Final Report for DOE Grant DE-SC0017883: Identification of Adaptive Fungal Pathogen Resistance Loci in Switchgrass

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Project Period: 9/1/2017 - 8/31/2021

ACCOMPLISHMENTS

1. What were the major goals of the project?

The primary goals of the project were to characterize the distribution of rust pathogens on switchgrass across central North America and to identify genetic loci that control resistance to those pathogens. The research leveraged common garden plantings of a switchgrass four-way F2 mapping population and a Genome Wide Association Study (GWAS) panel across multiple field sites from South Texas to South Dakota.

Aim 1 of the project was to identify the distribution of different rust pathogens across sites using morphological and molecular techniques. Analyses of local environmental variables, using Geographic Information System (GIS) techniques, were conducted to determine whether pathogen distributions and diversity can be predicted by climate and other environmental factors.

Aim 2 focused on identifying genetic loci contributing to rust pathogen resistance across field sites using Quantitative Trait Locus (QTL) mapping and GWAS techniques. Our goal was to determine whether the same or different sets of loci control resistance across particular geographic regions in which switchgrass is grown.

The goal of Aim 3 was to confirm that QTLs we have identified from the field control resistance to local rust pathogens. To accomplish this goal, we had planned to select individual genotypes from the four-way F2 mapping population that differ in predicted alleles for pathogen resistance.

2. What was accomplished under these goals?

Aim 1: Over the course of the grant period, we collected rust-infected plant material from the four-way mapping population across nine field sites from Brookings, South Dakota, East to Hickory Corners, Michigan, and South to Kingsville, Texas. In the first couple years of the grant period, we used internal transcribed spacer (ITS) and spore morphology for identification of rust at the species level. More recently, we have been conducting whole genome pooled-sequencing to assess the population genetic structure of the dominant rust species, *Puccinia novopanici*, across the geographic range of our field experiments. From the initial molecular and morphological analyses, we have identified three rust fungi: *P. graminicola* (11 samples)*, P. cumminsii* (1 sample)*,* and *P. novopanici* (161 samples). The latter species occurred at all sites examined to date, whereas, the former species, *P. graminicola*, was found primarily at northern field sites (Lincoln, NE and Brookings, SD). These early results confirm that *P. novopanici* is the predominant rust fungus affecting switchgrass in the central United States. Two other recently described switchgrass rust fungi, *P. pammelii* and *P. pascua*, appear to have geographic distributions limited to the south-central U.S. Our initial species-level geographic distribution data of rust species across the field sites was published with our QTL mapping results in *New Phytologist* (VanWallendael et al. 2020; See List of Products). The population genomic analysis of *P. novopanici* will be published with our results from the rust-resistance GWAS study, which is currently being completed.

In addition to surveying rust pathogens, we found the head smut fungus (Tilletiacae: *Tilletia maclaganii*) has now appeared at all of our field sites. This was surprising, as prior to this discovery, the purported southernmost geographic distribution of *T. maclaganii* was in central Oklahoma. Thus, these findings represent the first report of switchgrass head smut in TX and a significant range expansion of this important switchgrass pathogen. We published these findings as a report in *Plant Disease* (Kenaley et al. 2019). While rust pathogens have a greater impact on upland genotypes, lowland genotypes are equally susceptible to smut as upland. To facilitate future research on the population genetics of switchgrass smut, we have partnered with the DOE Joint Genome Institute (JGI) to develop a reference genome for *Tilletia maclaganii.*

Finally, the results from surveys of fungal pathogens led us to wonder how other members of the leaf fungal microbiome community could play a role in impacting disease severity. To better understand this dynamic, postdoc Dr. Acer VanWallendael led a collaborative survey project of the fungal microbiome over the course of the 2019 field season on a diverse set of switchgrass germplasm. This study has been published as a pre-print in bioRxiv and is currently under review. This study resulted in the following three major conclusions:
1) Pathogens and their hyperparasites are central members in microbial covariance networks. A key goal of the study of host-microbe interactions is to determine conditions that prevent disease development. Our study maps out microbe-microbe associations over time, showing that hyperparasites may play an important role in controlling pathogens. Further, this finding suggests that immunological changes that target specific pathogens will have downstream impacts on the entire microbiome.
2) Over time, microbial communities progress deterministically, not stochastically. Community assembly in leaf microbiomes may be more predictable than has been previously assumed, and can be understood using the theoretical framework of ecological succession.
3)Three linked genes are tightly associated to microbiome composition in switchgrass. To date, very few gene targets have been identified that control overall microbiome composition in plant leaves, especially in natural settings. Further study of the genes we identified may result in a greater understanding of the processes plants use to deter or recruit particular microbes. Since the majority of crops grown worldwide for food, turf, and bioenergy are in the same family as switchgrass (Poaceae), this may have important applications in numerous settings.

Aim 2: To identify QTLs for rust susceptibility, we surveyed the four-way F2 mapping population for pathogens from 2016-2021 at eight field sites where the mapping population was planted. Overall, we discovered two major QTL and many minor QTL for rust resistance. Interestingly, the two major QTL almost exclusively had effects on resistance at the northern four field sites, with inconsistent or no effects at the southern four field sites. This result suggests that either 1) there are different rust pathogen variants in the northern US or 2) that resistance loci are subject to strong genotype x environment interactions. This study was published in a special issue of *New Phytologist* (VanWallendael et al. 2020). In parallel with the QTL mapping of rust resistance, PI Lowry led a study of QTL mapping at the ten latitudinal field sites for other important plant traits (biomass, flowering time, spring emergence time, tiller number, and plant height). This paper was published in *PNAS* (Lowry et al. 2019). The results from this study were directly applicable to the results from the pathogen resistance study, as we found that both of the major loci for rust resistance overlapped major biomass QTLs. Thus, the same genes (pleiotropy) or a linked set of genes contributes to both disease resistance and biomass. Regardless of the mechanism, selecting for alleles through breeding at these two loci should lead to an increase in both pathogen resistance and biomass. We have recently published two papers (Lovell et al. 2021, MacQueen et al. 2021) that have results from the GWAS panel field experiments that will similarly be important for us interpreting the GWAS results for the rust/smut pathogens that are described below.

To identify more broadly identify loci involved in pathogen susceptibility, we surveyed our switchgrass diversity (GWAS) panel for pathogens from 2019-2021 at nine field sites. From these results, we have now identified multiple loci involved in rust and smut susceptibility. The most consistent region associated with rust susceptibility was the large pericentromeric region of chromosome 2K. This poses a particular challenge, since mapping genes in this low-recombination region is challenging. Further loci have been identified that co-locate with previously discovered QTL, and we are working to identify genes in these regions that may be associated to resistance. An additional locus identified through GWAS analysis of the leaf fungal microbiome (VanWallendael et al. 2021) contributes to substantial differentiation of rust as well. Genotypes with one or more alternate alleles at this locus exhibit nearly four times greater rust severity in the peak of the growing season. This locus is linked to cysteine-rich Receptor-Like Kinase genes that are often involved in the plant hypersensitive immune response, raising the possibility that these genes are important regulators of fungal pathogens.

We have also begun the process of integrating our surveys of pathogens on the diversity GWAS panel with hyperspectral data collected throughout the field season by drones. Dr. Nathan Emery is currently taking the lead on analyzing this data to determine if we can predict pathogen severity early in the season from hyperspectral data.

Aim 3: To propagate sterile individuals from the four-way F2 population that can be used for rust inoculation, we developed a node-based tissue culture method. We modified an existing protocol to remove problematic fungal endophytes through the application of Benomyl fungicide on culture plates. After overcoming the challenge of fungal endophyte contamination, we successfully regrew both upland and lowland switchgrass genotypes from tiller node cuttings to fully grown plants. However, we have found that this propagation procedure is too slow for propagation of genetic mapping populations, as each genotype requires the procedure to be optimized to be successful. Therefore, we clonally propagated 100 F2s with our standard horticultural procedures. 50 of these F2s are predicted to be more resistant than the other 50 F2s based on genotypes at disease resistance QTLs. We had planned to transport these propagated F2s from MSU to Cornell for confirmation of pathogen resistance QTLs. Unfortunately, high personel turnover at Cornell, as well as the COVID19 pandemic, prevented this research from going forward. Despite this major setback, the sterile propagation methods that we developed as part of this aim should be very useful for future studies on the switchgrass microbiome.

3. What opportunities for training and professional development did the project provide?

At MSU, the grant funding provided excellent training opportunities for two postdocs (Acer VanWallendael, Nathan Emery) and six technicians (Marissa VanDamme, Lisa Vormwald, Conner Lamb, Mauricio Swartzendruber, Darlene Brennan, Linnea Fraser). Throughout this project, VanWallendael has received training in QTL mapping, GWAS, the fundamentals of plant pathogen biology, and multiple statistical analyses. Based on VanWallendael's success, he is currently applying for academic faculty positions. Emery has applied his knowledge of plant physiology to this project by collecting paired sampling of leaf level physiology with pathogen infection throughout the 2019, 2020, and 2021 field seasons. Dr. Emery has recently begun working to integrate hyperspectral data collected from drones to predict pathogen severity. This represents a new direction for Emery's career path. As part of each technician's appointment, they must conduct an annual evaluation, through which they develop a set of training goals each year with PI Lowry.

At Cornell, this project played a significant role in the training and career development of Dr. Shawn Kenaley and Dr. Chathu Wijewardana. Kenaley, who conducted the phylogenetic characterization of *Puccinia* species and developed PCR protocols for molecular identification of *Tilletia maclaganii*, left the project and Cornell to take a faculty position at Finger Lakes Community College in Canadaigua, NY. Wijewardana carried on the *Puccinia* and *Tilletia* molecular work begun by Kenaley and was successful in culturing the biotrophic pathogen *T. maclaganii*. Since Wijewardana left in August 2020, Kevin Meyer has been the main researcher in the Bergstrom Lab working with *T. maclaganii*, including preparation of DNA for construction of a reference genome and characterization of genomic variation in U.S. populations of the pathogen. Meyer performed the crucial step of culturing isolates of *T. maclaganii* derived from single haploid basidiospores formed 24 hours after germination of dikaryotic teliospores. Meyer has increased his mycological and molecular skills through research on this project.

4. How have the results been disseminated to communities of interest?

PI Lowry has given presentations of research results from this project at the following venues in during the grant period 1) Switchgrass IV meeting at University of Nebraska (Lincoln, NE); 2) Tsukuba Global Science Week. T-PIRC Symposium: Leading University Forum on Plant Resilience and Innovation (Tsukuba, Japan); 3) DOE Genomic Sciences Program Annual Principal Investigator (PI) Meeting (Tysons, VA); 4) Midwest Population Genetics Meeting (East Lansing, MI); 5) Great Lake Bioenergy Research Center Environmental Performance Meeting (Lansing, MI); 6) UC Davis Department of Plant Sciences (Davis, CA); 7) DOE Genomic Sciences Program Annual Principal Investigator (PI) Meeting (Tysons, VA); 8) International Switchgrass V Meeting (Champaign, IL); 9) Switchgrass Collaborators Meeting, Noble Foundation (Ardmore, OK); 10) International Evolution Meeting (Providence, RI); 11) Purdue University. Department of Botany and Plant Pathology (West Lafayette, IN); 12) Keynote Speaker. 4th International *Brachypodium* Conference (Huesca, Spain); 13) Pennsylvania State University. Plant Biology Seminar (State College, PA); 14) Great Lake Bioenergy Research Center Annual Science Meeting. Switchgrass Ecology Symposium (Virtual Conference); 15) 2020 DOE Genomic Sciences Program Annual Principal Investigator (PI)

Meeting. (Washington D.C); 16) Keynote Speaker. DOE Joint Genome Institute. Genomics of Energy & Environment Meeting 2021. (Virtual Conference). Co-PI Bergstrom has provided research updates on this project in in each year to the agricultural extension audiences in New York State, and with collaborators at Ernst Conservation Seed Company (largest commercial supplier of switchgrass seed in the Eastern U.S.) in Meadville, PA. Postdoc Acer VanWallendael has presented results from this research to the 1) DOE Genomic Sciences Program Annual Principal Investigator (PI) Meeting (Tysons, VA); 2) Plant Biology Department, Michigan State University (East Lansing, MI); 3) MSU Annual Ecology, Evolutionary Biology, and Behavior Meeting (East Lansing, MI); 4) International Switchgrass V Meeting (Champaign, IL); 5) International Society of Molecular Plant-Microbial Interactions (Glasgow, Scotland); 6) International Evolution Meeting (Providence, RI); 7) Great Lakes Bioenergy Research Center Sustainability meeting (online); 8) the Ecological Society of America meeting (online); and 9) the Plant and Animal Genomes meeting (San Diego, CA).

IMPACT

1. What is the impact on the development of the principal discipline(s) of the project?

The finding that two major QTLs have a major effect on rust resistance in northern field sites, but little or no effect in the southern field sites is a major discovery and provides new insights into how pathogen resistance should be thought of in a geographic context. In addition, the finding that one of the resistance QTLs was conferred by a southern lowland allele, while the other was conferred by a northern upland one was surprising, but very important. It is important because it suggests that improving rust resistance might benefit most by combining variation from upland and lowland gene pools. Further, we have now identified loci for rust and smut resistance through GWAS. These loci could be used by breeders to improve resistance to these pathogens generally.

2. What is the impact on other disciplines?

Our research confirms that there are major differences in the nature of biotic interactions within a species based on latitude. Few other studies have had the level of replication across space to actually test this hypothesis. Our work also has implications for the fields of fungal biodiversity, ecology, systematics, and taxonomy. Scant morphological and molecular data presently exist for switchgrass rust and smut fungi, and hence, their phylogenetic relationships and life histories remain poorly understood. This research opens up new avenues of research in rust population genomics and the interaction of pathogens with other microbiome community members. The protocol developed in this project to initiate fungal cultures from a single haploid basidiospore (sporidium) of the obligate smut fungus, *Tilletia maclaganii*, opens up new avenues for characterization of genomic diversity in populations of this pathogen.

3. What is the impact on the development of human resources?

This grant was the first major extramural award to PI Dr. David Lowry of his career. The funding played a crucial for him in establishing his independent research program at Michigan State University and undoubtedly, was crucial for Lowry being awarded tenure in 2020. The grant also launched Dr. Acer VanWallendael's postdoc career and facilitated Dr. Nathan Emery to work in new areas of research.

4. What is the impact on physical, institutional, and information resources that form infrastructure?
Development of an understanding of the epidemiology of switchgrass pathogens could help in the development of robust energy and bioproduct supply chains.

5. What is the impact on technology transfer?
None

6. What is the impact on society beyond science and technology?
The development of high-yielding, disease resistant energy grasses is a crucial step in the development of a sustainable future bio-based economy.

CHANGES-PROBLEMS

1. Changes in approach and reasons for change

The finding of two major QTLs for resistance that are primarily present in the north, but not in the south has greatly influenced the direction of our projects. We became very interested in trying to understand why this pattern has occurred. Thus, we decided to increase out effort on understanding whether there is genetic variation within *P. novopanici* that could potentially explain this pattern. Also, finding out that a genome for *P. novopanici* had been assembled influenced our plans, as it opened up the possibility to conduct population genomic analyses of *P. novopanici* with whole genome pooled sequencing of spores from each of the field sites. In addition, it became clear to us in the past year that we could get a better understanding of the causes of rust resistance if we could characterize the other fungi that the rusts might interact with in the leaves of switchgrass. This has led to our new sub-aim focused on characterizing the microbiome of switchgrass among genotypes and field sites throughout the growing season.

The results from surveys of fungal pathogens led us to wonder how other members of the leaf fungal microbiome community could play a role in impacting disease severity. To better understand this dynamic, we conducted a survey project of the fungal microbiome over the course of the 2019 field season on a diverse set of switchgrass germplasm. This study has been published as a pre-print in bioRxiv and is currently under review.

High quality DNA has been prepared from a New York isolate of the switchgrass smut fungus for construction of a reference genome for *Tilletia maclaganii* by the Joint Genome Institute. This will be a great public resource for mycologists, plant pathologists, and plant breeders who conduct research on switchgrass smut. This will be followed by genomic analysis of 65 other isolates collected from populations across 10 U.S. locations. We expect to publish results of this population study in a plant pathology journal within the next year.

2. Actual or anticipated problems or delays and actions or plans to resolve them

Our biggest unexpected challenge was postdoc Dr. Shawn Kenaley moving to another position in July 2018. We had a great deal of difficulty refilling this open position at Cornell University due to the specific requirements of the job. As a result, the position remained vacant for a year and considerably slowed down progress on the characterization of the rust pathogen samples collected from the field. We hired a new postdoc, Dr. Chathurika Wijewardana, who took the lead on the Cornell objectives starting in summer 2019. Dr. Wijewardana ended up leaving the position early during the COVID19 pandemic. As a result, funding was redirected to a technician in Bergstrom's lab, Kevin Myers. The high level of turnover at Cornell and the COVID19 pandemic prevented us for conducting greenhouse studies to confirm the function of potential rust resistance loci, as we initially had planned. Overall, we completed Aims 1 and 2, but could not complete Aim 3. In its place, we launched new areas of inquiry (population genomics of rust pathogens, leaf fungal microbiome research, and a smut genome project) that we outlined above.

3. Changes that have a significant impact on expenditures

Without the full-time postdoc position being filled at Cornell for a year, not much spending was done at Cornell in year 2 of the grant. As a result, PI Lowry chose to retain the Cornell sub-award at MSU for year 2.

4. Significant changes in use or care of human subjects, vertebrate animals, and/or biohazards

None

5. Change of primary performance site location from that originally proposed

No changes have been made.

6. Carryover Amount

PRODUCTS

1. Publications

VanWallendael A, Benucci GMN, da Costa PB, Fraser L, Sreedasyam A, Fritschi F, Juenger TE, Lovell JT, Bonito G, Lowry DB. 2021. Host genetic control of succession in the switchgrass leaf fungal microbiome. BioRxiv. 2021.03.26.437207.

MacQueen AH, Zhang L, Bonnette J, Boe AR, Fay PA, Fritschi FB, Lowry DB, Mitchell RB, Rouquette FM, Wu Y, Juenger TE. 2021. Mapping of genotype-by-environment interactions in phenology identifies two cues for flowering in switchgrass (*Panicum virgatum*). BioRxiv. 2021.08.19.456975.

VanWallendael A, Lowry DB, Hamilton JA. 2021. One hundred years into the study of ecotypes, new advances are being made through large-scale field experiments in perennial plant systems. Current Opinion in Plant Biology. *In press*

*Lovell JT, MacQueen AH, Mamidi S, Bonnette J, Jenkins J, Napier JD, Sreedasyam A, Session A, Shengqiang Shu A, Barry K, Bonos S, Boston L, Chapman J, Daum C, Deshpande S, Ewing A, Grabowski P, Haque T, Harrison M, Healey A, Jiang J, Kudrna D, Lipzen A, Pendergast IV TH, Plott C, Qi P, Shakirov EV, Sims D, Stewart A, Singan V, Tang Y, Thibivillier S, Webber J, Weng X, Williams M, Wu GA, Yoshinaga Y, Zane M, Zhang L, Zhang J, Behrman KD, Boe AR, Fay PA, Fritschi FB, Jastrow JD, Lloyd-Reilley J, Matamala R, Mitchell RB, Rouquette Jr FM, Ronald P, Saha M, Tobias CM, Udvardi M, Wing R, Wu Y, Bartley LE, Casler M, Devos KM, Lowry DB, Rokhsar D, Grimwood J, Juenger TE, Schmutz J. 2021. Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass. Nature. 590: 438–444.

*PI Lowry was interviewed by the Joint Genome Institute Genome Insider podcast to discuss the implications of this paper: https://jgi.doe.gov/genome-insider-s2ep1-exploring-diversity-of-american-prairie-switchgrass/

VanWallendael A, Bonnette J, Juenger TE, Fritschi FB, Fay PA, Mitchell RB, Lloyd-Reilley J, Rouquette FM, Bergstrom GC, Lowry DB. 2020. Geographic variation in the genetic basis of resistance to leaf rust in locally adapted ecotypes of the biofuel crop switchgrass (*Panicum virgatum*). New Phytologist. 227: 1696-1708.

Lowry DB, Lovell JT, Zhang L, Bonnette J, Fay PA, Mitchell RB, Lloyd Reilley J, Boe AR, Wu Y, Rouquette FM, Wynia RL, Weng X, Behrman KD, Healy A, Barry K, Lipzen A, Bauer D, Sharma A, Jenkins J, Schumtz J, Fritschi FB, Juenger TE. 2019. QTL x environment interactions underlie adaptive divergence in switchgrass across a large latitudinal gradient. PNAS. 116: 12933-12941.

VanWallendael A, Soltani A, Emery NC, Peixoto MM, Olsen J, Lowry DB. 2019. A molecular view of plant local adaptation: Incorporating stress-response networks. Annual Reviews in Plant Biology. 70: 559-583.

Kenaley SC, Bergstrom GC, Montes Ortiz ZK, VanWallendael A, Lowry DB, Bonnette JE, Juenger TE. 2019. First report of the head smut fungus*, Tilletia maclaganii*, affecting switchgrass in Texas. Plant Disease. 103: 578.

2. Intellectual Property

None

3. Technology or Techniques

Switchgrass Micropropagation from Nodes. We modified the protocol of Alexandrova et al. (1996) for the micropropagation of switchgrass from nodes to produce clones of switchgrass for controlled inoculations with rust. The main modification was to introduce treatments to eliminate problematic fungal endophytes, which kill regenerating plantlets. In particular, we are treating the node cuttings with 75% bleach/Triton X-100 and adding10uM 6-benzylaminopurine and 5mg/L Benomyl to the shoot-induction medium, both in plates and PhytaTrays.

Citation:Alexandrova KS, Denchev PD, Conger BV. 1996. Micropropagation of switchgrass by node culture. Crop science. 36:1709-1711.

Producing *T. maclaganii* cultures free from endophytic and epiphytic bacteria. We modified the protocol of Eibel et al. 2005, which uses malt extract/peptone media for the isolation and propagation of *Tilletia* and *Ustilago* wheat smut pathogens, to include the addition of media antibiotics ciprofloxacin and gentamicin (Mondo et al. 2012) and also introduced a teliospore surface sterilization step with 0.165% sodium hypochlorite/0.05% Tween 20 to facilitate the isolation of *T. maclaganii* into culture.

Citation: Mondo SJ, Toomer KH, Morton JB, Lekberg Y, Pawlowska TE. 2012. Evolutionary stability in a 400-million-year-old heritable facultative mutualism. Evolution 66: 2564-2257

Isolation of single haploid basidiospores of *T. maclaganii* into culture. In addition to single dikaryotic teliospore cultures of *T. maclaganii*, a simple method was developed to isolate single haploid basidiospores into culture: surface-sterilized teliospores were suspended in sterile water and incubated for 24 hours at 20 C to stimulate production of basidiospores, followed by vigorous vortexing for several seconds to dislodge basidiospores from teliospores, then allowing the suspension to settle for 5 minutes to segregate larger and heavier teliospores into a lower layer from lighter basidiospores in an upper layer. Basidiospores were then harvested and used to develop downstream single spore cultures. This needs to occur promptly upon basidiospore formation and prior to basidiospores pairing to form H-bodies (dikaryotic mycelium).

Citation: Eibel, P, Wolf GA, Koch E. 2005. Development and evaluation of an enzyme-linked immunosorbent assay (ELISA) for the detection of loose smut of barley (*Ustilago nuda*). European Journal of Plant Pathology 111: 113-124.

4. Other Products

None