain production. With no effective control measures available, identifying genetic resistance to BLS is critical. However, resistance sources in durum remain largely unknown. To address this knowledge gap, we conducted association mapping using the Global Durum Panel (GDP), comprising 742 genetically diverse tetraploid *Triticum turgidum* comprised of nine subspecies. Greenhouse evaluations at the seedling stage utilized in-house standardized spray inoculation protocol, assessing disease severity on a 1-to-9 scale. The GDP panel was genotyped using the Illumina iSelect 90K SNP array, with 16,633 SNP markers (MAF <0.05) pre-filtered for genomic analysis. We identified 48 significant SNP markers associated with BLS resistance across multiple chromosomes, 1A (1), 1B (3), 2A (25), 2B (2), 3A (1), 3B (3), 4B (1), 5A (1), 5B (3), 6A (2), 6B (1), 7A (2), 7B (3) with GLM, MLM, FarmCPU and BLINK models. Ongoing field trials aim to validate these findings, paving the way for pre-breeding efforts to develop BLS-resistant durum varieties.

Genome-wide association and biparental mapping revealed a major quantitative trait locus associated with resistance to bacterial leaf streak in durum

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Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa*, remerged as an important disease on bread wheat and durum globally. Lack of sources of resistance and poor understanding of the genetics of host resistance have made BLS resistance breeding difficult, particularly in durum. In this work, we evaluated durum cultivars from North Dakota State University (NDSU) for the reaction to BLS, and the results showed that most cultivars were susceptible, with only a few exceptions. A subset of the Globe Durum Panel was used for genome-wide association analysis (GWAS) and the identification of source of resistance to BLS. Additionally, we conducted disease evaluations and quantitative trait locus (QTL) analysis on two durum recombinant inbred line (RIL) populations: one derived from resistant NDSU cultivar Ben and the other from resistant Ethiopian landrace PI 387336. Both GWAS and biparental mapping revealed a major QTL at the very distal end of 6A. The alignment of single nucleotide polymorphism (SNP) markers from both experiments strongly indicates that the same locus confers BLS resistance in these durum materials. The identified resistant accessions and SNP markers will be useful in transferring this major QTL into elite durum cultivars for disease management.

A decade of cooperative nursery data reveals QTLs for bacterial leaf streak resistance and breeding

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Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa* increased in prevalence in the north central U.S. spring wheat growing region in the mid 2000's and remains a challenge to Minnesota wheat production. With no effective cultural or chemical controls, breeding for resistance is the best strategy to reduce damage caused by this disease. This study used a decade of cooperative nursery data to identify marker-trait associations (MTAs) for BLS resistance and develop breeder-friendly markers. The association mapping panel included 110 lines screened in the Bacterial Leaf Streak Cooperative Nursery (2013-2023), with 74 additional Minnesota breeding lines incorporated in 2023-2024, increasing the panel to 184 lines. This expansion improved the statistical power and applicability of discovered MTAs by incorporating lines closely related to the breeding program targeted for improvement.
Genotyping-by-sequencing resulted in over 6K SNP markers. Due to the unbalanced phenotypic dataset, best linear unbiased predictions of BLS resistance were calculated from 7056 phenotypic records. Lines were evaluated in inoculated nurseries across at least three environments, with two to four locations per year and an average of nine environments per line. Association mapping in GAPIT identified MTAs, and breeder-friendly KASP markers were designed for the most significant MTAs (3BL and 5BL) to support the development of wheat varieties with improved BLS resistance.

Unraveling the Complexity of Bacterial Leaf Streak Resistance in Wheat

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Bacterial leaf streak (BLS) disease, caused by *Xanthomonas translucens pv. undulosa* (*Xtu*), is a major threat to wheat production and can lead to yield losses of up to 60% in the Northern Great Plains of the United States. Currently, no effective chemical control measures exist for BLS, and genetic resistance is the most reliable strategy for disease management. Therefore, our objectives are to identify sources of BLS resistance and DNA markers associated with resistance genes through genome-wide association studies (GWAS) on elite wheat germplasm. We used the Regional Germplasm Observation Nursery (RGON) wheat panel, a genetically diverse collection of winter wheat lines from nine different U.S. states, to discover BLS resistance loci. GWAS identified nine marker-trait associations (MTAs) for BLS resistance across nine different chromosomes, highlighting key genomic regions that may contribute to disease resistance. Our state-wide survey and robust infection assays initially focused on *Xtu* as the causal agent of BLS. However, our findings reveal that *Pantoea ananatis* and *P. agglomerans*, alongside *Xtu*, are also pathogenic to wheat, causing indistinguishable symptoms. To achieve sustainable BLS management in wheat, we need to unravel the host resistance mechanisms against all three bacterial pathogens. Our initial findings suggest that resistance effective against *Xtu* may not confer protection against *Pantoea spp.*, underscoring the challenges in managing BLS. Investigating these phyllosphere bacterial interactions is crucial for improving disease diagnosis and developing sustainable BLS management strategies for wheat production.

Wild and cultivated barley species as sources of resistance to bacterial leaf streak

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Bacterial leaf streak (BLS) is a serious disease affecting barley (*Hordeum vulgare* L.) in regions with moderate to warm temperatures, high humidity, strong winds and frequent rainfall or overhead irrigation. The disease is caused by the bacterium *Xanthomonas translucens* pv. *translucens*. Because there is no chemical method to control the disease, using genetic resistance appears to be the only way to limit the impact of BLS. However, only a limited number of barley accessions were identified with partial resistance to BLS. The present study assembled a panel of barley breeding lines (N = 144) from the USDA-ARS' Aberdeen barley breeding program and wild barley accessions (*H. spontaneum*; N = 405) from the National Small Grains Collection in Aberdeen, ID. All barley entries were assessed for their response to BLS under controlled environment and genotyped using the Barley 50K iSelect Illumina SNP chip. We will present preliminary findings from disease evaluation studies and the QTLs identified.

Identification of quantitative trait loci for bacterial leaf streak resistance in two biparental barley populations

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Bacterial Leaf Streak (BLS) is a foliar disease of barley caused by *Xanthomonas translucens* pv. *translucens* and has emerged as a serious production threat in the Upper Midwest over the past two decades. Deployment of resistant cultivars is the best method to reduce the disease impact. Several sources of resistance to BLS have been described from the Minnesota barley improvement program (i.e. Rasmusson and 2MS16_4175-069), but the genetic basis has not been characterized. To determine the genetics of BLS resistance, quantitative trait locus (QTL) analysis was conducted using two biparental populations: Rasmusson (moderately resistant)/PI531986 (susceptible) and TM17.302-07 (susceptible)/2MS16_4175-069 (resistant), with 62 and 126 progeny, respectively. These populations were phenotyped for BLS reaction across multiple locations and years, and genotyped using the multi-species 3K array. In Rasmusson/PI531986 population, three QTLs were identified: two on chromosome 2H and one on chromosome 7H. One QTL on chromosome 2H was co-located with the barley photoperiod response gene *PpdH1*, reflecting a close relationship between BLS reaction and heading date. In TM17.302-07/2MS16_4175-069 population, four QTLs were identified: two on chromosome 2H, and one each on chromosomes 6H and 7H. These findings provide insight into the genetic

architecture of BLS resistance and can be used for marker-assisted or genomic selection in barley breeding.

Session 3: Management and Epidemiology

Understanding and managing wheat bacterial diseases in Uruguay

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Bacterial diseases of wheat have progressively increased in importance in the last two decades in the Southern Cone of South America, particularly in Uruguay. The challenge of managing emergent diseases requires a better understanding of the pathogens involved, their biology and epidemiology to develop efficient management measures. Surveys conducted in 2017 to 2019 and 2024 indicated that Bacterial leaf streak (BLS) caused by *Xanthomonas translucens* pv. *undulosa* (Xtu) was the main bacterial disease affecting wheat. Yet, other *Xanthomonas* strains identified as *X. prunicola* (Xp) were obtained from dry necrosis (BDN) lesions in wheat leaves, different from the typical water soaking and subsequent brown necrosis of BLS. Studies have focused on a) characterizing the genetic and pathogenic diversity of both pathogens populations based on comparative approaches including optimization of specific qPCR methods for the detection and quantification of Xtu and Xp , use of multilocus sequence analysis (MLSA), MLS typing (MLST) and whole-genome sequencing (WGS); b) developing methodologies to inoculate pathogenic Xanthomonas in seedlings (controlled conditions) and adult plants (in the field); c) establishing two field nurseries (specific for BLS and BDN) to screen commercial and advanced wheat genotypes and identify and characterize potential sources of resistance that may eventually be used through INIA´s breeding program; d) epidemiological studies on the role of seed and hosts for Xtu and Xp, e) determining the ability of Xp to multiply and establish within host tissues during infection; f) establishing the effect of both Xtu and Xp on the microbiota of the phyllosphere, their interactions and eventually, identify potential biocontrol agents. Advances will be presented and discussed.

Comparison of Bacterial Leaf Streak Ratings from Natural Infection and Artificial Inoculation under Field Conditions

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Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa*, has been observed with increasing prevalence in Minnesota spring wheat since the early 2000s. The University of Minnesota (UMN) spring wheat breeding program has evaluated BLS in statewide yield trials since 2005. Differential BLS among wheat varieties following natural infection was observed in 53 environments from 2005-2024. An inoculated BLS trial, the BLSCN, has been conducted and evaluated in 44 environments from 2013 to 2024. These two datasets, which include many of the same varieties, enable a comparative analysis of varietal reactions under both natural and inoculated conditions. We will present the ranking of varieties based on their performance in these two scenarios.

Studying management strategies and host-pathogen interactions of bacterial leaf streak in Canada

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Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* (Xt), poses a significant threat to wheat and barley production worldwide, including North America. The recent rise of BLS in Canada has raised concerns about its impact on crop yields and the need for effective management strategies. To address this emerging issue, our research aims to: 1) Evaluate Canadian barley germplasm for resistance to BLS; 2) Compare the pathogenicity and virulence of historical and modern Xt isolates in Canada; 3) Identify and map resistance genes associated with BLS in wheat using a double haploid (DH) population 4) Assess the potential of biocontrol agents (BCAs) as a sustainable BLS management strategy; and 5) Understand the diversity of the pathogen in Canada using sequencing tools. Using BLS isolates from Canada and the United States, barley genotypes that are either moderate resistant or resistant to BLS were identified under greenhouse conditions. The virulence between historical and modern BLS isolates did not differ significantly although variation among isolates was observed. Works done on host resistance identified two QTLs associated with BLS resistance in 4A and 7D chromosomes in the DH population of wheat. To control BLS through BCAs, we have identified two potential biocontrol agents *Pseudomonas brassicacearum* DF41 and *Pseudomonas chlororaphis* PA23 effective in controlling Xt with *in vitro* assay. In addition, we have identified phenazine as the potential secondary metabolites involved in biocontrol in PA23. Our diversity analysis using housekeeping genes revealed that Canadian Xt isolates are not completely identical to those from the United States, indicating potential genetic variation between the populations. These findings will contribute to a better understanding of BLS dynamics and support the development of integrated management approaches to mitigate its impact on Canadian cereal crops.

Innovative approach to elucidate seed-to-seed transmission mechanism of *Xanthomonas translucens* in barley and wheat

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Xanthomonas translucens pv. *translucens* (Xtt) and *Xanthomonas translucens* pv. *undulosa* (Xtu)

are the two economically important bacterial pathogens that cause bacterial leaf streak (BLS) in cereal crops, particularly on wheat and barley. Xtu causes BLS on both wheat and barley, while Xtt causes disease on barley only. These bacterial pathogens are believed to be seedborne, which makes them a significant threat as infected seeds serve as the primary inoculum source of early infections in seedlings and long-distance transmission. Previous studies showed that these pathogens can be detected in seeds, however, there is no report on exact localization on seeds. Conflicting transmission rates have also been reported, yet, a full cycle seed-to-seed transmission has not also been fully elucidated on these pathosystems. To provide a more comprehensive understanding of seed transmissibility of *X. translucens* on wheat and barley in a seed-to-seed scale, we developed a technique that will allow us to monitor and detect the presence of Xtt and Xtu on barley and wheat in all stages of the plant. Xtt and Xtu were tagged with green-fluorescence protein (GFP) and DNA sequence-based tags. Combining these techniques could be a reliable way to speed up the detection and visualization of BLS-causing pathogens *in planta*. The developed method offers a more robust approach to investigate the seed-to-seed transmission of *X. translucens* in barley and wheat.

Session 4: Other Leaf Streak Pathogens

Elucidating the genetic control of bacterial wilt resistance in Italian ryegrass

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Xanthomonas translucens pv. *graminis* (Xtg) causes bacterial wilt, one of the most important forage grass diseases in temperate grasslands. Molecular genetic and genomic tools have the potential to significantly benefit resistance breeding and to enable targeted resistance management. In the past, a major QTL for bacterial wilt resistance was identified in *Lolium multiflorum* and Xtg was shown to rely on a non-canonical type III secretion system for plant infection. A number of candidate genes for bacterial wilt resistance were identified using a bulked segregant analysis approach. Whole genome sequencing of susceptible and resistant pools of a large biparental F_2 population revealed a ~330kb region associated to bacterial wilt resistance. Comparative genomics of different *X. translucens* pathovars allowed to identify virulence traits characteristic for Xtg. A high number of transposases were exclusively found in Xtg 25 and corresponded to 413 to 457 insertion/excision transposable elements per strain. These mobile 26 genetic elements are likely to be involved in the observed genomic plasticity and may play an important role in the adaptation of Xtg. The candidate plant resistance genes together with the bacterial virulence factors identified provide an invaluable resource for the development of genomics assisted selection strategies. In addition, the well characterised plant genotypes and bacterial strains serve as an ideal model system to fully understand the complex *L. multiflorum* - Xtg interaction.

It's not what you think: *Pantoea* as a model for diagnosing and discovering emerging bacterial pathogens

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In June 2021 and 2023, wheat leaves with yellow lesions running parallel to veins were found across eastern Colorado, suggestive of bacterial disease. To determine the causal agent, lysate was made from symptomatic leaf tissue from four field samples, macerated in sterilized water, and spread on YDC medium to isolate bacteria. Single yellow, mucoid colonies were selected for further streaking, and three genes, *16S rRNA*, *gyrB,* and *rpoB*, were amplified and Sanger sequenced from genomic DNA isolated from the colonies. A BLAST search against whole genomes identified three isolates as *Pantoea agglomerans,* and one isolate as *P. allii*. To complete Koch's postulates and confirm the *Pantoea sp.* as the causal agents, isolates were grown as lawns on NA medium, suspended in 10 mM $MgCl_2$ using a final optical density of 0.1 (~10^9 CFU/mL), and syringe-infiltrated into the entire leaf area of 10-day-old wheat seedling leaves (var. Hatcher). Inoculated plants were transferred to a growth chamber, and yellowing symptoms began developing at 7 dpi. Each of the four *Pantoea* isolates were re-isolated from symptomatic leaves, and a sample of each isolate was plated on NA. The colonies appeared phenotypically identical to the original isolates, and Sanger sequencing confirmed the identities as *Pantoea spp*. To our knowledge, this is the first report of *Pantoea* causing disease in wheat in the United States, and the first report of *P. allii* as a wheat disease-causing agent. We are currently investigating whether these *Pantoea* species can be transmitted through wheat seeds, their impacts on germination, and other disease outcomes that may affect wheat growers.

Decoding the bacterial leaf streak disease causing bacterial interactions in wheat

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Bacterial leaf streak (BLS) poses a significant threat to wheat production, with yield losses of up to 60% reported in the Northern Great Plains of the U.S. To assess the impact of BLS in South Dakota, we conducted field surveys across wheat-producing counties in 2022 and 2023. Although early reports identified *Xanthomonas translucens* (Xtu) as the primary pathogen, our findings show that *Pantoea ananatis* and *P. agglomerans*, along with Xtu, also infect wheat and cause similar symptoms. Additionally, we isolated a single strain of *Xanthomonas prunicola*, a species currently recognized as the predominant bacterial pathogen in wheat-growing regions of South America. With no effective chemical control options available, genetic resistance remains the most viable strategy for managing BLS. This highlights the urgent need to understand host resistance mechanisms against all four bacterial pathogens to support sustainable disease management. Notably, our preliminary data suggest that resistance to Xtu does not necessarily

provide protection against *Pantoea* species, emphasizing the complexity of BLS control. Investigating these interactions within the wheat phyllosphere is critical for improving diagnostic accuracy and developing long-term, effective management strategies.

Xanthomonas prunicola: pathogenicity and genomic features of a novel wheat pathogen

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Bacterial leaf streak of wheat, caused by *Xanthomonas translucens*, is a globally significant disease. Bacterial leaf necrosis (BLN), a newly described disease reported in South America and the United States, is caused by *Xanthomonas prunicola*, a species originally described as a nectarine pathogen. Unlike *X. translucens*, *X. prunicola* produces distinct dry necrotic lesions on wheat. Pathogenicity assays confirmed that all wheat-associated *X. prunicola* strains induced necrotic symptoms in wheat distinct from water soaking symptoms elicited by *X. translucens*, while *X. prunicola* from nectarine failed to infect this host. Field inoculations on wheat cv. INIA Tijereta confirmed *X. prunicola* pathogenicity under natural conditions, with severity reaching 40–60% at late grain filling stages. Comparative genomic analyses revealed key differences between wheat- and nectarine-associated *X. prunicola* strains, including a functional type VI secretion system (T6SS) present only in wheat isolates and the exclusive presence of type III effectors XopR and XopW in these strains. These findings highlight the host range expansion of *X. prunicola* and suggest a potential role of the T6SS in wheat pathogenicity. Further research is needed to elucidate epidemiological factors and management strategies for this emerging pathogen. This study provides the first field validation of *X. prunicola* as a wheat pathogen and identifies genomic adaptations distinguishing wheat-associated populations.

Emerging dynamics of *Xanthomonas prunicola* in wheat: epidemiology, ecological niches, and competitive exclusion with *Xanthomonas translucens*

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Bacterial Leaf Streak (BLS) is caused by *Xanthomonas translucens* (Xtu), while *Xanthomonas prunicola* (Xp), a newly identified wheat-associated species, was first described in 2018 as a *Prunus* pathogen. Xp has been recently reported in wheat fields in Uruguay, Argentina, and the U.S., suggesting an emerging global distribution in wheat production areas. Unlike Xtu, which causes water-soaked lesions, Xp causes dry necrotic symptoms, suggesting a different infection mechanism. Despite its growing prevalence, the role of Xp in wheat pathology and its interaction with Xtu remain poorly characterized. To investigate the epidemiology of *Xanthomonas* in wheat, we analyzed their co-occurrence, epiphytic potential, and presence in nearby grasses and weeds as alternative hosts. Field surveys in western Uruguay (Oct–Nov 2024) detected *Xanthomonas* in 26% of 54 sites. Among isolates, 42% were Xp and 21% were Xtu from wheat leaves, while 37% were Xp from alternative hosts. No co-infections of Xp and Xtu were detected, suggesting that these pathogens may occupy distinct ecological niches or have competitive interactions that limit their coexistence in individual infections. Our findings indicated that *Xp* has adapted to wheat and can persist in non-crop hosts, which may act as reservoirs for recurrent infections. Understanding the epidemiology and persistence of *Xp* is crucial for developing management strategies that address the diversity of *Xanthomonas* species affecting wheat.

Why is bacterial leaf streak absent from cereal crops in the EU while it is widespread on grass? Is cereal phytobiome playing a role?

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Bacterial leaf streak, caused by *Xanthomonas translucens* pv. *undulosa* (*Xtu*), poses a significant threat to wheat crops. Absent in Europe, it is hypothesized that winter wheat, dominant on the continent, is less susceptible than spring wheat. Among the factors potentially influencing such resistance, the wheat-associated microbiota remains poorly known but could play a key role to explain *Xtu* pathogenicity. To assess whether microbial diversity specific to winter and spring wheat explains differences in resistance to Bacterial leaf streak, this project aims at conducting a comparative study of wheat varieties. It will focus on three key pathogen entry points: seeds, leaf and stomata and hydathodes, using two complementary approaches. First, metagenomic analysis will characterize microbial communities at these entry sites, examining variations by location, wheat variety and responses to infection. Second, the functional role of the microbiota in resistance will be investigated by *in vitro* antagonistic assays, analysis of microbial compounds via HPLC, and confocal microscopy to study microbial niches. Significantly, *Xtu* uses T4SS and T6SS to mediate interbacterial competition. Understanding their role in *Xtu* interactions with the wheat microbiota and their contribution to pathogenicity will shed light on wheat defense mechanisms. Identifying key microbiota species could help disrupt *Xtu* life cycle and improve disease prevention strategies.

End