Report and Recommendations
NORTHEAST MULTISTATE ACTIVITIES COMMITTEE MEETING
May 25, 2022
10:00-11:00 am Zoom Teleconference

Members: Jan Nyrop (Cornell, chair), Matt Wilson (WVU), Puneet Srivastava (UMD), Jason White (CT-New Haven), Cindy Fitch (WVU/NEED), Ali Mitchell (NEED)

Request to approve FFY2023 Off-the-Top Budget (MAC recommendation to NERA)

- NE59: *Support for the Northeastern Regional Center for Rural Development* (Director: Stephan Goetz. See memo of May 12, 2022, Blair Siegfried to MAC, $40,788)
    - This funding request amount has stayed level since 2016 and the center remains very active on our collective behalf.
    - NERA should explicitly call out and record a statement on the leverage the NERCRD provides, upon approval of the FFY23 budget.
    - **The MAC unanimously moved to recommend the NE59/NERCRD budget request for FFY2023 at $40,788.**

Request to Write Multistate Coordinating Committee (MAC approval only)

- NE18862: *Legal Issues in Agriculture and Natural Resources,* 10/1/22-9/30/27. Submitted by Paul Goeringer – UMD, Proposed AA: Puneet Srivastava-UMD. (WVU collaborators are confirmed and WVU will serve as the second station supporting the request to write. Southern collaborators have been approached.)
    - This proposal was submitted as a coordinating committee but has potential to evolve into a multistate research project.
    - There are no existing multistate activities centered around legal issues in ag.
    - Paul Goeringer advises a team out of UMD and has been awarded two grants in back-to-back years on legal issues in ag.
    - There is a high potential for outreach connected to this project.
    - WVU has confirmed prospective participants for this project. Experiment Station funds are being offered to people outside the Davis College at WVU, including the law school and arts and sciences.
    - Matt Wilson recommends this team considers drafting a multistate research project.
    - Legal *and Legislative* issues in agriculture should be considered. Labor issues (e.g. overtime, pay requirements, etc.) are affecting the ag industry in New York.
    - This project mostly has value to the extent of which people who need to navigate the legal landscape, use the information.
    - **The MAC unanimously moved to approve this request to write coordinating committee proposal, with the recommendation to the technical team consider drafting a full multistate research proposal.**

Request to Approve Peer Reviewed Multistate Activities (MAC recommendations to NERA)

- NECC_TEMP2202: *Formal Structure for the Minor Use Animal Drug Program*, 10/2022 – 09/2027 [Renewal of NECC1702, AA: Margaret Smith – Cornell]
    - Longstanding project with broad coverage on minor use animal drugs.
- NE_TEMP2202: *The Equine Microbiome*, 10/2022 – 09/2027 [New project, AA: Eric Wommack – Delaware]
    - Equine nutrition is a challenge – this project hopes to characterize the equine microbiome to address nutrition, health, and well-being issues in horses.
- NE_TEMP2210: *Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Nutritive Value*, 10/2022 – 09/2027 [Renewal of NE1710, AA: Eric Bishop-von Wettberg, UVM]
    - Project is in good shape following three supportive reviews. New crop varieties are being put in the hands of practitioners.
- NE_TEMP2220: *Multi-state Coordinated Evaluation of Grape Cultivars and Clones*, 10/2022 – 09/2027 [Renewal of NE1720, AA: Margaret Smith – Cornell]
    - Reviews were supportive and no substantive changes were requested.
- NE_TEMP2227: *Contribution of Ovarian Function, Uterine Receptivity, and Embryo Quality to Pregnancy Success in Ruminants*, 10/2022 – 09/2027 [Renewal of NE1727, AA: Dave Townson – URI]
    - Reviews were supportive and this has been a longstanding and productive group.
- NE_TEMP2248: *Mastitis Resistance to Enhance Dairy Food Safety, Milk Quality, and Animal Welfare*, 10/2022 – 09/2027 [Renewal of NE1748, AA: Kumar Venkitanarayanan – UConn]
    - Reviews were supportive. Some comments had minor issues with the reference to food safety in the title, despite lack of effort on food safety in the proposal to food safety.
- **The MAC unanimously recommends to NERA the approval of the full slate of multistate activities above.**

Informational items

- NERA's nomination for the 2022 Experiment Station Section Award for Excellence in Multistate Research:
    - NE1962: *Outdoor Recreation, Parks and Other Green Environments: Understanding Human and Community Benefits and Mechanisms*, 10/01/2017 - 09/30/2022, AA: Matt Wilson. (Attached)

Discussion items for a future MAC meeting

- Outreach Plan discussion
    - At the request to write stage: What are the MAC's expectations on Extension/outreach plan? Can the MAC approach writing teams about this up front, so they don't move beyond the request to write stage without a robust plan?
    - What should be the stated role for Extension in a multistate research proposal? How is an Extension effort funded in this context?
    - How can technical teams incorporate Extension in a meaningful way, from the beginning of the project lifecycle? How do we ensure the outreach plan robustly

and intently extends the science that is being developed? We should promote Extension participation on multistate projects.

- Initial requirements for a request to write
    - A number of requests to write have been submitted to NERA as full project proposal drafts. Is this helpful? Should requests to write call for full proposal drafts as opposed to just the issues and justification section? Or should it be an option to the prospective writing team that they can submit either a request to write or a full proposal?

Attachments

- May 25, 2022 MAC Meeting agenda
    - NERCRD Request
    - Request to Write
        - Legal Issues in Agriculture and Natural Resources
    - Requests to Approve Peer Reviewed Multistate Activities
        - NECC_TEMP2202: Formal Structure for the Minor Use Animal Drug Program
        - NE_TEMP2202: The Equine Microbiome
        - NE_TEMP2210: Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Nutritive Value
        - NE_TEMP2220: Multi-state Coordinated Evaluation of Grape Cultivars and Clones
        - NE_TEMP2227: Contribution of Ovarian Function, Uterine Receptivity, and Embryo Quality to Pregnancy Success in Ruminants
        - NE_TEMP2248: Mastitis Resistance to Enhance Dairy Food Safety, Milk Quality, and Animal Welfare

Agenda
NORTHEAST MULTISTATE ACTIVITIES COMMITTEE MEETING
May 25, 2022
10:00-11:00 am Zoom Teleconference

https://zoom.us/j/5717743008
Password (all caps): NERA

Members: Jan Nyrop (Cornell, chair), Matt Wilson (WVU), Puneet Srivastava (UMD), Jason White (CT-New Haven), Cindy Fitch (WVU/NEED), Ali Mitchell (NEED)

Request to approve FFY2023 Off-the-Top Budget (MAC recommendation to NERA)

- NE59: *Support for the Northeastern Regional Center for Rural Development* (Director: Stephan Goetz. See memo of May 12, 2022, Blair Siegfried to MAC, $40,788)

Request to Write (requires MAC approval only)

- NE18862: *Legal Issues in Agriculture and Natural Resources*, 10/1/22-9/30/27. Submitted by Paul Goeringer – UMD, Proposed AA: Puneet Srivastava-UMD. (WVU collaborators are confirmed and WVU will serve as the second station supporting the request to write. Southern collaborators have been approached.)

Request to Approve Peer Reviewed Multistate Activities (MAC recommendations to NERA)

- NECC_TEMP2202: *Formal Structure for the Minor Use Animal Drug Program*, 10/2022 – 09/2027 [Renewal of NECC1702, AA: Margaret Smith – Cornell]
- NE_TEMP2202: *The Equine Microbiome*, 10/2022 – 09/2027 [New project, AA: Eric Wommack – Delaware]
- NE_TEMP2210: *Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Nutritive Value*, 10/2022 – 09/2027 [Renewal of NE1710, AA: Eric Bishop-von Wettberg, UVM]
- NE_TEMP2220: *Multi-state Coordinated Evaluation of Grape Cultivars and Clones*, 10/2022 – 09/2027 [Renewal of NE1720, AA: Margaret Smith – Cornell]
- NE_TEMP2227: *Contribution of Ovarian Function, Uterine Receptivity, and Embryo Quality to Pregnancy Success in Ruminants*, 10/2022 – 09/2027 [Renewal of NE1727, AA: Dave Townson – URI]
- NE_TEMP2248: *Mastitis Resistance to Enhance Dairy Food Safety, Milk Quality, and Animal Welfare*, 10/2022 – 09/2027 [Renewal of NE1748, AA: Kumar Venkitanarayanan – UConn]

Informational items

- NE nomination for the 2022 Experiment Station Section Award for Excellence in Multistate Research, NE1962: *Outdoor Recreation, Parks and Other Green Environments: Understanding Human and Community Benefits and Mechanisms*, 10/01/2017 - 09/30/2022, AA: Matt Wilson. (Attached)

PennState

Research and Graduate Education
Pennsylvania Agricultural Experiment Station
College of Agricultural Sciences
The Pennsylvania State University
217 Agricultural Administration Building
University Park, PA 16802-2600

814-865-3136
agsci.psu.edu/research
agsci.psu.edu/graduatestudents

DATE: May 12, 2022

FROM: Blair Siegfried, Associate Dean for Research and Graduate Education
Director, Pennsylvania Agricultural Experiment Station

TO: Members of the Northeast Multi-State Activities Committee (NE-MAC)
Jan Nyrop
Matt Wilson
Puneet Srivastava
Jason White
Andra Johnson
Cindy Fitch

RE: Action Item for NERA Meeting

By means of this email, I am requesting off-the-top funding in the amount of $40,788 for the Northeast Regional Center for Rural Development, for the period October 1, 2022 through September 30, 2023, for NE-59, Regional Research Coordination, Northeast Region. The regional funds are used to support the salaries of the Center directors and staff. Penn State pays all the fringe benefits associated with these personnel services. This means that the bulk of the USDA-NIFA special research funds for rural development are used to support the program.

The Center continues, through its Director and staff, Board of Directors, and Technical Advisory Committee, to provide excellent leadership, coordination, and financial assistance for rural development and land use research in the region. I strongly support the continuation of these regional research dollars for this purpose.

If you have any questions, please call me. Thank you.

cc: Directors of Agricultural Experiment Station, NE Region
S. Goetz
R. Unger
A. Raghavan

NE18862: Legal Issues in Agriculture and Natural Resources

Status: Draft

Duration	10/01/2022 to 09/30/2027
Admin Advisors:	[Puneet Srivastava]
NIFA Reps:	

Statement of Issues and Justification

Legal risks are pervasive in agricultural and natural resource industries. Stakeholders' decisions are often constrained by limited knowledge of laws and their impacts on operations, including environmental laws, contract laws, agricultural leasing, bankruptcy, estate planning, food safety, and others. Legal risk impacts vary across agricultural firm types, geographic regions, and government agencies ranging from local ordinances to state laws to federal law.

Legal risk impacts are becoming a growing concern of agriculture and natural resources operations. For example, in Maryland, 60 percent of agricultural producers and service providers responded that laws and regulations in the state affect farm businesses to a high degree. In this survey, respondents highlighted a wide range of issues impacting agricultural operations from environmental law issues, zoning and planning issues, estate planning, and USDA programs as potential areas of concern for producers (Millet-Williams, 2019). This is just one example in one state with other states having similar to truly unique issues depending on the conditions in that state.

While much progress has been made in understanding making decisions with legal risks, the knowledge base remains incomplete due to the continually evolving nature of U.S. law. There is a continuing need to examine both short- and long-term effects of legal changes in agriculture and other natural resource-based industries. The ever-evolving definition of waters covered under the Clean Water Act is a good example, highlighting the importance of understanding the changing nature of the law and legal risk management. A better understanding of how legal changes affect these businesses will improve and help firm-level decision-making in adapting to changes in the laws. Though proposed in the Northeast, one of the strengths of this project is it will bring a national scope of institutions represented by the participants and allow us to understand a breadth of the local, state, and federal laws impacting the agricultural and natural resource industries.

While many legal issues are initially driven by local and/or state interests, these may turn into concerns in other states as well. In response to legal challenges involving North Carolina's right-to-farm law, in 2018 the North Carolina legislature amended that law to provide additional statutory protections to agricultural operations. Following the North Carolina amendments, several other states looked at modifying their state's right-to-farm law to provide similar protections. Pulling together a coalition of national institutions working together on these issues will help all states' understanding of the effects of changes in agricultural law.

This proposed coalition would allow researchers to present work to a broader group of peers and allow for a more successful understanding of applying these legal issues to a broader range of legal risks impacting the agricultural and natural resource industries. In addition, the information exchange format creates opportunities for researchers to interact on issues of mutual interest, fostering extramural grant-writing efforts.

Objectives

1. Provide a scientific/professional forum to facilitate the exchange of theoretical and methodological approaches to agricultural law, and to develop original concepts and preliminary research related to agriculture and natural resources.
2. Develop and communicate legal analysis of contract law, succession planning, nuisance, and environmental legal issues and legal risk management strategies in agriculture, including analysis of how these laws impact firm-level decisions, technology adoption, and access to information.
3. Develop and communicate legal analysis of federal laws and regulations impacting agricultural and resource businesses.
4. Develop and communicate legal analysis of how state laws and regulations vary among the states can impact agricultural and resource businesses.

Procedures and Activities

The primary activity would be an annual meeting, allowing for the exchange of ideas and information related to legal issues surrounding agricultural and natural resources law. Project members will hold this meeting in conjunction with the American Agricultural Law Association's (AALA) annual meeting/Extension Risk Management Educators (ERME) Conference, with individual tracks for the project members to exchange ideas and information.

At quarterly virtual meetings, the project members would make presentations on current issues, allowing the project team to meet more regularly and discuss developing legal issues in our respective states and how to best address them.

Based on this increased collaboration, we would expect to increase the development of organized symposia sessions for the AALA's annual meeting, for meetings of agricultural economists and policy professionals, and for risk management conferences. We would also expect this collaboration to help us develop theme issues for interested law journals focusing on agricultural law, including the Drake Journal of Agricultural Law, Kentucky Journal of Equine, Agriculture, and Natural Resource Law, and the Texas A&M Law Review.

Expected Outcomes and Impacts

- Exchange of ideas, information/data, and research results at a multi-day professional meeting of project members held in the fall of each year and virtual quarterly meetings.
- Coordination of research and extension programs surrounding legal issues impacting agricultural and natural resources firms.
- Research results and insights to directly inform and evaluate federal and state laws and their impact on decisions in agriculture and natural resources and the future landscape.
- Development of themed publications about developing issues, as this group works with existing law journals, such as Drake Journal of Agricultural Law, Kentucky Journal of Equine, Agriculture, and Natural Resource Law, and the Texas A&M Law Review, and other outlets such as Choices Magazine.
- Formal interaction with private practice attorneys, governmental attorneys, in-house counsels, and other professionals. By holding our annual meeting in conjunction with the AALA's annual meeting/ERME conference, we will be able to interact with those professionals working on these issues and better develop academic research focused on current legal issues.

Educational Plan

We will disseminate research and extension information to stakeholder groups through publications and presentations. We will develop a website that will provide electronic copies of all publications, presentations, and recordings of virtual meetings. We will make presentations of specific issues addressed to clientele groups as requested. The project members will collaborate on organizing sessions for the AALA's annual meeting and for professional meetings of agricultural economists and Extension faculty as needed.

Organization/Governance

A three-member executive committee consisting of a past project chair, project chair, and program chair will govern the project. Administrative issues will be addressed during the business meeting held in conjunction with the annual meeting. The committee will conduct elections to fill the position of program chair during the business meeting. The program chair coordinates the program for the next annual meeting and the quarterly virtual meeting sessions. The outgoing program chair becomes the project chair and is responsible for conducting the business meeting, submitting an annual report on project activities, and maintaining communication with the administrative advisor and the Northeast Association of Agricultural Experiment Station Directors. The outgoing project chair will become the past project chair to provide additional support to the executive committee. Initially, at the first annual meeting, the project team would elect project team members to fill the three executive committee positions.

We would also incorporate into this project an advisory panel of key stakeholders such as attorneys, Experiment Station Directors, agricultural operators, and other ag service providers. This advisory panel would be integrated into our annual meetings to help provide additional feedback on research to ensure its timely and valuable to our target audience.

Literature Cited

Selected works focused on agricultural and natural resource laws

Ellixson, Ashley, et al. "Legal and Economic Implications of Farm Data: Ownership and Possible Protections." Drake J. Agric. L. 24 (2019): 49.

Ferrell, Shannon L., and Eric A. DeVuyst. "Decommissioning wind energy projects: An economic and political analysis." Energy policy 53 (2013): 105-113.

Ferrell, Shannon L., et al. "The Future of Agricultural Law: A Generational Shift." Drake J. Agric. L. 18 (2013): 107.

Ferrell, Shannon L., and Rodney Jones. "Legal Issues Affecting Farm Transition." Data Development and Policy Analysis Conference, Washington, DC. 2013.

Ferrell, Shannon L. "Legal Issues on the Farm Data Frontier, Part I: Managing First-Degree Relationships in Farm Data Transfers." Drake J. Agric. L. 21 (2016): 13.

Ferrell, Shannon L. "The Technical and Ethical Challenges for Lawyers in Evaluating Wind Energy Development Agreements." Drake J. Agric. L. 17 (2012): 55.

Goeringer, Paul. "Adapting to the Changing World of Biotechnology: Syngenta AG MIR162 Corn Litigation as Regulation by Litigation." Tex. A&M L. Rev. 4 (2016): 373.

Goeringer, L. Paul, and Harold L. Goodwin. "An Overview of Arkansas' Right-to-Farm Law." J. Food L. & Pol'y 9 (2013): 1.

Goeringer, L. Paul, H. L. Goodwin, and Michael Popp. "The New Fuel Frontier: Biomass Contracting." Ky. J. Equine Agric. & Nat. Resources L. 5 (2012): 71.

Goeringer, Paul, Ashley Ellixson, and Jon Moyle. "Privacy Issues and the Use of UASs/Drones in Maryland." Drones in Maryland (July 30, 2015) (2015).

Goeringer, Paul, et al. "Understanding the Diverse Legal Needs of the Maryland Agricultural Community." (2014).

Lashmet, Tiffany Dowell. “Eminent Domain in Texas: A Landowner’s Guide.” Texas A&M AgriLife Extension (March 2020).

Lashmet, Tiffany Dowell. “Impact of Conversion of Land from Agricultural Use Property Tax Valuation to Wildlife Use Valuation on the Livestock Industry.” No. 1459-2016-120536. 2015.

Lashmet, Tiffany Dowell. “Owning Your Piece of Texas: Key Laws Texas Landowners Need to Know” Texas A&M AgriLife Extension (May 2019).

Lashmet, Tiffany Dowell, Shannon Ferrell, Rusty Rumley, & Paul Goeringer. “Ranchers Agricultural Leasing Handbook: Grazing, Hunting, & Livestock Leases.” Texas A&M AgriLife Extension (June 2016).

Lashmet, Tiffany Dowell, and Amber Miller. "Texas exempt wells: Where does fracking fit?." Natural Resources Journal 55.2 (2015): 239-268.

Millet-Williams, Nerice, et. al. “2019 Ag Law Education Assessment Evaluation in Maryland.” University of Maryland (August 2019).

Suri, Mayhah, and Paul Goeringer. "Community Supported Agriculture: How do Maryland Operators Manage Legal Risks." Ky. J. Equine Agric. & Nat. Resources L. 9 (2016): 211.

Land Grant Participating States/Institutions

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
Grand Total:	0	0	0

Program/KA	Total FTE
Grand FTE Total:	0

NECC_TEMP2202: Formal Structure for the Minor Use Animal Drug Program

Status: Submitted As Final

Duration	10/01/2022 to 09/30/2027
Admin Advisors:	[Margaret E. Smith]
NIFA Reps:	

Statement of Issues and Justification

The need, as indicated by Stakeholders.

The Minor Use Animal Drug Program (MUADP) currently has eight active projects in therapeutics and production devices for game birds, goats, sheep, and fish that have been requested and are supported by representative stakeholders. Through the nearly 39 years of the Program, stakeholders have requested work on 40 drug/species combinations. During this period the Program has published 43 Public Master Files (PMF) supporting 59 new label claims and has published 211 peer-reviewed, scientific articles. Moreover, 18 stakeholder drug requests continue to represent needy projects. Thus, the stakeholder needs exceed the current resources available to the Program.

The importance of the work, and what the consequences are if it is not done.

United States gross annual farm-gate income from the production of specialty animal species has been estimated by producer groups at over $4.8 billion. Further, these farm gate revenues produce an economic stimulus to the US Gross Domestic Product estimated at another $37 billion. Federal regulations over the concerns of human food safety, inappropriate drug usage, and antimicrobial resistance underscore the need for research in the approval of additional safe and effective therapeutics for our sheep, goat, farmed shrimp and fish, venison, honey, and game birds.

Moreover, recent increasing consumer demand for minor species in the diet will engender greater scrutiny of minor species foodstuffs. Much of our current commercial minor species food and fiber products, however, are imported. One-third of the lamb and 82% of venison consumed in the US comes from Australia and New Zealand. Nearly 90% of the commercially farmed shrimp are imported from Asia. Additionally, two-thirds of the honey consumed in the US is imported and half of that honey comes from China. The MUADP is the only initiative that endeavors to provide the US minor species animal producers with safe and effective therapeutics to compete in a global market while assuring US consumers a safe and wholesome food supply.

Improper drug use in animal agriculture has been identified as one of several major forces that are likely to impact the security of the US agriculture and food system enterprise in the coming years. The National Institute of Food and Agriculture (NIFA) has recognized that a major initiative to protect the biosecurity of our nation's food systems is timely, necessary, and worthwhile. The data provided by the MUADP is an essential part of the NIFA initiative and funding of the Program is critical to performing this function.

Loss of the MUADP will adversely affect the biosecurity and agricultural diversity of our nation's food systems, and create potentially serious disruptions to business and trade. Further, it will also diminish protections for producers, consumers, the food and agricultural system, and the national economy as a whole.

The technical feasibility of the research.

The Program has over 39 years of experience in the development of data to satisfy the U.S. Food and Drug Administration's Center for Veterinary Medicine (FDA/CVM) regulatory requirements for a minor species drug approval. Currently, the Program is comprised of a knowledgeable team with the skills and reputations to network with stakeholders, industry groups, and regulatory agencies. Its physical assets include university-based research facilities with up-to-date technology and a loyal stakeholder base with additional access to commercial production facilities for research support. The university-based structure of the Program provides personnel policies that establish quality performance. Its direct interaction with FDA/CVM personnel ensures adequate protocol design and timely review.

The MUADP accomplishments speak to the technical feasibility of the proposed research, which is structurally similar to its past. The Program has published 43 Public Master Files (PMF) supporting 59 new label claims and has published 211 peer-reviewed, scientific articles during the 39 years of the program.

The advantages for doing the work as a multistate effort.

There are several advantages for the MUADP to function as a multistate effort. These advantages emerge from the requirements of FDA/CVM to perform efficacy studies at multiple regional locations, and the geographically dispersed nature of commercial minor species farming throughout the country. As currently organized, the MUADP is staffed through regional laboratories at colleges of veterinary medicine in each of two regions, the Northeast and North Central. These regions are recognized centers of excellence for minor species. The Northeast is a center of excellence for aquaculture, while the North Central coordinates research in game birds, sheep, and goats. The Regional Coordinators and associated faculty at their colleges have professional relationships with commercial minor species farms in their respective regions. The Cornell University College of Veterinary Medicine has an excellent facility and have conducted many therapeutic trials with marine finfish species including three AQUI-S20E (10% eugenol) target animal safety studies over the last three summers utilizing artificial seawater. In 22Q3 the Northeast Region is funded to test the margin of study for AQUAFLOR (florfenicol) medicated feed on marine finfish as well. Thus, through these relationships, a multistate network of minor species stakeholders exists. Utilizing these networks, stakeholders provide input as to needed therapies and in-kind support for clinical trials. These professional, multistate networks have provided the basis for the dramatic efficiency of the Program.

What the likely impacts will be from completing the work.

Continued maintenance of a formal structure for the MUADP will prevent the loss of the 88 Investigational New Animal Drug files (INADs) at the FDA/CVM that will be scheduled to terminate for lack of a sponsor. The loss of these INADs will result (1) in a collective loss of work estimated at over $5 million and (2) loss of stakeholder incentive to provide support for the Program.

Continued research on the current eight active projects in the program will introduce safe and effective therapeutics for gamebirds, goats, sheep, and fish. The resulting increase in domestic production will decrease the US reliance on imported foodstuffs and help protect the safety and food security of the domestic food supply.

Likely impacts of our current and anticipated projects include (1) protecting human food safety and security; (2) preventing and controlling local zoonotic diseases in both major and minor species; (3) stimulating small farm economy; (4) increasing the diversity of our agricultural base to include important specialty species; and (5) maintaining the health and welfare of agriculturally important specialty animal species in the US.

Objectives

1. Provide the formal structure necessary to maintain the 88 investigational new animal drug INAD files held in the name of the MUADP.
   Comments: Accomplishments to date: The 88 INAD files continue to remain active and support needed drug/species combinations for minor species. The MUADP is currently working with the Minor Use Minor Species (MUMS) program of CVM to obtain sponsors for these outstanding INAD files.
2. Continue Animal and Plant Health Inspection Service, U.S. Department of Agriculture funded research on cattle fever tick and sheep drug approvals.
   Comments: Accomplishments to date: The cattle fever tick project has been completed and data have been shared with Animal and Plant Health Inspection Service, U.S. Department of Agriculture and the manufacturer is working with Food and Drug Administration/Center for Veterinary Medicine on an acceptable manufacturing process. Positive Feeds (The Sponsor) now needs to complete the Chemistry and Manufacturing Controls (CMC) Technical Section of the application. The CMC is only in its initial stages of preparation and should have been done significantly earlier in the study, but due to delays in sponsor submissions, this program has failed to advance. Both CVM and the Program are working to maintain project momentum. It is ideal to have the CMC approved prior to submission of the efficacy study report. The work of the Program is complete for this study. See attached Table 1 for a summary of MUADP active projects.
3. In conjunction with NIFA and stakeholders, identify a stable funding source to work with the FDA/CVM to facilitate their approval of animal health products and provide information for the safe and efficacious use of these materials in specialty animal species.
   Comments: Accomplishments to date: Over the past 18 months, the Program has participated in the formation of the Tactical Sciences Network (TSN) under the direction of Dr. Martin A. Draper, Associate Dean, Research and Graduate Studies, College of Agriculture, Director, Research, K-State Research and Extension, Kansas State University. The TSN is intended to bring together independent projects that are all closely linked to directly benefit end users. The projects are largely associated with biosecurity, touching on plant health, animal health, antibiotic resistance, animal drugs for less common livestock species, tracking drug residues in livestock, food safety, education for disaster response and various levels of crop pest management which include researchers, regional clearinghouses or centers and state programs. Through this new association, the Program has submitted a budget of $2.5M per year to complete work on several of the current active projects. Funding is anticipated through NIFA.

Procedures and Activities

Grants

Dr. Rodman G. Getchell has obtained funding of $200,000 over two years through U.S. Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA), and Agriculture and Food Research Initiative (AFRI): USDA-NIFA-AFRI-Funding Opportunity Number 007052 in the animal health program area under “Therapeutic interventions for disease reduction or treatment.” Researchers within the Cornell University Aquatic Animal Health Program and USFWS Aquatic Animal Drug Approval Partnership (AADAP) program will continue a collaboration to conduct studies to increase the number of safe and effective drugs that can be used to benefit the U.S. aquaculture community. Trials will be conducted with Florfenicol (AQUAFLOR®), Oxytetracycline (TM200), and Hydrogen peroxide (Perox-Aid®), which are used to treat diseases and parasites of fish.

Drs. Griffith and Kreuder have been granted $500,000 over two years: Kreuder AJ, Plummer PJ, Smith JS, Griffith RW. Efficacy of tulathromycin for treatment of respiratory disease in goats: a final step towards label approval. USDA NIFA. 7/1/21 - 6/30/23. $499,872.

Work accomplished under the original project

- As part of the coordinated effort to generate data to support approval by FDA/CVM, Cornell University researchers has conducted a series of studies under a recently submitted protocol to FDA/CVM (AQS20E-18-SEA-TAS.2b) to evaluate the safety of AQUI-S®20E (10% eugenol) used to sedate marine fish to the handleable stage of anesthesia in saltwater. The objective of these studies is to evaluate the safety of AQUI-S®20E to saltwater-reared marine finfish when overexposed to either the HEC (1×) or 1.5× the highest proposed efficacious dose. The results of these safety studies will be used to complete the target animal safety (TAS) technical section data requirements for marine finfish and ultimately support a New Animal Drug Application to FDA/CVM to approve the use of AQUI-S®20E for sedation in all finfish.
- The Striped Bass Final Study Report has been prepared and submitted to the FD/CVMA "drug sponsor", which is different from the NIFA funding support. The report, photos, and summary tables are 160 pages long and the appendices with additional tables, figures, data analyses, and histopathology and quality assurances reports are included in the next 700 pages. This large document is required as part of the technical section for addressing animal safety, which is needed for eventual licensing of the new sedative under study in this project.
- In the second year of our USDA NIFA study titled, “The Safety of AQUI-S®20E as a Sedative on a Variety of Marine Finfish at Cornell University” we have made major progress responding to FDA requests for information about our data from the striped bass trials we collected in 2019 and we were able to conduct the Florida pompano trials even with the delays getting access to our laboratories because of COVID-19. We have completed the histological evaluations, most of the data analysis, final study report writing, and presenting our results to our stakeholders during the virtual AADAP Meeting that occurred August 27-29, 2021. Manuscript preparation has started, but is waiting for all the second-year data to be tabulated and analyzed.
- The Target Animal Safety technical section entitled, The Safety of AQUI-S® 20E as a Sedative on a Variety of Marine Finfish” at Cornell University. (Study Number 2019-001) was accepted at FDA/CVM and the Summary Freedom of Information document is being prepared at the Agency.
- Strontium Chloride immersion for salmon: MUADP continues collaboration with our research partner, Syndel USA, to generate the data necessary to obtain FDA approval of strontium chloride hexahydrate immersion for the skeletal marking of freshwater salmonid fry and fingerlings.Fishery managers need a method to chemically mark salmon to determine migration patterns while avoiding the stress of tagging or other physical markings. While there are other drug products approved for skeletal marking in salmon, this project provides a non-antimicrobial alternative.
    - Target Animal Safety: Dr. Rod Getchell, Northeast Regional Coordinator for the MUADP, completed a GLP Target Animal Safety study evaluating strontium chloride hexahydrate immersion for the skeletal marking of freshwater salmonid fry and fingerlings.
    - In May 2019, CVM determined that the Target Animal Safety technical section was complete for this indication.
- Fenbendazole in quail: MUADP continues collaboration with our research partners, Dr. Ron Kendall of Texas Tech University and Merck Animal Health, to generate the data necessary to obtain FDA approval of fenbendazole-medicated feed for the treatment of nematodes in quail. This project has impact for both wild quail and farmed quail. Wild quail populations have declined by more than 85% in the last five decades. While many causes for the decline have been proposed, this project addresses the potential role of parasitism. Field studies conducted by Dr. Kendall have shown that fenbendazole is effective against common parasites in quail. The ongoing research will also support FDA approval for the use of fenbendazole in farmed quail. There are currently no anthelmintics approved for use in quail. Successful completion of this project will provide access to a class of therapeutic drugs previously unavailable to quail farmers.

Other work completed or ongoing

Fenbendazole/pheasants:

Human Food Safety = COMPLETE

Target Animal Safety = COMPLETE

Environmental Impact = FDA/CVM reviewing the Environmental Assessment

Controlled Internal Drug Release (CIDR) device that releases progesterone /goats:

Human Food Safety = COMPLETE

Target Animal Safety = COMPLETE

Environmental Impact = COMPLETE

Effectiveness = Current plan is to write up the study and submit to FDA/CVM

Erythromycin/salmonids:

Human Food Safety = COMPLETE

Target Animal Safety = COMPLETE

Environmental Impact = Environmental assessment requires administrative revisions

Effectiveness = Work ongoing on White Paper to reaffirm the technical section complete letter received in 1998, so that the indication can be broadened to all salmonids, as opposed to only Chinook

Work that is incomplete, or areas in need of further investigation

- The first year of our AQUI-S 20E target animal safety study with Yellow Clownfish is underway.
- Tulathromycin study to demonstrate efficacy in sheep and goats.
- Lasalocid develop residue method and resubmit target animal safety.
- The Program will continue to review and prioritize active projects in conjunction with stakeholder needs.
- Establish stronger outreach to stakeholders as drug approvals continue to increase; and coordinate with CVM Minor Use Minor Species (MUMS) program to work with new, interested sponsors to address some of the 88 outstanding files.

Publications

Getchell, Rodman G., D.M. Scott, B.M. Chambers, N. Wandelear, P.R. Bowser, P. Baneux, D. Kirby, H. Marquis, and M. Blair. 2020. The Safety of AQUI-S® 20E (10% Eugenol) as a sedative on marine fish. Aquaculture America 2020, February 9-12, 2020, Honolulu, HI.

Getchell, Rodman G., Danielle M. Scott, Brian M. Chambers, Niccole Wandelear, Paul R. Bowser, Philippe Baneux, Drew Kirby, Hélène Marquis, and Marilyn Blair. 2019. The safety of AQUI-S® 20E (10% Eugenol) as a sedative on a marine finfish. Aquatic Animal Drug Approval Partnership (AADAP) Program U.S. Fish and Wildlife Service,August 31, 2019 Bozeman, MT

Gorden P, Ydstie J, Kleinhenz M, et al.: Comparative plasma and interstitial fluid pharmacokinetics and tissue residues of ceftiofur crystalline free acid in cattle with induced coliform mastitis. Journal of Veterinary Pharmacology and Therapeutics. 2018;41.

Smith J, Borts D, Griffith R, Mochel J: Pharmacokinetics of tulathromycin in healthy goats and goats with induced Pasteurella multocida pneumonia2018.

Smith J, Mochel J, Borts D, Griffith R: Effects of experimentally induced respiratory disease on the pharmacokinetics and tissue residues of tulathromycin in meat goats. Journal of Veterinary Pharmacology and Therapeutics. 2019;42.

Smith J, Mochel J, Seo Y-J, Ahrens A, Griffith R: Evaluation of a Pasteurella multocida Respiratory Disease Induction Model for Goats (Capra aegagrus hircus). Vol 702020.

Smith J, Viall A, Breuer R, et al.: Preliminary Investigation of Bovine Whole Blood Xenotransfusion as a Therapeutic Modality for the Treatment of Anemia in Goats. Frontiers in Veterinary Science. 2021;8:1-7.

Xia J, Pang J, Tang Y, et al.: High prevalence of fluoroquinolone-resistant Campylobacter in sheep and increased Campylobacter counts in the bile and gallbladder of sheep medicated with tetracycline in feed. Applied and Environmental Microbiology. 2019;85.

Yaeger M, Mochel J, Wu Z, et al.: Pharmacokinetics of tulathromycin in pregnant ewes (Ovis aries) challenged with Campylobacter jejuni. PLOS ONE. 2021;16:e0256862.

Yaeger M, Wu Z, Plummer P, et al.: Experimental evaluation of tulathromycin as a treatment for Campylobacter jejuni abortion in pregnant ewes. American journal of veterinary research. 2020;81:205-209.

Publication in preparation

Getchell, Rodman G., et al.: Three AQUI-S20E (10% eugenol) target animal safety studies are in preparation for publication in 2022, and the AQUAFLOR (50% florfenicol) marine finfish safety research will be submitted for publishing in 2023-2024.

Expected Outcomes and Impacts

- The Program will continue to work with the TSN under the direction of Dr. Martin A. Draper, to establish a more reasonable and secure source of funding through National Institute of Food and Agriculture.
- The continued development of drug approvals for minor species through the MUADP will contribute to addressing critical issues as noted below. Comments: • Protect human food safety and biosecurity • Prevent and control local zoonotic diseases in both major and minor species • Stimulate small farm economy • Increase the diversity of our agricultural base to include important specialty species • Ensure the health and welfare of agriculturally important specialty animal species in the US.

Educational Plan

Education of stakeholder groups through peer-reviewed publications and presentations of data generated by the Program are used to combat the disinformation on minor species therapeutics and husbandry generated through social media. These efforts create feedback loops enabling robust stakeholder interactions thereby increasing cross collaborations/coordination between different minor species organizations.

The Program also provides educational opportunities for graduate students, interns and summer fellows. For example, the 2019 AQUAVET® Summer Fellow, DVM student Danielle Scott returned for a second summer and took on evenmore responsibility conducting the target animal safety study utilizing Good Laboratory Practices. Ms. Scott has drafted the materials and method section of the upcoming journal publication. A second DVM, Kyara Moran Cornell Class of 2023, completed many of the 2020 data tables during COVID from her home in Miami.

Organization/Governance

The recommended Standard Governance for multistate research activities includes the election of a Chair, a Chair-elect, and a Secretary (Dr. John Babish). All officers have maintained their positions over the course of the Program and will continue through the next term to provide continuity. Administrative guidance is provided by an Administrative Advisor, Dr. Margaret Smith, and a NIFA Representative, Dr. Tim Sullivan.

The interrelationship of the groups involved is represented schematically in Figure 1 (see attachments). The Coordinating Committee in Figure 1 consists of representatives of the universities, and stakeholders (FDA/CVM, producers and pharma). Responsibilities of the Coordinating Committee include the selection of drug/species research and consulting on progress of the studies. Members also disseminate information on the program through presentations, publications and social media. Zoom meetings of AA, NIFA, FDA/CVM and university personnel are held roughly every six to eight weeks. Program representatives also participate in Zoom meetings of the Tactical Sciences Network under the direction of Dr. Martin A. Draper, Associate Dean, Research and Graduate Studies, College of Agriculture, Director, Research, K-State Research and Extension, Kansas State University.

All MUADP studies are conducted under a series of government regulations and standards. These include FDA Good Laboratory Practice for Nonclinical Laboratory Studies (GLP), Good Clinical Laboratory Practice (GCLP), and Institutional Animal Care and Use Committee (IACUC) regulations. Few academically-based programs have qualified to meet such an array of regulations successfully.

Literature Cited

Tactical Sciences Network proposal: [https://portal.nifa.usda.gov/web/crisprojectpages/1019623-tactical-sciences-coordination-network-to-enhance-plant-and-animal-health-and-biosecurity.html]

U.S. total lamb and mutton imports and exports from 2006 to 2021[https://www.statista.com/statistics/194707/us-total-lamb-and-mutton-imports-and-exports-since-2001/]

US Shrimp Imports - Study of Shrimp Market with Shrimp Buyers in US[https://www.exportgenius.in/blog/us-shrimp-imports-study-of-shrimp-market-with-shrimp-buyers-in-usa285.php#:~:text=Shrimp%2C%20which%20comes%20under%20HS%20Code%20030617%2C%20is,metric%20tons%20of%20shrimps%20from%20the%20global%20countries]

USA imports 60% of its honey [https://soltegra.com.mx/en/the-united-states-leads-the-world-in-honey-imports/]

Land Grant Participating States/Institutions

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
Grand Total:	**0**	**0**	**0**

Program/KA	Total FTE
Grand FTE Total:	**0**

Appendix J1: CC Evaluation (Submitted)

Status: Complete
Project ID / Title:
NECC_TEMP2202: Formal Structure for the Minor Use Animal Drug Program

Questions

1. Goals and objectives clearly stated and appropriate to committee activity(s)	**Excellent**
2. There is a good potential to attain the objectives and plan identified in the activity.	**Excellent**
3. Activity addresses priority research and is not duplicative with existing activities.	**Excellent**
4. Activity has moved beyond individual activity(s) and ideas to a collective, interdependent activity.	**Excellent**
For renewal projects only:	
5a. Attendance of the preceding project has been adequate and reflects broad participation by designated project participants.	**Excellent**
5b. The project has developed and demonstrated technology transfer to clientele.	**Excellent**

Recommendation
Approve/continue with normal revision.
Comments:
The proposed multi-state project NECC_TEMP2202 (Formal Structure for the Minor Use Animal Drug Program) should be continued because of the importance of the proposed work: 1) to the protect the integrity of the US food supply, 2) to ensure the health and well-being of agriculturally important minor food animal species, 3) to enhance the diversity of food sources available in the US (thus minimizing reliance solely on animal source foods from cattle, hogs, and poultry; think of the impact COVID-19 had on those industries), and 4) to stimulate rural economic development. There is an impressive track record of this group of scientists, and stopping the project now would not only represent a waste of scarce financial resources previously invested in the program but also cause an immediate cessation to projects that are nearing completion. This project is vitally important to US agriculture, and additional approvals of products for use in minor species should lead to growth in those industries and concomitant decrease in reliance on food imports into the US.

Appendix J1: CC Evaluation (Submitted)

Status: Complete
Project ID / Title:
NECC_TEMP2202: Formal Structure for the Minor Use Animal Drug Program

Questions

1. Goals and objectives clearly stated and appropriate to committee activity(s)	**Excellent**
2. There is a good potential to attain the objectives and plan identified in the activity.	**Excellent**
3. Activity addresses priority research and is not duplicative with existing activities.	**Excellent**
4. Activity has moved beyond individual activity(s) and ideas to a collective, interdependent activity.	**Excellent**
For renewal projects only:	
5a. Attendance of the preceding project has been adequate and reflects broad participation by designated project participants.	**Excellent**
5b. The project has developed and demonstrated technology transfer to clientele.	**Good**

Recommendation
Approve/continue with normal revision.
Comments:
The objectives are well stated and are clear and concise. Portions of the objectives have been achieved and there is a good to excellent chance to attain most if not all of the objectives and plan as written. This work definitely addresses a critical need generating information for the approval of drugs for the use in minor species. The dearth of approved drugs that are available for used in minor species is a critical control point that continues to stymie the growth and threatens the survival of these relatively small but important agricultural elements of our food system. The work outlined in this program is not duplicative and is sorely needed. Researchers involved with this program should be lauded for having successfully obtained USDA-NIFA grants to help fund projects but additional funding is likely to be needed and is noted in the expected outcomes and impacts. In addition, these grants have allowed for the movement from individual activity(ies) to development of collective interdependent activity with that involvement collaborators from other institutions/agencies/pharmaceutical companies. Besides the publications listed, presentations based on the data generated from this program have been used to battle the misinformation about therapeutics/drug use in minor species that is often present in social media. There has also been a modest but successful educational effort and there are plans for this to continue in the immediate future.

Appendix J1: CC Evaluation (Submitted)

Status: Complete
Project ID / Title:
NECC_TEMP2202: Formal Structure for the Minor Use Animal Drug Program

Questions

1. Goals and objectives clearly stated and appropriate to committee activity(s)	**Excellent**
2. There is a good potential to attain the objectives and plan identified in the activity.	**Good**
3. Activity addresses priority research and is not duplicative with existing activities.	**Good**
4. Activity has moved beyond individual activity(s) and ideas to a collective, interdependent activity.	**Excellent**
For renewal projects only:	
5a. Attendance of the preceding project has been adequate and reflects broad participation by designated project participants.	**Excellent**
5b. The project has developed and demonstrated technology transfer to clientele.	**Excellent**

Recommendation
Approve/continue with revision (provide specific recommendations in Comments below).
Comments:
Overall, the project is done many things with a minimalistic budget. It may be very helpful to consider involving the product owners (NADA Sponsors) more fully in your activities. Being responsible for providing updates to CVM for several of these projects and to maintain their MUMS designation, it would be extremely helpful to be more fully updated on the progress of these projects. It is only through information received from CVM's MUM's group that I have any idea of project progress up until I read this report. It might be helpful for sponsors to be invited during reviews of their projects. I have been included in several meeting for one project and as the owner of the NADA, i have access to a great deal of information on the approval in the major species and therefore I am able to provide information that can be helpful to the research team that could both minimize cost and help speed these projects. I believe that the NADA owner could greatly assist these projects. I also suggest clarification about CIDR. This is an intravaginal device that release progesterone. The current description seems to indicate to the contrary. .

Appendix J1: CC Evaluation (Submitted)

Status: Complete
Project ID / Title:
NECC_TEMP2202: Formal Structure for the Minor Use Animal Drug Program

Questions

1. Goals and objectives clearly stated and appropriate to committee activity(s)	**Excellent**
2. There is a good potential to attain the objectives and plan identified in the activity.	**Excellent**
3. Activity addresses priority research and is not duplicative with existing activities.	**Excellent**
4. Activity has moved beyond individual activity(s) and ideas to a collective, interdependent activity.	**Excellent**
For renewal projects only:	
5a. Attendance of the preceding project has been adequate and reflects broad participation by designated project participants.	**Excellent**
5b. The project has developed and demonstrated technology transfer to clientele.	**Excellent**

Recommendation
Approve/continue with normal revision.
Comments:
The Minor Use Animal Drug Program (MUADP) has been a critical part of moving drug approvals forward with US FDA-CVM (Center for Veterinary Medicine) for minor species. These include all species other than the seven major species such as cattle, horses, dogs and cats. Very few approved pharmaceuticals are in the minor species tool box. MUADP's research, coordination, and contribution have been critical to moving forward with new pharmaceuticals and/or extending claims for the few pharmaceuticals that do exist for these species. This proposal will address the ongoing and growing need for approved therapeutants in minor species raised for food. Included are therapeutants such as antibiotics as well as sedatives, analgesics and anesthetics necessary for humane handling and welfare. Over the past years MUADP has clearly demonstrated the capacity to conduct regulatory research and to coordinate efforts required to meet the high standards of GLP and GMP now required for drug approvals. Peer reviewed publications have helped to educate end about safe and efficacious use of these therapeutants. This solid foundation, plus the capacity to work with diverse academic institutions, is much needed to continue to address health and welfare of minor species raised for food.

NECC_TEMP2202: Formal Structure for the Minor Use Animal Drug Program
Responses, Revisions and Comments to Peer Reviews

The Minor Use Animal Drug Program would like to thank all of the reviewers for their considered evaluation of our proposal NECC_TEMP2202. Our responses, revisions, and comments are presented below and, where applicable, are incorporated into the revision.

GK Review (Annotated)

Comment GK: It might be repetitive below but I would suggest making reference to the number of label claims in which MUADP was involved, since the number of technical sections and Public Master Files completed and accepted by CVM represent the measurable, objective endpoint.

Response: The following sentences were added: "During this period the Program has published 43 Public Master Files (PMF) supporting 59 new label claims and has published 211 peer-reviewed, scientific articles. Moreover, 18 stakeholder drug requests continue to represent needy potential projects.

Comment GK: Inserted "food"

Response: The insert was changed to "food and fiber" to reflect wool-producing sheep and angora goats.

Comment GK: This paragraph doesn't really address what MUADP has been able to do - the next paragraph only talks about what happens if MUADP does not get this support. MUADP is an essential part of this important initiative by ensuring a safe food supply.

Response: Response: The following sentence was inserted: "The data provided by the MUADP is an essential part of the NIFA initiative and funding of the Program is critical to performing this function".

Comment GK: Does the MUADP group have facilities to do marine (saltwater) studies? Offshore aquaculture appears to be getting the green light so it might be good to add this in as part of the networking effort.

Response: The following was added: "The Cornell University College of Veterinary Medicine has an excellent facility and have conducted many therapeutic trials with marine finfish species including three AQUI-S20E (10% eugenol) target animal safety studies over the last three summers utilizing artificial seawater. In 22Q3 the Northeast Region is funded to test the margin of study for AQUAFLOR (florfenicol) medicated feed on marine finfish as well."

Comment GK: ...and help protect the safety/food security of the domestic food supply

Response: The suggested wording was added.

Comment GK: Might want to say how MUADP intends to move these forward - e.g., is the plan to look for sponsors to support these products?

Response: This sentence was added: "The MUADP is currently working with the Minor Use Minor Species (MUMS) program of CVM to obtain sponsors for these outstanding INAD files."

Comment GK: This is a problem with the sponsor not MUADP. Might be helpful to say MUADP has kept the project on track.

Response: This wording was added: "but due to delays in sponsor submissions, this program has failed to advance. Both CVM and the Program are working to maintain project momentum."

Comment GK: To enhance the impact of MUADP's role can you say something here about how the MUADP and Sponsor's efforts are complementary for the TAS section. They are both key to the ultimate end goal which is to get all the technical sections approved for a drug approval. MUADP has been very important in coordinating this effort.

Response: This sentence was added: "The efforts of the MUADP and Sponsor are complementary for the TAS section. They are both key to the completion and approval of all the technical sections required for a drug approval. MUADP has been essential in coordinating this effort."

Comment GK: Is the funding group to whom this application is being sent aware of how much time and effort is required for each of these Technical Sections? Typically they take 1-2 years of hard work and a lot of back-and-forth'ing with FDA CVM. It might be helpful to say that somewhere in the text - you have a lot of successes listed here that shouldn't be undersold!

Response: Yes, from the beginning each sponsor is familiar with the time and effort required for the interactions with the FDA/CVM. Since Program eligibility requires a major species approval, the sponsors have previously been through the system.

Comment GK: Explain the acronym (CIDR)

Response: "Controlled Internal Drug Release (CIDR) device that releases progesterone /goats" was added

Comment GK: Maybe also say something about continuing to work with new/interested sponsors to address some of the outstanding files?

Response: The following was added to the final bullet point under incomplete work: "Coordinate with CVM Minor Use Minor Species program to work with new, interested sponsor to address some of the 88 outstanding files."

Comment GK: Should this one be listed above under "Other work completed or ongoing"

Response: The lasalocid residue method development work is technically incomplete and "on hold" awaiting funding. What is required is a bridging, validation study from the major species.

Comment GK: Are there publications in preparation for release in 2022 that could also be listed?

Response: The following was added: "In preparation: Getchell, Rodman G., et al.: Three AQUI-S20E (10% eugenol) target animal safety studies are in preparation for publication in 2022, and the AQUAFLOR (50% florfenicol) marine finfish safety research will be submitted for publishing in 2023-2024."

Comment GK: General comment - do you want to say something about standards to which the FDA-CVM studies are held? Specifically, MUADP studies have to meet GLP and GMP, which are difficult, document heavy standards, so you might want to indicate that meeting these is a real achievement. Also these studies at academic institutions need to meet IACUC standards for animal welfare so that might be worth mentioning too. Not everyone can meet both of these sets of standards.

Response: The following was inserted: "All MUADP studies are conducted under a series of government regulations and standards. These include FDA Good Laboratory Practice for Nonclinical Laboratory Studies (GLP), Good Clinical Laboratory Practice (GCLP), and Institutional Animal Care and Use Committee (IACUC) regulations. Few academically-based programs have qualified to meet such an array of regulations successfully."

Reviewer 1

Comments: There is an impressive track record of this group of scientists, and stopping the project now would not only represent a waste of scarce financial resources previously invested in the program but also cause an immediate cessation to projects that are nearing completion. This project is vitally important to US agriculture, and additional approvals of products for use in minor species should lead to growth in those industries and concomitant decrease in reliance on food imports into the US.

Response: Thank you for the recognition of our uniqueness and importance to US agriculture. As noted above, the Program operates under a spectrum of regulations that, in combination, are generally not found in academic institutions. Continued functioning of the Program should not only lead to growth in minor species industries and a decrease in reliance on food imports, but enhance biosecurity for the US food supply.

Reviewer 2

Comments: Researchers involved with this program should be lauded for having successfully obtained USDA-NIFA grants to help fund projects but additional funding is likely to be needed and is noted in the expected outcomes and impacts. In addition, these grants have allowed for the movement from individual activity(ies) to development of collective interdependent activity with that involvement collaborators from other institutions/agencies/pharmaceutical companies. Besides the publications listed, presentations based on the data generated from this program have been used to battle the misinformation about therapeutics/drug use in minor species that is often present in social media. There has also been a modest but successful educational effort and there are plans for this to continue in the immediate future.

Response: As the Reviewer has pointed out, our education efforts have been modest. The Program has been working with the Tactical Sciences Network to enhance our outreach and educational activities through them.

Reviewer 3

It may be very helpful to consider involving the product owners (NADA Sponsors) more fully in your activities. Being responsible for providing updates to CVM for several of these projects and to maintain their MUMS designation, it would be extremely helpful to be more fully updated on the progress of these projects. It is only through information received from CVM's MUM's group that I have any idea of project progress up until I read this report. It might be helpful for sponsors to be invited during reviews of their projects. I have been included in several meeting for one project and as the owner of the NADA, i have access to a great deal of information on the approval in the major species and therefore I am able to provide information that can be helpful to the research team that could both minimize cost and help speed these projects. I believe that the NADA owner could greatly assist these projects. I also suggest clarification about CIDR. This is an intravaginal device that release progesterone. The current description seems to indicate to the contrary.

Response: The Reviewer correctly identified a significant shortfall of the program over the last three years. Communications with shareholders have been left to Regional Coordinators and CVM liaisons. This format does little to educate shareholders about the breadth of the operations. The Program is working with the Tactical Sciences Network to establish a more formal system to enhance shareholder and public knowledge of our work. As noted above, the following was added to the description: "Controlled Internal Drug Release (CIDR) device that releases progesterone /goats."

Reviewer 4

Over the past years MUADP has clearly demonstrated the capacity to conduct regulatory research and to coordinate efforts required to meet the high standards of GLP and GMP now required for drug approvals. Peer reviewed publications have helped to educate end about safe and efficacious use of these therapeutants. This solid foundation, plus the capacity to work with diverse academic institutions, is much needed to continue to address health and welfare of minor species raised for food.

> *Response: Thank you for your recognition of one of the hidden features of the Program - the MUADP operates under a spectrum of regulations that, in combination, are generally not found in academic institutions. As such we are unique and we would be difficult and expensive to replace or replicate.*

NE_TEMP2202: The Equine Microbiome

Status: Submitted As Final

Duration	10/01/2022 to 09/30/2027
Admin Advisors:	[K. Eric Wommack]
NIFA Reps:	

Statement of Issues and Justification

The stakeholders who will be served by the Equine Microbiome Multistate Research Project include: microbiome researchers, undergraduate and graduate students, horse owners/managers, veterinarians, equine industries (e.g., feed producers, nutritionists, behaviorists, competitors) equine enthusiasts (e.g., youth groups and spectators), and underserved communities (e.g., rural horse owners, undergraduate and graduate students with limited access to bioinformatics, biostatistics, or data resources).

The impact of the gut microbiome has been a popular press topic for human and animal care. Despite the popularity and availability of products purporting to positively impact health through modulating the gut microbiome, research to understand the details of the functional impact of the microbiome on horse health and disease is still in its infancy. A search of PubMed using the terms “equine” and “microbiome” yielded only 320 results. Publication in this area has increased exponentially since 2007.

This project will address the following needs to bridge the gaps between equine microbiome researchers, equine industries, students, and the public:

- Advance the knowledgebase of equine microbiome science with robust, statistically significant sample numbers and diversity.
- Communicate advances in microbiome science among researchers, students, and the public.
- Refine and test protocols and standardized methods for researchers to enable collaboration, data sharing, and greater utilization of samples and data.
- Provide translational connections between the equine gut microbiome and health parameters, nutrition, management, and performance that are based on research specific to horses.

The importance of this work is to establish a coordinated and collaborative network of equine microbiome researchers and to provide resources for students and the public that reflect the state of the science.

The consequences of not establishing the Equine Microbiome Multistate Group are:

- Confusion regarding the state of equine microbiome science among stakeholders leading to the spread of misleading information, poor purchasing and management decisions, uncertainty regarding efficacy of products and research strategies.
- The continued lack of coordination among researchers, which is slowing the progress and efficiency of the science due to smaller sample sizes (horsekeeping is expensive relative to other species), inconsistent sampling and analysis protocols, inconsistent training of students, inability to share data.
- Lack of understanding of the unique aspects of the equine microbiome and continued application of therapeutic and nutritional strategies from research on other species such as ruminants and humans that may be ineffective or have adverse effects on the unique equine gastro-intestinal tract.

The Equine Microbiome Multistate Group will leverage the depth of technical expertise, range of laboratory, sequencing, and animal facilities, diversity of horse herds, extensive archival sampling efforts, training capacities, and stakeholder support of each member institution. As a nation-wide effort, this group will maximize impact of data/samples for multiple studies, reach numbers needed for statistical power, mitigate geographic bias, and coordinate host and microbial connections.

As a Multistate effort, this project will be able to:

- Investigate the role of the microbiome in equine health by describing the structural and functional properties of the “normal” equine microbiome.
- Test hypotheses of microbiome structure and function related to management, life stages, and disease states.
- Build and test statistically rigorous models of host-microbiome interactions.
- Establish a network for training students, outreach to the public, veterinary and equine industry education in microbiome science.
- Create print and web-based resources for the equine community (public, education, veterinary and equine industry) to increase understanding of microbiome science and its applications to improving horse health and management.
-

The likely impacts of successful completion of this work are:

- For Researchers,

    1. The opportunity to work together to develop grant proposals and experimental collaborations to explore research questions focused on understanding the structure and function of the equine microbiome in health and disease. Through this multistate we will share expertise, horse samples, and technical assistance for basic and translational experimental approaches.
    2. The establishment of a shared database of metadata, sequence data and samples will increase the statistical power and efficiency of hypothesis testing and enable a baseline for the “normal” equine gut microbiome.

- For Students,

    1. The shared database will provide opportunities to formulate and test hypotheses and increase research participation, especially for students from institutions with limited access to research facilities.
    2. Training in bioinformatics, biostatistics, microbial ecology, and host physiology will enable students to apply knowledge and techniques to real-world questions in the equine industry.
    3. The opportunity to participate in conferences and workshops around equine microbiome science will enable students to widen their network, practice presentation skills, and gain confidence in their futures as scientists.

- For Veterinarians and Nutritionists,

    1. Equine microbiome insights will inform clinical practice, specifically understanding the impact of the microbiome on horse health and welfare and advice given to horse owners regarding management, feeding, and modulating the microbiome via pre/probiotics or antibiotics.
    2. Observations made by clinical veterinarians will provide essential questions and insights for microbiome researchers.
    3. Conference and workshop participation will enable veterinarians to share insights, clinical strategies, questions, and case studies.
    4. Creation of evidence-based web and print resources to translate equine microbiome science for better health outcomes.
    5. Equine nutritionists and feed manufacturers will gain insight to how the diet, microbiome, and equids interact with the potential to guide feeding recommendations and feed formulation for improved outcomes (health, welfare, and performance).

- For Horse Owners and Enthusiasts,

    1. Greater understanding of the impacts of diet, management, and other factors on microbiome health, and ultimately the welfare of horses.
    2. Deeper knowledge of specific feed ingredients, pre/pro/postbiotics will enable horse owners to make informed decisions in purchasing horse care products, supplements, and feeds.
    3. Conference and workshop participation will help to dispel myths about the impacts of feeding and management practices on the microbiome, and promote equine welfare through communication of research based findings.
    4. Creation of evidence-based web and print resources to translate equine microbiome science for better management decisions.
    5.

Related, Current and Previous Work

The equine gut microbiome plays a crucial role for the horse in accessing nutrients and energy. As hindgut fermenting animals, horses have limited endogenous enzyme capacity in the foregut to digest and absorb nutrients, instead relying on communities of microbes in the caecum and colon to break down complex plant material and ferment simple sugars and amino acids to short chain fatty acids that can be used by the horse for energy. Unlike ruminants that are known for digestive efficiency (50-60% DM digestibility of forage), the horse is less successful at extracting nutrients (39-48% DM digestibility of forage)[1]. To maintain energy levels for their large body size, a high rate of intake is required. Since approximately 70% of the equine energy budget is supplied by microbial fermentation products [2,3], this activity is a crucial component of the horses' energy budget.

Studies to characterize the structure of the equine gut microbiome show that the equine gut microbiome is dominated by Firmicutes and Bacteriodetes, with lesser abundance of other groups such as Spirochaeta, Proteobacteria, Fibrobacter, and Archaea [4–7]. Relative abundances of members of equine gut microbiome are sensitive to diet [4–7], stress [8–10], immune status [11], and age [12,13], and prebiotics and probiotics formulated and marketed to modulate the equine gut microbiome show variable results [14–17].

Most published equine microbiome studies are based on relatively few samples. Efforts to combine datasets to increase statistical power are constrained by inconsistent sampling and/or sequencing methods, confounding factors (diet or environment), and differences in duration or other conditions. Efforts such as the Human Microbiome Project [18,19], the American Gut Project [18–20], and the development of the Dog Dysbiosis Index [21] demonstrate the power of a coordinated, collaborative approach to microbiome research.

As equine microbiome science moves from compositional surveys to functional studies seeking to understand the details of the conversations between the microbiome and the horse host, the formation of this Multistate Research group will enable us to expand our sampling and experimental design possibilities through leveraging the diversity of horse herds, environmental conditions and dietary strategies available to our members. At the same time, we bring a depth and breadth of expertise to bear in microbial ecology, genomics, genetics, bioinformatics, clinical veterinary practice, equine management, nutrition, and biostatistics. The coordination of sampling protocols and the establishment of a shared database of samples and metadata will provide resources and lasting benefits to the equine science community.

Objectives

1. Identify the components and functionalities of the "normal" equine gut microbiome.
2. Identify how specific factors: diet, age, metabolic diseases, inflammation, stress and others impact the structure and function of the equine microbiome, and examine the role of the equine microbiome in management, maintaining horse health, and disease prevention.
3. Explore how the genetic factors of horses are related to and interact with the gut microbiome.
4. Disseminate results and applications of microbiome science for equine welfare and management through stakeholder outreach and education, including youth groups, horse owners, nutritionists, and veterinarians.
5. 5. Create print and web-based resources for the equine community (public, education, veterinary and equine industry) to increase understanding of microbiome science and its applications to improving horse health and management.

Methods

Objective 1: Identify the components and functionalities of the “normal” equine gut microbiome through comparative surveys, *in vitro* and *in vivo* approaches.

Core community structure will be explored through sequence-based 16S and shotgun metagenomic surveys. Culture-based approaches will be used to investigate functional aspects of equine microbiome communities including in vitro challenge trials and in vivo measurements of nutrient acquisition, digestibility, and metabolite production.

To leverage the maximum number of horses and regions, we will establish methods for equine microbiome sampling, reporting metadata, and data analysis to enable robust comparisons between studies, efficiency in sample utilization and synthesis of datasets. Datasets will be provided to the research community through an equine microbiome database to facilitate sharing of data from a range of samples. Sampling and reporting methods will be with established metadata standards and ontologies tailored to the unique aspects of equine management and use.

Objective 2: Identify how specific factors: diet, age, metabolic diseases, inflammation, stress, environment and others impact the structure and function of the equine microbiome and examine the role of the equine microbiome in management, maintaining horse health, and disease prevention.

Associations between microbiome differences and horse health parameters will have greater statistical power as the database grows. Sequence and culture-based experiments based on hypotheses generated from the survey data will seek to understand the roles of the microbiome in maintaining equine health. This effort will focus on three areas: a) Normal life stage factors such as age, breed, gender and others b) Management factors such as diet, workload, stress, environment and others, c) Disease factors such as parasitism, metabolic diseases, inflammation, and others.

Objective 3: Study how the genetic factors of horses are related to and interact with the gut microbiome.

To explore the horse side of the host-gut microbiome conversation, genotype and phenotype analysis of equine samples including feces, blood, hair, saliva, gastric fluid, gastric endoscopy results, vaginal swabs, semen and others will be correlated with sequence and culture-based data to develop and test models of host-gut microbiome interactions.

Objective 4: Disseminate results and applications of microbiome science for equine welfare and management through stakeholder outreach and education, including youth groups, horse owners, nutritionists and veterinarians.

We will develop and share workshop series/other educational events for stakeholders in the area of equine microbiome science. Additionally, we will organize a yearly conference segmented to target multiple stakeholder audiences to develop collaborations, share data analysis strategies, disseminate and interpret findings.

Objective 5: Create print and web-based resources for the equine community (public, education, veterinary and equine industry) to increase understanding of microbiome science and its applications to improving horse health and management.

As understandings of the impacts of the equine microbiome on horse health are shown by the data (generated by this project or elsewhere), we will create and disseminate print and web based materials to enable each stakeholder group to translate findings into more informed decision making for care and management.

Measurement of Progress and Results

Outputs

- 1. Written protocols (SOPs) for Equine Microbiome studies: sampling, metadata collection and analysis
- 2. Database of samples/ metadata that is searchable and shared with the equine microbiome research community.
- 3. Organization of workshops for stakeholders and a biyearly Equine Microbiome Conference for the scientific, general and clinical community.
- 4. To support grant writing and experimental design efforts, compilation of a list of available horses, environments, and facilities to provide a diversity of samples as well as available technical resources and facilities to provide analytical depth and strength for cutting edge research plans.
- 5. Collaborative grant proposals and manuscripts identifying the structure and functionality of the core microbiome for horses.
- 6. Collaborative grant proposals and manuscripts reporting the role of the microbiome in equine health, management, and disease prevention.
- 7. Collaborative grant proposals and manuscripts reporting the interactions between the equine gut microbiome and host genetics, behavior, and immune function.
- 3. Informational materials (print and web-based) for non-academic stakeholders to translate microbiome science into better care and management decisions.

Outcomes or Projected Impacts

- 1. Increased communication, collaboration, networking among scientists working on Equine Microbiome topics.
- 2. Increased rate of progress in equine microbiome science from survey-based studies to a more functional understanding
- 3. Greater depth and breadth of communication of equine microbiome topics to the equine science community at large.
- 4. Greater level of funding for equine microbiome grant proposals.
- 5. Increased integration of microbiome insights into equine nutrition and veterinary publications and products.
- 6. Increased integration of knowledge of the equine microbiome and the host.

Milestones

(**2022**):Establishment of protocols (SOPs) for Equine Microbiome Studies.

(**2022**):Compilation of lists of horses/facilities and technical/analytical capabilities.

(**2022**):Identification of questions and hypotheses for collaborative study and submission of collaborative grant proposals.

(**2022**):Identify stakeholder questions and confusions around the equine gut microbiome.

(**2023**):Organization and establishment of the Equine Microbiome Database.

(**2023**):Initial addition of data/samples and metadata to the database.

(**2023**):Planning and executing the first Equine Microbiome Conference

(**2023**):Studies and grant proposals to identify the role of the microbiome in equine health, management, nutrition, and disease prevention.

(**2023**):Studies and grant proposals to identify host/microbiome factors related to behavior, immune health, performance.

(**2023**):Development and dissemination of educational materials about the equine microbiome in health, management, and nutrition.

(**2024**):Evaluation of depth and breadth of Equine Microbiome Database.

(**2024**):Expansion of the Equine Microbiome Database in sample types, horses and numbers as identified.

(**2024**):Continue studies to identify the role of the microbiome in equine health, management, nutrition, and disease prevention.

(**2024**):Continue studies to identify host/microbiome factors related to behavior, immune health, performance.

(**2024**):Development and dissemination of educational materials about the equine microbiome in health, management, and nutrition.

(**2025**):Planning and executing the second Equine Microbiome Conference

(**2025**):Continue identification of underrepresented sample groups and expansion of Equine Microbiome Database.

(**2025**):Summarize outcomes and determine directions for new and ongoing research questions.

(**2025**):Complete and write materials summarizing collaborative studies.

(**2025**):Make practical recommendations about management decision-making and maintaining horse health through microbiome interventions

(**2025**):Development and dissemination of educational materials about the equine microbiome in health, management, and nutrition.

Outreach Plan

1. Identification of questions and misinformation about the equine microbiome by all stakeholder groups.
2. Creation of education materials (webinars, fact sheets, speaker series, infographics) accessible to the general public regarding equine management in relation to the microbiome. These would be freely available to the public and accessible to under-served and underrepresented communities.
3. Opportunities for stakeholder submission of samples and contribution of metadata to the Equine Microbiome Database.
4. The Equine Microbiome protocols and database will be made available to researchers and the public through an accessible web portal.
5. Biyearly conference will have segments targeted to the more general equine owning/managing public.
6. Workshops for youth groups (e.g. FFA, 4-H, and Pony Club) and undergraduate students on microbiome science.
7. Manuscripts of experimental results submitted to peer review journals.

Organization/Governance

Organization/Governance

This regional project NE-18869 was initiated by the University of Delaware and the University of Connecticut. The following are the current members of the project:

Amy Biddle, University of Delaware

Jenifer Nadeau, University of Connecticut

Sarah Reed, University of Connecticut

Aaron Ericsson, University of Missouri

Robert Coleman, University of Kentucky

Stephen Coleman, Colorado State University

Carissa Wickens, University of Florida

Samantha Brooks, University of Florida

Carolyn Hammer, North Dakota State University

Sara Mastellar, The Ohio State University’s Agricultural Technical Institute

Sarah Springer-White Texas A&M University

The recommended Standard Governance for multistate research activities including the election of a Chair, a Chair-elect, and a Secretary. All officers will be elected for at least two-year terms to provide continuity. Administrative guidance will be provided by an assigned Administrative Advisor and a CSREES Representative. As of this date, Amy Biddle, University of Delaware, has been serving as chair and will continue so until the initiation of the next project. Subcommittee/research teams will be chosen from the objective areas: 1. Evaluate existing methods for equine microbiome sampling, reporting metadata, and data analysis to enable robust comparisons between studies, efficiency in sample utilization and synthesis of datasets. 2. Establish an equine microbiome database to facilitate sharing of data from a range of samples. 3. Compile a list of available expertise, horses, and facility resources at participants’ locations to leverage institutional strengths to address microbiome questions using cutting edge techniques. 4. Compile a list of available technical and analytical resources: Core facilities and expertise for sequencing, diagnostic labs, specialized analytical tools to leverage institutional strengths to address microbiome questions using cutting edge techniques. 5. Identify the components of the “normal” equine gut microbiome. 6. Disseminate results and applications of microbiome science for equine welfare and management through stakeholder outreach and education, including youth groups, horse owners, nutritionists and veterinarians. 7. Examine the role of the equine microbiome in management, maintaining horse health and disease prevention. 8. Study how the genetic, immunological, and behavioral factors of horses are related to and interact with the gut microbiome.

Literature Cited

1. Cymbaluk NF. Comparison of forage digestion by cattle and horses. Canadian Journal of Animal Science. 1990;70: 601–610. doi:10.4141/cjas90-072
2. Argenzio RA, Southworth M, Stevens CE. Sites of organic acid production and absorption in the equine gastrointestinal tract. American Journal of Physiology. 1974;226: 1043–1050.
3. Hintz HF, Argenzio RA, Schryver HF. Digestion coefficients, blood glucose levels and molar percentage of volatile acids in intestinal fluid of ponies fed varying forage-grain ratios. Journal of Animal Science. 1971;33: 992–995.
4. Diether N, Willing B. Microbial Fermentation of Dietary Protein: An Important Factor in Diet–Microbe–Host Interaction. Microorganisms. 2019;7: 19. doi:10.3390/microorganisms7010019
5. Daly K, Proudman CJ, Duncan SH, Flint HJ, Dyer J, Shirazi-Beechey SP. Alterations in microbiota and fermentation products in equine large intestine in response to dietary variation and intestinal disease. British Journal of Nutrition. 2011;107: 989–995. doi:10.1017/S0007114511003825
6. Dougal K, de la Fuente G, Harris PA, Girdwood SE, Pinloche E, Geor RJ, et al. Characterisation of the Faecal Bacterial Community in Adult and Elderly Horses Fed a High Fibre, High Oil or High Starch Diet Using 454 Pyrosequencing. Ibekwe AM, editor. PLoS ONE. 2014;9: e87424. doi:10.1371/journal.pone.0087424
7. Dougal K, Harris PA, Girdwood SE, Creevey CJ, Curtis GC, Barfoot CF, et al. Changes in the Total Fecal Bacterial Population in Individual Horses Maintained on a Restricted Diet Over 6 Weeks. Frontiers in Microbiology. 2017;8. doi:10.3389/fmicb.2017.01502
8. Alexander S, Irvine CHG. Stress in the Racing Horse: Coping vs Not Coping. JES. 1998;9: 77–81. doi:10.1294/jes.9.77
9. Destrez A, Grimm P, Julliand V. Dietary-induced modulation of the hindgut microbiota is related to behavioral responses during stressful events in horses. Physiology & Behavior. 2019;202: 94–100. doi:10.1016/j.physbeh.2019.02.003
10. Ayala I, Martos NF, Silvan G, Gutierrez-Panizo C, Clavel JG, Illera JC. Cortisol, adrenocorticotropic hormone, serotonin, adrenaline and noradrenaline serum concentrations in relation to disease and stress in the horse. Research in Veterinary Science. 2012;93: 103–107. doi:10.1016/j.rvsc.2011.05.013
11. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nature Reviews Immunology. 2009;9: 313–323. doi:10.1038/nri2515
12. Elzinga S, Nielsen BD, Schott HC, Rapson J, Robison CI, McCutcheon J, et al. Comparison of Nutrient Digestibility Between Adult and Aged Horses. Journal of Equine Veterinary Science. 2014;34: 1164–1169. doi:10.1016/j.jevs.2014.06.021
13. Ireland JL, McGowan CM, Clegg PD, Chandler KJ, Pinchbeck GL. A survey of health care and disease in geriatric horses aged 30years or older. The Veterinary Journal. 2012;192: 57–64. doi:10.1016/j.tvjl.2011.03.021
14. Ishizaka S, Matsuda A, Amagai Y, Oida K, Jang H, Ueda Y, et al. Oral Administration of Fermented Probiotics Improves the Condition of Feces in Adult Horses. Journal of Equine Science. 2014;25: 65–72. doi:10.1294/jes.25.65
15. Tanabe S, Suzuki T, Wasano Y, Nakajima F, Kawasaki H, Tsuda T, et al. Anti-inflammatory and Intestinal Barrier–protective Activities of Commensal Lactobacilli and Bifidobacteria in Thoroughbreds: Role of Probiotics in Diarrhea Prevention in Neonatal Thoroughbreds. Journal of Equine Science. 2014;25: 37–43. doi:10.1294/jes.25.37
16. Wynn SG. Probiotics in veterinary practice. Journal of the American Veterinary Medical Association. 2009;234: 606–613.
17. Bachmann M, Glatter M, Bochnia M, Greef JM, Breves G, Zeyner A. Degradation of Monosaccharides, Disaccharides, and Fructans in the Stomach of Horses Adapted to a Prebiotic Dose of Fructooligosaccharides and Inulin. Journal of Equine Veterinary Science. 2021;105: 103731. doi:10.1016/j.jevs.2021.103731
18. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The Human Microbiome Project. Nature. 2007;449: 804–810. doi:10.1038/nature06244
19. Proctor LM. The Human Microbiome Project in 2011 and Beyond. Cell Host & Microbe. 2011;10: 287–291. doi:10.1016/j.chom.2011.10.001
20. McDonald D, Hyde E, Debelius JW, Morton JT, Gonzalez A, Ackermann G, et al. American Gut: an Open Platform for Citizen Science Microbiome Research. 2018;3: 28.
21. AlShawaqfeh M, Wajid B, Minamoto Y, Markel M, Lidbury J, Steiner J, et al. A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. FEMS Microbiology Ecology. 2017;93. doi:10.1093/femsec/fix136

Land Grant Participating States/Institutions

DE,CT,ND

Non Land Grant Participating States/Institutions

Ohio State University

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Biddle, Amy S		Delaware - University of Delaware	1,2,3,4,5,6,7,8	302 305 901	3810 3130 4099	1010 1103 1110	0.60	0.00	0.00	0	0
Hammer, Carrie	Yes	North Dakota - North Dakota State University	1,2,4,5	305 311	3810 3810	1020 1090	0.10	0.00	0.00	0	0
Mastellar, Sara		Ohio State University	1,2,3,4,5	0	0	0	0.10	0.00	0.00	0	0
Nadeau, Jenifer A	Yes	Connecticut -Storrs	1,2,4,5	305	3810	1010	0.10	0.00	0.00	1	305

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
302-3810-1010	0.2	0	0
305-3130-1103	0.2	0	0
901-4099-1110	0.2	0	0
305-3810-1010	0.1	0	0
0-0-0	0.1	0	0
206-1131-1060	0.1	0	0.1
305-3810-1020	0.05	0	0
311-3810-1090	0.05	0	0
Grand Total:	**0.90**	**0.00**	**0.00**

Program/KA	Total FTE
0	0
305	0.33
0	0
0	0
0	0
Grand FTE Total:	**1**

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2202: The Equine Microbiome

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Good
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
This is an excellent overview of the need for additional research regarding the equine microbiome and methodology to increase this knowledge base. My only comment is that a major feed company (Purina) is already collecting samples for microbial analysis, with several hundred (or more?) already collected. It would be wise to initiate communication with Purina and discuss options to share data, as there is a common goal.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2202: The Equine Microbiome

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
The proposed study will provide critical information for ongoing research into equine health and the microbiome. As the investigators point out, equine microbiome research has consisted of small projects with diverse methodology leading to a lack of definitive information on "normal" and the influence of animal and environmental factors on "normal". As a clinician with strong interest in equine gastrointestinal disease and the microbiome, the findings from these collaborators will be vital for ongoing work.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2202: The Equine Microbiome

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project with revision
2. Achievable goals/objectives:
Good
3. Appropriate scope of activity to accomplish objectives:
Good
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Good
Comments
This is a great, well needed multi-state project that will benefit the stakeholders listed for sure. My few specific comments on the project are below, some more specific than others.

- For the PubMed search maybe put the date that the search was done. I just tried and now get 348, so it is increasing rapidly!
- Under the first set of bullets for 'needs' what about exposing those stakeholders to new ways of analyzing the data? There is always something new coming out (from work in other species) and it might not always be wrong to try something new.
- For the horse owners and enthusiasts, what about the group creating publications or other web-based materials targeted to the lay person to help with your goals of dispelling myths and increasing communication?
- In the details of Objective 4, recommend adding in a bit on publications for your various stakeholders (i.e. what is mentioned above for targeting lay people as well as other groups, like vets.)
- Again, in Outputs, other pubs, not just manuscripts.
- There is only the author under 'projected participation' are there members in mind that the group hopes to target? Should these individuals be listed? I see them listed under the 'current members' but they are not in Appendix E, does that mean they are not really members?
- Outreach plan #2 is the first time educational materials for the general public or horse owning public is mentioned. As mentioned above, it should be at least briefly mentioned before this point.

More generally, some of the objectives were a bit brief so hard to determine exactly what would be happening under these objectives. Understandably there will be a wide variety of studies accomplished under these objectives, but some more details would be helpful.
Your Recommendation:
Approve/continue project with revision

Project ID/Title: NE_TEMP2202: The Equine Microbiome

Response to Reviewers

Reviewer #1:

We appreciate the suggestion to reach out to Purina as potential collaborators. I met with Robert Jacobs and Mary Beth Gordon from Purina in 2019 as they were just developing their equine microbiome project. They offered to collaborate with sample collection and sequencing but were not interested in sharing data. As the availability of data is a key element and value proposition of this effort, I did not reach out to them to join in the formation of this Multistate group. That said, it would be of great interest to invite leaders in veterinary, nutritional and pharma industries to join and benefit from the efforts of this group.

Reviewer #2:

Thank you for your encouragement and support. Your comments provide rationale to include additional communications for clinicians and the general public as recommended by Reviewer #3 (addressed below).

Reviewer #3: Each point is addressed directly below.

- For the PubMed search maybe put the date that the search was done. I just tried and now get 348, so it is increasing rapidly!

The date of the PubMed search has been added (10/18/2021).

- Under the first set of bullets for 'needs' what about exposing those stakeholders to new ways of analyzing the data? There is always something new coming out (from work in other species) and it might not always be wrong to try something new.

This is a really important point. Tools and strategies for data analysis have been rapidly changing in this area across species and questions. I have revised the third bullet point to include data analysis and the development of protocols.

- For the horse owners and enthusiasts, what about the group creating publications or other web-based materials targeted to the lay person to help with your goals of dispelling myths and increasing communication?

While this goal is implied elsewhere, I agree that it should be more explicitly stated. I have added this goal: 5) Create print and web-based resources for the equine community (public, education, veterinary and equine industry) to increase understanding of microbiome science and its applications to improving horse health and management."

- In the details of Objective 4, recommend adding in a bit on publications for your various stakeholders (i.e. what is mentioned above for targeting lay people as well as other groups, like vets.)

In addition to adding Objective #5 (above), specific goals have been added for stakeholders:

For Veterinarians and Nutritionists

Creation of evidence-based web and print resources to translate equine microbiome science for better health outcomes.

For Horse Owners and Enthusiasts

Creation of evidence-based web and print resources to translate equine microbiome science for better management decisions.

- Again, in Outputs, other pubs, not just manuscripts.

These have been added as described above and in Output #3:

3. Informational materials (print and web-based) for non-academic stakeholders to translate microbiome science into better care and management decisions.

- There is only the author under 'projected participation' are there members in mind that the group hopes to target? Should these individuals be listed? I see them listed under the 'current members' but they are not in Appendix E, does that mean they are not really members?

The current members of the project are listed below and in the Organization/Governance section. Appendix E was not available to submit for the Proposal

Amy Biddle, University of Delaware
Jenifer Nadeau, University of Connecticut
Sarah Reed, University of Connecticut
Aaron Ericsson, University of Missouri
Robert Coleman, University of Kentucky
Stephen Coleman, Colorado State University
Carissa Wickens, University of Florida
Samantha Brooks, University of Florida
Carolyn Hammer, North Dakota State University
Sara Mastellar, The Ohio State University's Agricultural Technical Institute
Sarah White-Springer, Texas A&M University

- Outreach plan #2 is the first time educational materials for the general public or horse owning public is mentioned. As mentioned above, it should be at least briefly mentioned before this point.

These have been added as described above and in Output #3:

More generally, some of the objectives were a bit brief so hard to determine exactly what would be happening under these objectives. Understandably there will be a wide variety of studies accomplished under these objectives, but some more details would be helpful.

Clarifying language has been added. There will be a wide range of studies under each objective, focusing on the dynamics of the microbiome as well as the host. The survey data will be used to generate hypotheses regarding normal microbiome structure and functionality, response to stress, interventions, and health factors.

NE_TEMP2210: Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Nutritive Value

Status: Submitted As Final

Duration	10/01/2022 to 09/30/2027
Admin	[Eric Bishop-von
Advisors:	Wettberg]
NIFA Reps:	

Statement of Issues and Justification

The economics of producing food, fiber, and energy products is a major issue in providing food security. Forage crops are the foundation of livestock and dairy enterprises in North America. According to the USDA National Agricultural Statistics Service, in 2019, 30.6 million acres of hay and haylage were harvested, worth more than $20.6 billion. These figures are conservative estimates of forage production since significant acreage is devoted to pastures and rangelands for which no estimates of economic value are readily available.

Use of leguminous forages minimizes nitrogen fertilization because they fix atmospheric N, thus reducing inputs and the risk of environmental contamination from fertilizer usage. The fibrous roots of grass species reduce soil erosion and capture environmental contaminants. Because these forage species are perennial, land disturbance is minimized, thus reducing the potential for soil erosion. In addition, they sequester carbon and reduce greenhouse gas emissions.

Breeding of perennial forage crops has resulted in improved cultivars that make livestock and dairy production more economical by reducing inputs and increasing outputs. Forage species, such as switchgrass, have potential for energy production. Compared to other types of crop species, perennial forage species enable more sustainable agricultural systems. Improved forage cultivars translate to benefits to agricultural producers of animal and energy products. The seed industry benefits from the production and marketing of improved cultivars. All Americans benefit from food security, reduced costs of food and energy, and protection of the environment by reduced use of pesticides, herbicides, and fertilizers.

During the last few decades, the number of forage breeders in North America has been decreasing. The number of forage researchers in the USA decreased by 60% between 1984 and 2009. The number of extension workers declined by 30% (Rouquette et al., 2009). In some state experiment stations, as a forage scientist has left their position, they were not replaced. For example, forage breeding positions have been lost at Iowa State University, Oklahoma State University, and Kansas State University. The number of forage scientists at USDA-ARS also has declined. When the forage breeder at USDA-ARS in Mandan, ND, retired, his position was not replaced. The Noble Foundation, a private research institute, ceased research activities in alfalfa genetics in 2021.

There are few private breeding companies, with ongoing consolidations further reducing their numbers. Currently, three main companies constitute the bulk of private alfalfa breeding in the USA (Forage Genetics, Intl., S&W Seed Company, and Corteva Agriscience). These companies work on few perennial forage species, primarily alfalfa with minor efforts on a few grass and clover species. Several major international forage seed companies focus on cool-season grasses and clovers, but most of that breeding is done outside the USA. Many forage species of importance in North America are receiving no attention by private breeders.

As budgets and the number of scientists have been reduced, the need for cooperative research is more essential than ever. Most forage breeders work on multiple forage species, thus diluting efforts on individual species. Because these forage species are perennial, establishing fields is less frequent than with annual crops. Therefore, seed is sold less frequently per unit land area compared to that of annual crops. Unless forage cultivars are broadly adapted for use across a large range of environments, seed companies are not interested in new cultivars because of the limited market. All of these factors point to the need for cooperative research to make significant advances in developing improved forage cultivars adapted to a wide range of environments. The current NE-1710 project fosters the interactions necessary to achieve goals with diminishing resources without unnecessary duplication.

This project aligns with the following NIFA Priority Science Areas:

1. **Agroclimate science.** There is a need for forage crops that will be productive under abiotic stresses, including drought, flooding, cold and warm temperatures, and soil salinity and acidity.
2. **Bioeconomy-Bioenergy-Bioproducts.** Cooperative research is needed for developing cultivars of bioenergy crops with improved biomass and quality, while protecting these crops from biotic and abiotic stress conditions.
3. **Human nutrition.** Breeding crops with higher forage yield and quality, longevity, and resistance or tolerance to biotic and abiotic stress conditions will ensure an ample supply of good quality feed to animals, an essential step in securing food for consumers.
4. **Sustainable agricultural production systems.** Diverse, perennial forage systems and increased cover crop use in annual crop production are important for increasing agricultural sustainability.

Without cooperative research through the multistate project, the ability to accomplish these priorities would be severely curtailed. In the absence of a cooperative research network of forage researchers, new forage cultivars would be more narrowly adapted and the scope and scale of research outputs would be more limited. Farmers rely on forage breeders to improve the productivity of these crops, especially when new diseases, insects, and other problems arise, and the impact on providing feed for the livestock industries, especially for beef and dairy production, would be huge.

The impacts of the proposed research will be significant. Germplasm with new traits will be available to private and other public breeders to use in their programs for developing improved cultivars. Improved forage cultivars directly released from the multistate project scientists will make seed and forage production more economical for farmers and seed companies. Development of breeding methods, both traditional and molecular methods, will enhance efficiencies and effectiveness of improving forages for traits of low heritability or from unadapted genetic backgrounds. Data from forage yield trials across multiple locations and years will be available for breeders to use for selecting experimental populations that will be released as cultivars and available for licensing, for the seed industry in advertising seed of the cultivars, and for extension educators and farmers when selecting cultivars for their locations. These data also will help breeders to better understand the broad adaptation and resilience of forage cultivars. Development of forage species as feedstocks for the biofuel industry ultimately will contribute toward more secure and sustainable energy production. Between CO2 driven climate change and rising prices of petroleum products, long-term focus needs to be on renewable, sustainable energy sources. The overall impact will be more economical food and energy production while reducing negative environmental impacts in the agricultural systems.

The scientists cooperating in this project have the ability to accomplish the proposed research. The current NE-1710 project consists of most of the North American forage breeders, who have cooperated in research for many years. In addition to scientists at state agricultural experiment stations, the multistate cooperative research among forage scientists has evolved over the years to include more scientists from USDA-ARS, Agriculture and Agri-Food Canada, Canadian universities, and the Land Institute. These forage breeders and scientists have extensive experience in research on forages.

Many accomplishments have already been realized in the form of improved germplasm and cultivars; information on breeding methods for improving forage yield; and data on forage yield of multiple species for use by breeders, the seed industry, farmers, and extension educators. Extension presentations and information on the web have informed various stakeholders of the new information and cultivars developed by this project. Other scientists have been informed of the research results through professional publications and presentations at professional conferences. The scientists have the equipment along with field, greenhouse, and laboratory facilities to accomplish the proposed work.

Because of the long-term nature of research on perennial forage species, some of the research initiated in the last few years will continue into the next project period. Some of the research, however, will be new as a result of the collaborative efforts and discussions during our technical committee meetings. The research includes both traditional breeding and new molecular genetic technologies, and will enhance adaptation and resilience to changing environments. In addition, emphasis will continue in cooperative research on plant species for biofuel use as well as use for the livestock industry.

Funding for these collaborative efforts would be only partially covered by the multistate-Hatch funding. Most of the funding would come from other sources such as the seed industry, royalties from seed sales of cultivars, private sources, and various public funding sources at the state and federal levels (primarily competitive grants). In the past, the existence of the NE-1710 project and its predecessors have been a key factor in helping to secure other grant funds, such as USDA-NIFA grants, for accomplishing the research goals.

This proposal continues a long-term, continent-wide research project that has provided multi-location testing and selection environments to a number of forage and biofuel breeding projects. The current multistate Hatch project, NE1710, which ends in 2022, has grown to include forage and cover crop grass and legume breeders and bioenergy researchers throughout the US and Canada. Due to this geographical breadth, we have been able to implement experiments with nation-wide benefit and cannot be accomplished by any breeder individually. Individual participants in the proposal have their own research projects narrowly focused on species adapted to their regions, and the needs of producers at their locations. Our goal with this multi-location research project is to identify several major objectives that complement each location's individual research projects but that, through the collaborative arrangements provided by the umbrella of this project, provide a larger geographical context in order to ensure new cultivars will have broad adaptability in different geographies, flexibility in a range of different cropping systems or uses, and resilience to shifting weather patterns.

In this renewal, we have developed projects related to the major perennial herbaceous crop groups, viz., legumes (alfalfa, birdsfoot trefoil, clovers), annual cover crops, cool-season grasses (tall fescue, orchardgrass, meadow bromegrass, intermediate wheatgrass), and warm-season grasses (switchgrass and giant miscanthus). Our sub-projects vary in size, from ongoing major breeding efforts in alfalfa to smaller projects that are the only coordinated breeding and improvement efforts for certain crops like birdsfoot trefoil. While numerous other forage crops are of importance, particularly in certain regions or specific niches, these crops represent major species of interest across broad regions of the continent. Therefore, we focus on these projects which require multi-location collaboration.

Furthermore, acknowledging that funding methods have changed over time, most of the collaborators, even those at state AES, do not receive funding for this regional research. Consequently, these projects are ones that complement existing research and can reasonably be tied with ongoing goals each collaborator has at their location. This also means that expensive objectives (e.g., those involving large-scale DNA sequencing or genomic selection) cannot be proposed within these research projects. However, by combining our programs, we can develop a framework to ask interesting questions about topics of broad interest, e.g., genotype ×environment interaction, that we can then use to attract external funding.

Related, Current and Previous Work

Previous research results from this project (NE1710)

The previous multistate project NE1710 pursued two overarching objectives, to first improve legumes (alfalfa, birds-foot trefoil, and red clover), cool-season grasses (orchardgrass, tall fescue, and meadow bromegrass), and warm season grasses (switchgrass), and second to draw on the power of multi-site trials to understand patterns of genotype by environment variation.

Legumes:

Alfalfa:

In NE1710 we developed germplasm pools from NPGS germplasm for multi-site trials. We divided the germplasm into northern and southern pools to evaluate material most likely to be climatically adapted. The Northern germplasm was divided into four populations based on its provenance: Central Asian, Russian, Ottoman and Siberian. Populations have been kept separate for seed increase. Multi-site trialling of northern material occurred at Cornell, NY Madison WI, Nova Scotia, and Southern Quebec. Southern material was trialed in California's central valley, Georgia, and Florida. Western sites have experienced extreme drought in recent years, conditions climatic models expect to become more common. Prebreeding activities in NE1710 are essential to meeting these challenges.

Birds Foot Trefoil:

With a growing market for grassfed milk and meat, legume forages that do not cause bloat are essential. The leading candidate for this is birdsfoot trefoil, *Lotus corniculatus.* Birdsfoot trefoil from different regions were combined, in a five-location trial with sites in Nova Scotia, New York (Ithaca), Rhode Island (West Kingston), Wisconsin (Madison), and Utah (Logan). The work examined bioactive compounds that can control ruminant intestinal parasites. Although the sites are established, COVID-induced laboratory shutdowns, particularly at USDA ARS labs and at partner labs in Canada, have created a sample backlog. We propose to expand the multi-site trials in coming work, as COVID delays abate.

Cool Season grasses

Developing resilient cool-season grasses adapted to variable climatic conditions has involved multi-site trials with selections at six locations, including two in Utah, two in Saskatchewan, and two in Quebec. At each of these sites, selections have been made on several cool season grasses, specifically tall fescue, meadowbrome, timothy, and orchardgrass. At the more northern sites, winter hardiness has been an issue, particularly in Saskatchewan. The Saskatchewan group is looking at sugar accumulation as an indicator of cold tolerance, using RNA-seq during cold acclimation. At more southern sites, trials are needed to develop grasses that thrive in cool seasons, which is essential for some management systems.

Warm Season Grasses

Warm season grasses are of great importance in pasture-based systems across the Southern US, are key biofuel crops, and a 10 site switchgrass trial has been performed by David Lowry (Michigan State University) and Tom Jeunger (University of Texas). Supported by material coming from this trial, work has progressed in characterizing head-smut fungus, a major pathogen of this warm season grass. As there is no known sexual cycle, evidence may emerge that the disease is caused by a single clone of the fungus.

Miscanthus

With climate change bringing more extreme summer heat to many northern locations, there is a need for more work on warm season grasses in locations once considered too cool for them. As part of this need, more work is needed to understand variation in nutrient mobilization and cold adaptation in this species. There are currently three commercial lines of giant miscanthus (*Miscanthus* x *giganteus*; Mxg) planted in the U.S. Two (Freedom and Nagara) are patented and licensed to AGrow Tech. The third, Illinois clone, exists in the public domain. Giant miscanthus is a tall, perennial, deciduous C4 grass with annual tonnage that makes it valuable as a biomass crop. It is derived from crosses between *M. sinensis* (2n=2x=38) and *M. sacchariflorus* (2n=4x=76). Since it is derived from parents of different ploidy levels, and has an odd number of chromosome sets, it is seed sterile (2n=3x=57). Being seed sterile precludes breeding of any of the Mxg cultivars except for somaclonal manipulations. The only way to generate new Mxg cultivars is by reconstituting the novel Mxg triploids is through crosses of new *M. sinensis* and new *M. sacchariflorus* crosses. Several Mxg seedlings have been generated at the University of Illinois and are being tested at various locations in the U.S.

2.0 G X E for Tall Fescue, orchardgrass, Red Clover

Much of the power of a multistate Hatch is the capacity to lookat differences in forage cultivar performance at different locations/environments. NE1710 has examined multisite G*E in tall fescue and orchardgrass. Work with red clover has been delayed due to logistical challenges of moving seed across the US-Canadian border. To date, 14 entries of tall fescue, made by germplasm from Georgia and Nova Scotia have been evaluated. The panel was planted at 3 locations, including the University of Georgia, Cornell University, and Nova Scotia. Two years of data were collected at UGA and Cornell, and one year at Nova Scotia. The objective is to have a minimum of 3 years of field data per location to assess GxE interactions. Analyses will be performed by sub-project lead A. Missaoui in Georgia.

Relevant background for research planned in this project

Although participants in this project individually conduct breeding, pre-breeding, genetics, and agronomic research on various forage and biofuel crops, the specific research projects we are proposing here represent aspects of these improvement programs that can be enhanced through regional and continent-wide collaboration. These projects are not meant to be comprehensive programs covering all, or even a few, forage and biofuel crops. They also reflect areas of continued interest in the group, covering repeated cycles of this multi-state Hatch group.

Alfalfa Improvement

Alfalfa breeders have been highly successful at improving alfalfa over the past 80 years, so that most new cultivars today have high levels of resistance to multiple pathogens or pests (e.g., Bouton 2021). However, the variable and extreme weather brought by climate change and the ongoing movement of pests and pathogens creates the need for increased resilience. Further, the improvement of alfalfa yield, other than that resulting from improved resistances, has been slow or non-existent over the past 30 years (Brummer and Casler, 2014). A broad goal for alfalfa improvement is to ensure breeding germplasm is available to assist breeders dealing with these challenges in the future, which can be accomplished by pre-breeding (e.g. Scotti and Brummer, 2010).

The U.S. National Plant Germplasm System (NPGS) maintains over 3000 alfalfa (*Medicago sativa* L.) germplasm accessions (Irish and Greene, 2021). This collection is a rich resource for North American alfalfa breeders. However, insufficient work has been expended in prebreeding the germplasm in this collection to develop new sources of variation for quantitative traits, such as yield and adaptation. Prebreeding can be defined as the process of converting a large number of germplasm accessions into a few adapted breeding pools that could be incorporated into commercial breeding programs (e.g., Egan et al., 2021).

With rapid changes in climate, prebreeding offers the potential of providing breeders with new, semi-improved populations to incorporate into their programs. At its 2010 meeting, the North American Alfalfa Improvement Conference (NAAIC, an international organization of public and private alfalfa researchers) made the systematic germplasm improvement program one of the highest priority research projects. Past examples using non-North American alfalfa germplasm to enhance elite alfalfa germplasm pools include the development of leafhopper resistant alfalfa (Elden and McCaslin, 1997), lodging tolerant alfalfa (Lamb et al., 2000), disease resistance (Elgin et. al, 1988). Numerous studies have also show that genetically distinct alfalfa germplasm could be used as the basis for obtaining yield heterosis in hybrid or semi-hybrid alfalfa breeding schemes (Dudley and Davis, 1966; Busbice and Rawlings, 1974; Riday and Brummer 2006; Bhandari et al., 2007; Parajuli et al., 2021). Over the past decade and a half, a program to pre-breed pure yellow flowered alfalfa germplasm (subsp. falcata) has been underway (e.g., Riday and Wagner, 2012), falling within the NE1710 regional project.

Historically, alfalfa germplasm was classified into pools based on region of origin (e.g., Barnes et al. (1977). However, defining pools of extant breeding germplasm in 2021 is more difficult, given the extensive mixing over the past century; if anything, work with genome-wide SNPS suggests cultivars are structured according to fall dormancy classification (e.g., Li et al., 2014; Qiang et al., 2015; Ilhan et al., 2016), possibly reflecting past introgression of *M. falcata* germplasm. Recently genome assemblies have been published for diploid (Li et al., 2020) and tetraploid (Shen et al., 2020, Chen et al., 2020) alfalfa. Across all these experiments and past marker based assessments (reviewed by Li and Brummer 2012), the differentiation of yellow and purple flowered taxa is well established. Among tetraploids, however, a clear gradation is obvious with the hybrid subsp. *xvaria* intermediate between the two ends, effectively creating a continuum between true sativa and true falcata types. More fully understanding overall genetic variation would help to structure future breeding programs. For all these reasons this project is necessary to more fully exploit the NPGS germplasm system to pre-breed alfalfa. Work was started under NE1710 to develop a yellow-flowered ‘falcata’ pool. The project described here would expand this work by developing multiple geographic based germplasm pools in subsp. sativa.

Winter annual cover crop improvement

In 2017, 15.4 million acres of cover crops were planted in the US, representing a 50% increase in acreage over the previous five years (Census of Agriculture, 2017). The most common species planted as cover crops include small grains (e.g., cereal rye, oats, winter wheat), legumes (e.g., clovers, hairy vetch, winter pea), and brassicas (e.g., radish, turnip) (CTIC, 2020). To date, cover crop breeding efforts have been quite limited, and few improved varieties are available to farmers. Farmers often purchase seed as “variety not stated” (VNS), and VNS seed has unknown and suboptimal performance for traits of importance to growers, including winter hardiness, maturity, nitrogen fixation, and biomass production (Wayman et al, 2017). Starting in 2015 a national Cover Crop Breeding Network was established, including universities, USDA, and farmers, and currently breeds four winter annual cover crops. Cover crop variety trials have also been conducted at universities and by USDA (USDA-NRCS Plant Materials Centers, 2020) to a limited extent. However, there is not currently a coordinated variety trialing system in the public or private sector with high resolution in the Northeast or in other regions across North America.

Root traits are a critical component of the soil organic carbon (SOC) contributions of cover crops (Austin et al., 2017; Mazzilli et al., 2015; Kong and Six, 2010), driving the potential contribution of cover crops to climate change mitigation and provide rotational value (e.g. Marques et al., 2020). However, root traits remain poorly characterized both among and within cover crop species, and the few cover crop breeding and variety characterization efforts have focused on a limited suite of economical traits, for example nitrogen fixation (Moore et al., 2020; Muller et al., 2021). If cover crops are to reach their potential as a tool to fight climate change, a major focus on breeding for root traits is needed.

Switchgrass Improvement

Switchgrass (*Panicum virgatum* L.) is a native warm-season C4 perennial range grass that is a target

feedstock for U.S. production of sustainable cellulosic biofuels, electricity, and synthetic gas (Boateng et

al, 2007; Schmer et al., 2008). The principal objective of switchgrass breeding programs has been to

develop cultivars with high biomass production (Parrish et al., 2012). However, high yield depends on the cultivar's ability to tolerate environmental and biotic stresses. Large monoculture biofuel plantings have not yet been sown, but when they are, disease pressure will increase (Crouch et al., 2009; Uppalapati et al., 2013; Zhu et al., 2013; Serba et al., 2015, Sykes et al., 2016, Bowen et al., 2021). Therefore, understanding genetic variation for resistance to the most serious disease pathogens needs to be undertaken now (Stewart and Cromey 2011).

Switchgrass harbors substantial natural genetic variation for many traits, and that variation appears to

extend to disease resistance. Much genetic variation within switchgrass is partitioned between upland

and lowland ecotypes, driven by adaptation to habitats differing in soil water availability and other factors

(e.g., Milano et al., 2016; Lowry et al., 2019, 2021). In addition, many genetically-based abiotic stress responses and disease susceptibility vary by latitude (e.g. McMillan, 1964; Meyer et al., 2014; Lovell et al., 2016; Lowry et al., 2019; Lovell et al., 2021; Gustafson et al., 2003; Uppalapati et al., 2013; Serba et al., 2015, VanWallendael et al., 2021). Due to recent work, breeders have a growing understanding of the genetics of resistance to the multiple microbial pathogens of switchgrass (VanWallendael et al., 2021). However, we still require an evaluation of pathogen diversity across geographically distinct switchgrass populations at a national scale. Given that pathogen diversity differs by region, the success of the proposed work will necessitate multistate collaboration.

Cool-season Grass Improvement

Cool-season (C3) grasses comprise a major forage source in both the United States and Canada. Cool-season grasses are grown both in monoculture plantings and mixed with legumes and stands are

managed for grazing, hay, and/or silage production. Meadow bromegrass (*Bromus riparius* Rehm.), tall

fescue *(Lolium arundinaceum* (Schreb.) Darbys.) and orchardgrass (*Dactylis glomerata* L.) are three

important cool-season grasses used in temperate regions of North America. Tall fescue and

orchardgrass are widely grown and are well known to farmers, and meadow bromegrass has been

recommended across Canada for some time (Knowles et al., 1993). There is interest in the U., with new cultivars being developed in Montana and Utah. The increased consumer demand for grass-fed and

organic animal products necessitate further improvement of cool-season grasses. Additionally, the effects of ongoing climate change necessitate selection for increased drought, heat, cold, and salinity tolerance.

The former NE1010 and NE1710 project developed a meadow bromegrass breeding population by crossing superior plants selected at four locations across North America. However, this germplasm has not been tested widely. Tall fescue is recommended across North America; however, improving its palatability and nutritive value would expand its use. New soft-leaf tall fescue populations developed in Utah may be useful if they prove to be adapted across the tall fescue growing regions of North America and the soft leaf trait, which improves palatability, is expressed in all environments. Orchardgrass is cultivated in the northern regions of the United States and in all Canadian provinces. In Europe, increased concentrations of water soluble carbohydrates (WSC) in grasses provided increased animal production and decreased environmental impacts (Miller et al., 2001; Lee et al., 2001, Tubritt et al., 2018). Increased WSC may also buffer plants against various abiotic stresses, including drought and freezing (Volaire and Thomas, 1995; Sanada et al., 2007; Robins and Lovatt 2016). No cultivars have been developed in North America for higher WSC, despite the value of the trait. The development of cultivars with increased levels of WSC is a new breeding objective of forage breeding programs in recent years.

Birdsfoot trefoil improvement

Birdsfoot trefoil, as a non-bloating legume, is a desirable component of grazing systems. Breeding

programs have focused on improving persistence and seedling germination. One of the characteristics that makes trefoil a useful forage legume is their condensed tannin (CT) content. The quantity of CT present in legumes has been shown to correlate with animal productivity as well as impact gastrointestinal parasites in forage-fed ruminants (Waghorn and McNabb, 2003; Min et al, 2003; Ghelichkhan et al., 2017). Condensed tannin is a term that includes a group of diverse secondary compounds. The specific compounds associated with the above improved animal performance were not identified. Research into the impact of CT on the parasitic gastrointestinal nematodes that are parasitic to livestock has suggested the possibility that higher CT content of forage legumes will reduce the levels of infection (Hoste et al., 2006; Marley et al., 2006; Waghorn and McNabb, 2003). Assessing the CT profiles among currently available birdsfoot trefoil cultivars will aid in estimating available genetic variability for this trait.

Relation to other CRIS/REEIS research

The proposed work is the only multi-state umbrella for forage grass and legume breeding. As such it serves as an umbrella, supporting multi-site work that cannot be done otherwise. For those with Hatch assignments at their home institutions, this project aligns with their local projects. These all show the need for a multistate umbrella to link individual research projects.

1. NE1710 complements several mixed pasture projects, such as NH.W-2020-02151, a northern tier pasture management project, and TEN2020-05081, Keyser et al, supplementing cool season grasses in warmer Southeastern US.
2. Among warm season grasses, several projects are looking at switchgrass (ALAZ11192020MA (PI Aspinwall, switchgrass improvement and PENW-2018-05922, PI Carlson northern switchgrass improvement) and Miscanthus (ILLU-802-628, PI Sacks on Miscanthus genomics and 8042-13611-029-08S PI McCarthy, focused on Miscanthus in the Delmarva peninsula). A new NIFA award to PI Rios at UF (2022-67013-36252) complements these efforts with molecular breeding in bermudagrass (Cynodon spp.).
3. NE1710 supports several alfalfa breeding and improvement projects, including CA-D-PLS-2557-CG (PI Brummer), WNP03692 (Zhang et al, hybrid breeding with purging), WIS04059 on cutting time web platform development, CA-D-PLS-2482-CG (Putnam and Hutchmaker, alfalfa tolerance of saline soils).
4. As we have noted, there has been a multi-decadal decline in the number of forage breeders and agronomists. Consequently training new breeders is critical. WISW-2020-11303 (PI Newman) is a training grant on forage breeding and agronomy.
5. Finally, NE1710 is an umbrella for a number of individual Hatch projects. These include the following as examples. SD00R669-18 (Boe, alfalfa and switchgrass), CA-D-PLS-2246-RR (Brummer, alfalfa), NYC-149948 V. Moore Cornell, alfalfa, NYC-149946 (Robbins and Hansen, alfalfa breeding), MIS-162160 (Baldwin on switchgrass and Miscanthus), KY006108 (Phillips, grasses), MAS00557 Nusslein UMASS on cool season grasses, UTA-01480 Miller on forages, UTA-01482 (Creech on alfalfa breeding), FLA-AGR-005761 (Rios, alfalfa), ND06141 on grazing, VT-H02603 (Greenwood, cattle diets), VT-H02501MS (von Wettberg, cover crops), NH00665 (Smith, weeds of pastures), TEX0-1-6324 (Smith and Smith, forage grasses and clovers), ND01514 (Berti, forage management), SD00H740-22 (Wu, alfalfa phenotyping), NM-RAY-17H (Ray, alfalfa), OKL03137 (Wu, warm seasons), MIN-13-117 (Ehkle, cool seasons), NJ12182 (Bonos, switchgrass), TEN00518 (Bhandari, Switchgrass).

Objectives

1. Developing broadly adapted, climate resilient forages for sustainable cropping systems.
   Comments: This objective has six sub-objectives. 1.1. Developing regionally adapted, resilient alfalfa germplasm pools. Cooperating locations: AES: Cornell Univ., Univ. Florida, Univ. Vermont, and Univ. California, Davis [co-lead]; USDA-ARS: Logan, UT and Madison, WI [co-lead]; AAFC: Québec, QC, Saskatoon, SK, 1.2. Evaluating annual cover crops for regional adaptation and climate resilience and mitigation. AES: Cornell Univ. [Lead], Univ. Rhode Island; Univ. Vermont, AAFC: Truro, NS. 1.3. Developing switchgrass germplasm with improved fungal pathogen resistance. Cooperating locations: AES: Cornell Univ. [lead], Mississippi State Univ., Rutgers Univ., South Dakota State Univ.; USDA-ARS: Madison, WI. 1.4. Developing resilient cool-season grasses adapted to variable climatic conditions. Cooperating locations: AES: Cornell Univ.; South Dakota State Univ.; Univ. California, Davis; Univ. Kentucky, and Univ. Minnesota; USDA-ARS: Logan, UT [co-lead] and Madison, WI; AAFC: Québec, QC and Saskatoon, SK [co-lead]. 1.5. Determining the extent of genetic variability of CT among currently available birdsfoot trefoil cultivars and elite lines. Cooperating locations: AES: Cornell Univ., Univ. Rhode Island; Univ. Vermont USDA-ARS: Logan, UT and Madison, WI; AAFC: Truro, NS [lead]. 1.6. Evaluating Miscanthus for forage and bioenergy across warm season locations. AES: Univ. Mississippi [co-lead], Univ. Illinois [co-lead].
2. Understanding genotype by environment interactions across multiple forage species
   Comments: Cooperating locations: AES: Auburn Univ., Cornell Univ., Mississippi State Univ., Rutgers Univ., South Dakota State Univ., Univ. California, Davis, Univ. Florida, Univ. Georgia, Univ. Kentucky, Univ. Minnesota, Univ. Rhode Island, Univ. Tennessee; Univ. Vermont; USDA-ARS: Logan, UT and Madison, WI; AAFC, Lethbridge, AB, Québec, QC, Saskatoon, SK, and Truro, NS.

Methods

1. 1. *Developing broadly adapted, climate resilient forages for sustainable cropping systems*

The six sub-objectives described here are natural outgrowths of the existing breeding programs of the participants. These six projects complement and extend individual programs and could not be conducted without regional (or continent-wide) participation.

1.1 Developing regionally adapted, resilient alfalfa germplasm pools

The overarching goal of this project is to use the NPGS alfalfa germplasm collection to enhance genetic

diversity in elite North American alfalfa breeding pools, continuing work started in NE1710. Selection from the NPGS pools will be organized into region of origin germplasm pools that can be useful for long-term genetic improvement of alfalfa and potentially valuable for the creation of heterotic groups and hybrid cultivars. The project consists of a series of related experiments that by nature are collaborative across multiple North American locations.

Germplasm pool development.

Based largely on accessions that are documented as landraces or cultivars from regions outside North America, we have been developing pools based on Northern (fall dormancy levels from 1-5) and Southern adaptation (fall dormancy 5- 12). These populations are derived from discrete ecogeographic regions, with four Northern and four Southern pools. The Northern pools include Siberia/Mongolia, Central Asia, Balkans-Turkey-Black Sea Region, and North Eastern Europe to the Ural Mountains; Southern pools are from South America, North Africa, Southern Asia (India, Iran), and the Arabian Peninsula. In conjunction with those regional pools, in proposed work we plan to develop two broadly based populations by pooling the four regional pools.

Evaluation/selection within new germplasm pools for broad adaptation

We expect that multiple cycles of selection will be necessary for these pools to be both broadly adaptable to North American climates and useful in commercial breeding programs. Therefore, we established multi-location breeding nurseries to select plants within populations to develop improved versions of these germplasms in NE1710, and expect to continue them to allow sufficient generations for broad adaptation. In addition to scientists involved at the outset of this project, we will also invite other breeders to participate either by becoming members of the regional project itself or by conducting evaluation trials. Trials will include comparisons of new germplasm against “check” varieties.

Diversity evaluation of germplasm pool

In order to validate our germplasm pool approach we will conduct genomic studies of developing pools using falcata as outgroup to determine how distinctive the eco-geographic regional germplasm pools are in relationship to North American elite alfalfa germplasm. This analysis, led by Rios, Brummer, and colleagues, could help us also define less utilized regional germplasm sources and guide enhancement efforts.

Nondormant cultivars have been released and are widely adopted in the southeastern United States (Bouton, 2021). In Florida, nondormant cultivars have been developed for improved adaptation to the state's subtropical agroecosystem ['Florida 66' (Horner, 1970), 'Florida 77' (Horner and Ruelke, 1981), and 'Florida 99'], but these cultivars are not commercially available. PD Rios resumed the breeding program at UF using Florida-adapted germplasm as the basis for the program. Advanced breeding lines have shown improved yield in Florida, Mississippi and Oklahoma, and a new non-dormant cultivar bred through conventional approaches was released in 2021, and the invitation to negotiate is underway (closes on May 2 2022). . Besides working with existing non-dormant germplasm pools, 121 diverse populations (cultivars, breeding lines and landraces) from various sources showed significant phenotypic diversity for biomass yield across several harvests in Florida. Plant selection was performed within populations and used in crosses to generate a training population consisting of 145 full-sib and 36 half-sib families. These families were established in the field to phenotype for biomass production and nutritive value (Acharya et al., 2020). This breeding population represents the training set for ongoing genomic prediction and high-throughput phenotyping studies for biomass yield and nutritive value (Biswas et al., 2021).

Seed increases and yield/performance trials of new germplasm pools

Seed of the germplasm pools will be increased to provide sufficient seed quantities for yield evaluation at multiple locations. New germplasms are expected as part of this project that will be freely available to commercial alfalfa breeding interests through the NPGS system. We will characterize the new pools for various traits, including (but not limited to) fall dormancy, insect/disease resistance, salinity, winter hardiness, and waterlogging tolerance. We will work with other US alfalfa scientists in the public and private sectors to evaluate key insect and disease resistances of most importance nationally and within specific regions.

Release of germplasm to the public

All prebreeding populations developed through this project will be made publicly available through the National Plant Germplasm System, accessible through the Germplasm Resources Information Network. More advanced material will be published as appropriate in the Journal of Plant of Registrations.

1.2: Evaluating annual cover crops for regional adaptation and climate resilience and mitigation.

The overarching goal of this project is to evaluate winter annual cover crop varieties for suitability for cropping systems in the Northeast US and Eastern Canada. The project is new to NE2210, and builds on existing cover crop breeding collaborations between researchers both within and beyond this group. The project includes evaluation of cover crop species and varieties within species for regional adaptation and suitability for cover crop and forage uses and potential for climate resilience/mitigation. In the proposed study, a trial including commercial cultivars of hairy vetch, winter pea, and crimson and bersem clover will be planted at each study location, including New York, Vermont, Rhode Island, and Nova Scotia. Advanced breeding material from project collaborators will also be included in the trial as available. Trials will be planted on a fall date appropriate for each location. Experiments will be planted in a randomized complete block design with four replications. Plots will be drilled in multi-row plots, approximately five feet wide and 15 feet long, with exact size and row spacing depending on available equipment at each site, including checks.

Plots will be evaluated for key cover crop and forage traits including emergence, fall and spring vigor, winter survival, and maturity at termination. Fall emergence and winter survival will be evaluated on a visual percentage basis. Vigor evaluation will be conducted on a 1-9 visual rating scale. Maturity will be assessed on the scale developed by Kalu and Fick (1983) and modified as appropriate for each crop. Biomass will be harvested, dried, and weighed at a regionally appropriate time in the spring before typical cash crop planting. Data will be analyzed with a focus on G x E and identification of stable, broadly adapted varieties for the Northeast.

In addition, root traits will be evaluated at a subset of locations (University of Vermont and Cornell) using “shovelomics” methods developed for legumes (Burridge et al., 2016) and adapted to species of interest as needed. Four plants per plot will be excavated, washed, and evaluated for key root system traits using a combination of manual and image-based evaluation methods. These data will provide an assessment of the diversity of root structural traits both within and among cover crop species, and will be used to determine potential to select for cover crops with improved root characteristics and the potential for climate mitigation and adaptation.

1.3 Developing switchgrass germplasm with improved fungal pathogen resistance.

As biofuel production expands, planting of large monocultures of switchgrass will likely result in

increased disease, especially those caused by fungal pathogens. In order to develop switchgrass

germplasm and cultivars with resistance to fungal pathogens, breeders first need to understand which

pathogens are present in potential biofuel growing regions. Because no previous study has evaluated pathogen diversity at the national scale, this project presents an unparalleled opportunity to evaluate germplasm for resistance to these disease agents. Given that pathogen diversity differs by region, the success of the proposed work requires multistate collaboration. This project builds on efforts started in NE1710, but that were disrupted by the pandemic.

Quantify important switchgrass pathogens across the US

We will characterize the geographic distribution and severity of economically important/yield-reducing diseases caused by phytopathogenic fungi in the north-central, eastern, and south-eastern United States through surveys of common cultivars and breeding lines. Fungi can cause a range of mild to severe disease in plant hosts. However, disease symptoms or signs of phytopathogenic fungi may not immediately reveal the specific identity of a pathogen. Thus, we will first continue to quantify the severity of fungal diseases in switchgrass with an annual survey (2022-2025) of all commercially important and promising new lines of switchgrass in yield trials and nurseries in Mississippi (Mississippi State University), New Jersey (Rutgers University), New York State (Cornell University), South Dakota (South Dakota State University), and Wisconsin (USDA). This will build on preliminary data collected from 2017-2019. Disease surveys will focus on determining the severity of four diseases: 1) rust (*Puccinia emaculata* and *Uromyces graminicola*); 2) anthracnose (*Colletotrichum navitas*); 3) *Bipolaris* leaf spot; and, 4) head smut (*Tilletia* species). For each survey, collaborators will score only promising new lines and commercially important cultivars for infection using established, standardized visual disease severity rating systems (e.g., for rust, 0-9 *Puccinia emaculata* system). From these surveys, we will establish how disease severity varies by geographic location, differs between northern upland and southern lowland populations, and differs among germplasm within ecotypes.

Identify and quantify specific host-pathogen relationships

We will identify patterns of phenotypic variation for switchgrass resistance and, hence, susceptibility to fungal pathogens across common cultivars, breeding lines, and field sites. We have noted previously that varying fertilization regimes (esp. nitrogen application rate) and summer rainfall can significantly influence the incidence and severity of switchgrass disease, particularly anthracnose and head smut (S.C. Kenaley, Cornell Univ. unpub. data). To identify environmental factors associated with the severity of disease(s), we will compile a set of environmental covariates for each field site including: (i) 2022-2025 weather data, obtained from PRISM; (ii) soil fertility (established by collaborators); (iii) proximity of alternate hosts for foliar pathogens of switchgrass; and (iv) extent of vegetation types within 1 km, as determined from field observation and the USDA National Agricultural Statistics Service Cropland Data Layer. To determine how landscape features predict genotype-specific disease severity, we will conduct stepwise general linear model fitting. Here, the response variable will be disease severity and the landscape environmental factors will be predictor variables. Model comparison using Akaike information criterion (AIC) will be executed to identify best-fit models.

Pathogen resistance in switchgrass

We will quantify pathogens across common cultivars and breeding lines to identify switchgrass germplasm possessing tolerance and/or resistance to regional phytopathogenic fungi. We will compare results among field sites to identify regionally adapted switchgrass plants for possible cultivar release and further downstream genetic analyses. We will continue to collect fungi with switchgrass leaves annually in mid-summer to early fall 2022-2025 at all sites, continuing intermittent collections that were made from 2017-2019 and hindered by the pandemic. Culturable fungi will be identified by morphological analysis, whereas for rust and smut fungi, will be identified based on teliospore measurements and DNA marker results. Drs. Shawn Kenaley and Gary Bergstrom (Cornell Univ.) have conducted the only morphological and phylogenetic analyses of switchgrass rust fungi to date and, hence, have the expertise and procedures to successfully complete the aforementioned molecular and morphometric analyses.

New resistant switchgrass germplasm

Through the evaluations of fungi populations described above, we will be able to identify switchgrass populations and/or genotypes that are resistant or tolerant to one or more fungal pathogens. Resistant germplasm will be evaluated for biofuel characteristics and considered for release, based on the release protocols of the institutions involved in the project. Further selection, intercrossing, and population development will be conducted by the partners to generate highly productive, disease resistant cultivars. New cultivars will be exclusively licensed through Roundstone Native Seed Company. In addition to release, switchgrass cultivars are being stored/released through GRIN. Recent cultivars ‘Tusca’ (imazapic resistant lowland switchgrass), ‘Espresso” – high germination, high velocity of germination lowland switchgrass, and “Robusto’– high germination, high velocity of germination upland switchgrass.

1.4 Developing resilient cool-season grasses adapted to variable climatic conditions.

This project will develop cultivars and breeding lines of important cool-season grasses widely used in multiple North American forage breeding programs, including meadow bromegrass, tall fescue, orchardgrass, and timothy. Specific objectives are: 1) Evaluate new elite populations of cool-season grasses for potential cultivar release and commercialization 2) Select individual genotypes from the elite populations to develop new elite germplasm for future genetic improvement. 3) Identification of candidate genes associated with cold acclimation in orchardgrass.

Experimental approach: From 2017-2021 (Phase I), 10 populations of meadow bromegrass, 7 populations of tall fescue, 9 populations of orchardgrass, and 10 populations of timothy grass were characterized for biomass production, nutritive value, and survival for three production years at six locations. These included sites at: Logan and Pantuitch, Utah, Saskatoon and Swift Current, Saskatchewan, and Normandin and St-Augustin, Québec. Superior genotypes for each species were selected by each test site for new population development in 2021. In the proposed study (Phase II), plots of new elite populations of meadow brome, tall fescue, orchardgrass, and timothy grass developed by Phase I study will be established at the above mentioned experimental sites. The experimental design at each site was randomized complete block design with 4 replications. Plot size will be 1.4 x 2 m, with 4 rows containing 12 plants/row. Biomass and nutritive value (ADF, NDF and CP) will be collected during the first two production years. Survival data will be collected each year. Based on the multiple year data, the best populations at each site will be identified and selected for seed increase. The elite populations will potentially be released as commercial cultivars (Ampac Seed). In addition, new plant selection will be made for each species to create new elite germplasm for future breeding of the four cool-season grasses.

To determine water soluble carbohydrate (WSC) content, orchardgrass cultivar Killarney was cloned and a new nursery was transplanted in early spring 2019 at Saint-Augustin, Quebec and Saskatoon, SK Canada. At each site, the experimental design was a randomized complete block with four replications. The clones will be grouped into three sampling dates of 1-wk before a killing frost, immediately after (day temp. -2oC for more than 5 hours), and 1-wk after a killing frost. At each sampling, clones will be dug out and rinsed off before the stem bases (2-3g) were collected for WSC. The crown samples for RNAseq will also be collected from the field and stored at -80oC in the laboratory. RNA will be extracted from the samples, and RNAseq analysis will be carried out to determine differentially expressed genes and unique alleles for cold tolerance in orchardgrass.

1.5 Determining the extent of genetic variability of condensed tannins (CT) among currently available birdsfoot trefoil cultivars and elite lines

We will assess the CT profile and content of birdsfoot trefoil germplasm across diverse climatic conditions in northern latitudes. This investigation will be initiated by establishing small, replicated plots of birdsfoot trefoil cultivars and elite lines in regions where the investigators are located. In 2019 and 2020, prior to harvesting, the plots were assessed for development of stage. Plots will be sampled for CT profile and content and harvested to determine forage yield. We plan to repeat and expand this work to have a third year, and to bring in a new site in Vermont. Although fairly small in scope, this work is essential as the only continent wide work on this versatile and nutritious forage legume.

NEW: 1.6 Miscanthus

Preliminary trials in Illinois and Mississippi are planned to evaluate Giant miscanthus giant Miscanthus (*Miscanthus* x *giganteus*; Mxg). Several Mxg seedlings have been generated at the University of Illinois and will be tested at these climatically extreme sites. Evaluation will focus on biomass, agronomic characteristics, and disease. In Mississippi, cool season tolerance will be evaluated. This work, although preliminary in scope, is essential to scale up activities to a full nationwide scale.

2.0. *Understanding genotype by environment by management interactions across multiple forage species*

To provide the information needed for broadly adapted germplasm suitable to a range of production systems, multi-location and multi-production trials are needed. The concluding NE1710 has a component of G × E analysis that we intend to expand and deepen with similar methods but expanded datasets and an increased focus on the interacting effects of management decisions (G × E × M). Multi-location evaluation data for broadly adapted as well as location specific germplasm developed by members of the NE1710 committee will be generated either jointly or independently. Cooperating locations where testing will be conducted will be identified for each species. An individual from one of the locations will act as the coordinator. Seed from each cooperating location and the core set of cultivars will be distributed by the coordinator to each cooperating location. Seed of each entry will be submitted by each cooperating location to the coordinator of the trial, entries will typically not exceed four to six entries. For each species a core set of check cultivars will be included for testing.

Entries will be established following local practices in small plot trials and will be maintained for two to three years following the establishment year. In some locations this may include multiple management approaches. Each location will collect a core set of data, such as seedling vigor and plant stand in the establishment year, forage yield from a minimum of one harvest in the two to three years following establishment, forage quality data where the local cooperator has that capability and persistence following the last harvest in the second year following establishment. Each location will also collect data unique to the biotic and abiotic stresses for their specific environment. Individual locations may choose to include additional check cultivars when a test is specific to their location. At the conclusion of the final harvest year, raw/rep data will be sent from each cooperating location to the coordinator of that crop. This should occur no later than mid November. The coordinator for that crop will analyze and summarize the data, reporting G × E × M interactions for establishment, forage yield, and persistence. All data, including that unique to specific locations, will be summarized in mean tables, which will then be distributed to all cooperators.

Measurement of Progress and Results

Outputs

- Increase in improved forage cultivars that enhance the economics of livestock producers. Comments: Seed sales of new cultivars, when available.
- Increase in the alfalfa germplasm available to breeders in North America to develop improved cultivars more resilient and better adapted to climate stress in the future. Comments: We will release germplasm to NPGS when appropriate. We are also working on public releases.
- Increase in scientific knowledge through journal papers that will assist other scientists in future plant breeding activities.
- Increase preliminary data to enable future grant proposal development (e.g., alfalfa germplasm structure and use; switchgrass QTL mapping and marker development for pathogen resistance; water soluble carbohydrate analysis in grasses; bioactive compounds in trefoil)

Outcomes or Projected Impacts

- Increased acreage of cultivars with high forage yield and quality will result in enhanced economic vitality of forage and livestock production operations.
- Increase in improved germplasm will be provide material for further breeding and/or immediate commercialization with improved adaptation and resilience for production and persistence under pasture and hay production systems.
- Data that provide producers with options for cultivar selection, planting and harvesting to optimize production.

Milestones

(**2022**):Establishment of new cover crop sites

(**2023**):Establishment of Miscanthus trial plots

(**2024**):Assemble of multi-year G*E data

(**2024**):Among our milestones, we will include germplasm releases. These releases will include submissions to NPGS. Some of our material is in queue for submission to Journal of Plant of Registrations (JOPR). Several releases from our past project are currently in backlogs, awaiting state Agriculture Experiment Station approval due to COVID issues.

Outreach Plan

All members of the technical committee are involved in outreach to the scientific community, the seedndustry, and the farm community in their region. The primary means of outreach to the scientific community include publications in peer reviewed journals and presentations at conferences. Many of the members of the technical committee have active breeding programs that release cultivars, and these members typically have connections with seed companies to market their cultivars. The technical committee will work to enhance communication between scientists and industry colleagues to more effectively transfer results to industry and also to ensure research is being conducted on topics of relevance to the industry. In the current proposal, the alfalfa germplasm project already has S&W Seeds as a collaborating member and will involve other alfalfa companies as the project continues. Similar efforts to engage with seed companies will be made for the other objectives. For all objectives, the ultimate goal is to develop cultivars that can be licensed to seed companies and sold to farmers or other users. Finally, all members of the committee routinely speak at extension or grower meetings in their respective locations and work to ensure that extension personnel know about and are conversant ontheir research programs.

Throughout the life of the project, we will regularly invite other forage breeders, pathologistsentomologists, physiologists, and agronomists in the public and private sectors to collaborate on aspects of the projects, as needed. Any of these participants are also welcome to join the project as official members. Our goal is to be as inclusive as we can be to ensure we reach our objectives.

Organization/Governance

This project is organized by objective, with each objective having one or more lead scientists. Like the current NE1710 project, the lead scientists will prepare annual summaries of research in their objective (or sub-objective) and lead the discussion at the annual meeting. These scientists are tasked with keeping the objective moving forward, meeting the objectives in a timely manner, and tracking to ensure that each participant is keeping their part of the project going according to plan. All other participants contribute updates on their work.

The annual meetings have a chair and a secretary, who typically rotates to chair the succeeding year. The secretary for the next meeting is elected by the membership each year.

Literature Cited

Acharya, J.P., Lopez, Y., Gouveia, B.T., de Bem Oliveira, I., Resende, M.F., Muñoz, P.R. and Rios, E.F., 2020. Breeding alfalfa (Medicago sativa L.) adapted to subtropical agroecosystems. Agronomy, 10(5), p.742.

Arthur JC. 1934. Manual of the rusts in United States and Canada. Purdue Research Foundation,

Lafayette, IN. The Science Press Printing Co., Lancaster, PA. 438 p.

Austin, E.E., K. Wickings, M.D. McDaniel, G.P. Robertson, and A.S. Grandy. 2017. Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. GCB Bioenergy 9(7): 1252–1263. doi: 10.1111/gcbb.12428.

Barnes, D. K., Bingham, E. T., Murphy, R. P., Hunt, O. J., Beard, D. F., Skrdla, W. H., & Teuber, L. R. 1977Alfalfa germplasm in the United States: genetic vulnerability, use, and maintenance. USDA-ARS Tech. Bull,1571.

Biswas A, Andrade MHML,Acharya JP, de Souza CL, Lopez Y,de Assis G, Shirbhate S, Singh A,Munoz P and Rios EF (2021)Phenomics-Assisted Selectionfor Herbage Accumulation in Alfalfa(Medicago sativa L.).Front. Plant Sci. 12:756768.doi: 10.3389/fpls.2021.756768

Boateng, A.A., Daugaard, D.E., Goldberg, N.M. and Hicks, K.B., 2007. Bench-scale fluidized-bed pyrolysis of switchgrass for bio-oil production. *Industrial & Engineering Chemistry Research*, *46*(7), pp.1891-1897.

Bouton, J.H., 2021. Alfalfa. International Grasslands Proceedings Conference. https://uknowledge.uky.edu/cgi/viewcontent.cgi?article=4439&context=igc

Bowen, K.L., Hagan, A. and Miller, H.B., 2021. Rust (Puccinia emaculata) management and impact on biomass yield on switchgrass. *Plant Disease*, (ja).

Brummer, EC. and Casler, M.D., 2014. Cool season forages. *Yield gains in major US field crops*, *33*, pp.33-51.

Burrill TJ. 1884. New species of Uredineae (collection made by A. B. Seymour). Botanical Gazette 9:4, 187–191.

Burridge, J.D., Rangarajan, H. and Lynch, J.P., 2020. Comparative phenomics of annual grain legume root architecture. Crop Science, 60(5), pp.2574-2593.

Busbice, T.H. and Rawlings, J.O., 1974. Combining ability in crosses within and between diverse groups of alfalfa introductions. *Euphytica*, *23*(1), pp.86-94.

Casler, M.D., Tobias, C.M., Kaeppler, S.M., Buell, C.R., Wang, Z.Y., Cao, P., Schmutz, J. and Ronald, P., 2011. The switchgrass genome: tools and strategies. *The Plant Genome Journal*, *4*(3), p.273.

Chen, H., Zeng, Y., Yang, Y., Huang, L., Tang, B., Zhang, H., Hao, F., Liu, W., Li, Y., Liu, Y. and Zhang, X., 2020. Allele-aware chromosome-level genome assembly and efficient transgene-free genome editing for the autotetraploid cultivated alfalfa. *Nature communications*, *11*(1), pp.1-11.

Cornelius, D.R. and Johnston, C.O., 1941. Differences in plant type and reaction to rust among several collections of Panicum virgatum L. Journal of the American Society of Agronomy.

Crouch, J.A., Beirn, L.A., Cortese, L.M., Bonos, S.A. and Clarke, B.B., 2009. Anthracnose disease of switchgrass caused by the novel fungal species Colletotrichum navitas. *Mycological research*, *113*(12), pp.1411-1421.

CTIC, Conservation Technology Innovation Center Report. 2021. https://www.ctic.org/files/CoverCropSurvey%202020%2024mb.pdf

Cummins GB. 1971. The Rust Fungi of Cereals, Grasses and Bamboos, 2 edn. Springer-Verlag, New York. 570 p.

Dudley, J.W. and Davis, R.L., 1966. Preliminary Groupings of Plant Introductions of Alfalfa (Medicago sativa, L.) for Heterosis Studies 1. *Crop Science*, *6*(6), pp.597-600.

Egan, L.M., Hofmann, R.W., Ghamkhar, K. and Hoyos-Villegas, V., 2021. Prospects for Trifolium improvement through germplasm characterisation and pre-breeding in New Zealand and beyond. *Frontiers in Plant Science*, *12*, p.1056.

Elden, T.C. and McCaslin, M.A.R.K., 1997. Potato leafhopper (Homoptera: Cicadellidae) resistance in perennial glandular-haired alfalfa clones. *Journal of economic entomology*, *90*(3), pp.842-847.

Elgin Jr, J.H., Welty, R.E. and Gilchrist, D.B., 1988. Breeding for disease and nematode resistance.*Alfalfa and alfalfa improvement*, *29*, pp.827-858.

Fajolu, O.L., Vu, A.L., Dee, M.M., Zale, J., Gwinn, K.D. and Ownley, B.H., 2012. First report of leaf spot and necrotic roots on switchgrass caused by Curvularia lunata var. aeria in the United States. *Plant disease*, *96*(9), pp.1372-1372.

Farr DF, Rossman AY. 2021. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. http://nt.ars-grin.gov/fungaldatabases/. Accessed 26 November, 2021.

Frazier, T., Shen, Z., Zhao, B. and Bush, E., 2013. First report of Puccinia emaculata infection on switchgrass in Virginia.*Plant disea*

Ghelichkhan, M., Eun, J.S., Christensen, R.G., Stott, R.D. and MacAdam, J.W., 2018. Urine volume and nitrogen excretion are altered by feeding birdsfoot trefoil compared with alfalfa in lactating dairy cows. *Journal of animal science*, *96*(9), pp.3993-4001.

Gilley, M. and Tomaso-Peterson, M., 2011, June. Foliar diseases identified on switchgrass in Mississippi. In *PHYTOPATHOLOGY* (Vol. 101, No. 6, pp. S61-S61). 3340 PILOT KNOB ROAD, ST PAUL, MN 55121 USA: AMER PHYTOPATHOLOGICAL SOC.

Gravert, C.E., Tiffany, L.H. and Munkvold, G.P., 2000. Outbreak of smut caused by Tilletia maclaganii on cultivated switchgrass in Iowa. *Plant disease*, *84*(5), pp.596-596.

Gravert, C.E. and Munkvold, G.P., 2002. Fungi and diseases associated with cultivated switchgrass in Iowa.*Journal of the Iowa Academy of Science: JIAS*, *109*(1-2), pp.30-34.

Gustafson, D.M., Boe, A. and Jin, Y., 2003. Genetic variation for Puccinia emaculata infection in switchgrass.*Crop Science*, *43*(3), pp.755-759.

Hirsch, R.L., TeBeest, D.O., Bluhm, B.H. and West, C.P., 2010. First report of rust caused by Puccinia emaculata on switchgrass in Arkansas. *Plant disease*, *94*(3), pp.381-381.

Horner, E.S., and O.C. Ruelke. 1981. Registration of Florida 77 alfalfa..Crop Sci. 21:797.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M. and Hoskin, S.O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends in parasitology, 22(6), pp.253-261.

İlhan, D., Li, X., Brummer, E.C. and Şakiroğlu, M., 2016. Genetic diversity and population structure of tetraploid accessions of the Medicago sativa–falcata Complex. *Crop Science*, *56*(3), pp.1146-1156.

Irish, B.M. and Greene, S.L., 2021. Germplasm Collection, Genetic Resources, and Gene Pools in Alfalfa. In*The Alfalfa Genome* (pp. 43-64). Springer, Cham.

Kalu, B. A., & Fick, G. W. (1981). Quantifying morphological development of alfalfa for studies of herbage quality. Crop Science, 21(2), 267–271. https://doi.org/10.2135/cropsci1981.0011183X002100020016x

Kenaley, S.C., Hudler, G.W. and Bergstrom, G.C., 2016. Detection and phylogenetic relationships of Puccinia emaculata and Uromyces graminicola (Pucciniales) on switchgrass in New York State using rDNA sequence information. *Fungal biology*, *120*(5), pp.791-806.

Knowles, R.P., Baron, V.S., McCartney, D.H.: Meadow bromegrass. – Agriculture Canada Publication 1888/E, Ottawa 1993.

Lamb, J.F.S., Samac, D.A., Barnes, D.K. and Henjum, K.I., 2000. Increased herbage yield in alfalfa associated with selection for fibrous and lateral roots. *Crop Science*, *40*(3), pp.693-699.

Kong, A.Y.Y., and J. Six. 2010. Tracing Root vs. Residue Carbon into Soils from Conventional and Alternative Cropping Systems. Soil Science Society of America Journal 74(4): 1201–1210. doi: 10.2136/sssaj2009.0346.

Lee, M.R.F., L.J. Harris, J.M. Moorby, M.O. Humphreys, M.K. Theodorou, J.C. McRae, and N.D.Scollan. 2002. Rumen metabolism and nitrogen flow to the small intestine in steers offered Lolium perenne containing different levels of water-soluble carbohydrate. Animal Science 74:587-596.

Lenné, J.M., 1990. Rust on the tropical pasture grass Brachiaria humidicola in South America.*Plant disease*, *74*(9).

Li, X. and Brummer, E.C., 2012. Applied genetics and genomics in alfalfa breeding.*Agronomy*, *2*(1), pp.40-61.

Li, X., Wei, Y., Acharya, A., Jiang, Q., Kang, J. and Brummer, E.C., 2014. A saturated genetic linkage map of autotetraploid alfalfa (Medicago sativa L.) developed using genotyping-by-sequencing is highly syntenous with the Medicago truncatula genome. *G3: genes, genomes, genetics*, *4*(10), pp.1971-1979.

Li, A., Liu, A., Du, X., Chen, J.Y., Yin, M., Hu, H.Y., Shrestha, N., Wu, S.D., Wang, H.Q., Dou, Q.W. and Liu, Z.P., 2020. A chromosome-scale genome assembly of a diploid alfalfa, the progenitor of autotetraploid alfalfa. *Horticulture research*, *7*(1), pp.1-12.

Lovell, J.T., Shakirov, E.V., Schwartz, S., Lowry, D.B., Aspinwall, M.J., Taylor, S.H., Bonnette, J., Palacio-Mejia, J.D., Hawkes, C.V., Fay, P.A. and Juenger, T.E., 2016. Promises and challenges of eco-physiological genomics in the field: tests of drought responses in switchgrass. Plant Physiology, 172(2), pp.734-748.

Lovell, J.T., MacQueen, A.H., Mamidi, S., Bonnette, J., Jenkins, J., Napier, J.D., Sreedasyam, A., Healey, A., Session, A., Shu, S. and Barry, K., 2021. Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass. *Nature*, *590*(7846), pp.438-444.

Lowry, D.B., Lovell, J.T., Zhang, L., Bonnette, J., Fay, P.A., Mitchell, R.B., Lloyd-Reilley, J., Boe, A.R., Wu, Y., Rouquette, F.M. and Wynia, R.L., 2019. QTL× environment interactions underlie adaptive divergence in switchgrass across a large latitudinal gradient. *Proceedings of the National Academy of Sciences*, *116*(26), pp.12933-12941.

Marley, C.L., Fychan, R. and Jones, R., 2006. Yield, persistency and chemical composition of Lotus species and varieties (birdsfoot trefoil and greater birdsfoot trefoil) when harvested for silage in the UK. Grass and Forage Science, 61(2), pp.134-145.

Mazzilli, S., A. Kemanian, O. Ernst, R. Jackson, and G. Piñeiro. 2015. Greater humification of belowground than aboveground biomass carbon into particulate soil organic matter in no-till corn and soybean crops. Soil Biology and Biochemistry 85. doi: 10.1016/j.soilbio.2015.02.014.

McMillan C. 1964. Ecotypic differentiation within four North American prairie grasses. I. Morphologicalvariation within transplanted community fractions. American Journal of Botany 51: 1119–1128.

Meyer, E., Aspinwall, M.J., Lowry, D.B., Palacio-Mejía, J.D., Logan, T.L., Fay, P.A. and Juenger, T.E., 2014. Integrating transcriptional, metabolomic, and physiological responses to drought stress and recovery in switchgrass (Panicum virgatum L.). *BMC genomics*, *15*(1), pp.1-15.

Milano, E.R., Lowry, D.B. and Juenger, T.E., 2016. The genetic basis of upland/lowland ecotype divergence in switchgrass (Panicum virgatum). *G3: Genes, Genomes, Genetics*, *6*(11), pp.3561-3570.

Miller, L.A., J.M. Moorby, D.R. Davies, M.O. Humphreys, N.D. Scollan, J.C. McRae, and M.K.Theodorou. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.): milk production from late-lactation dairy cows. Grass and Forage Science 56:383-394.

Min, B.R., Barry, T.N., Attwood, G.T. and McNabb, W.C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal feed science and technology*, *106*(1-4), pp.3-19.

Moore, V., B. Davis, M. Poskaitis, J.E. Maul, L. Kissing Kucek, et al. 2020. Phenotypic and Nodule Microbial Diversity among Crimson Clover (Trifolium incarnatum L.) Accessions. Agronomy 10(9): 1434. doi: 10.3390/agronomy10091434.

Muller, K.E., J. Guinness, M. Hecking, and L.E. Drinkwater. 2021. Estimating agronomically relevant symbiotic nitrogen fixation in green manure breeding programs. Crop Science 61(5): 3314–3330. doi: 10.1002/csc2.20517.

Qiang, H., Chen, Z., Zhang, Z., Wang, X., Gao, H. and Wang, Z., 2015. Molecular diversity and population structure of a worldwide collection of cultivated tetraploid alfalfa (Medicago sativa subsp. sativa L.) germplasm as revealed by microsatellite markers. *PLoS One*, *10*(4), p.e0124592.

Parajuli, A., Yu, L., Peel, M., See, D., Wagner, S., Norberg, S. and Zhang, Z., 2020. Self Incompatibility, Inbreeding Depression, and Potential to Develop Inbred Lines in Alfalfa: A Review.

Parrish, D.J., Casler, M.D. and Monti, A., 2012. The evolution of switchgrass as an energy crop. In*Switchgrass* (pp. 1-28). Springer, London.

Ramachar P, Cummins G. 1963. The species of Uromyces on the tribe Paniceae. Mycopathologia etMycologia Applicata 19: 49–61.

Ramachar P, Cummins G. 1965. The species of Puccinia on the tribe Paniceae. Mycopathologia etMycologia Applicata 25: 7–60.

Riday, H. and Brummer, E.C., 2002. Heterosis of agronomic traits in alfalfa. Crop science, 42(4), pp.1081-1087.

Riday, H. and Brummer, E.C., 2006. Persistence and yield stability of intersubspecific alfalfa hybrids.

Riday, H., and S. Wagner. 2012. Improvements in alfalfa subspecies Falcata germplasms and their hybrid performance. Jul. 10-12, Proc. 2012 Joint Conference NAAIC, Trifolium, and Grass Breeders, Ithaca, NY.

Robins, J.G. and Lovatt, J.A., 2016. Cultivar by environment effects of perennial ryegrass cultivars selected for high water soluble carbohydrates managed under differing precipitation levels. Euphytica, 208(3), pp.571-581.

Rouquette, F.M., Jr., L.A. Redmon, G.E. Aiken, G.M. Hill, L.E. Sollenberger, and J. Andrae. 2009. ASAScentennial paper: Future needs research and extension forage utilization. J. Anim. Sci. 87:438-446.

Sanada, Y., Takai, T. and Yamada, T., 2007. Ecotypic variation of water-soluble carbohydrate concentration and winter hardiness in cocksfoot (Dactylis glomerata L.). Euphytica, 153(1), pp.267-280.

Schmer, M.R., Vogel, K.P., Mitchell, R.B. and Perrin, R.K., 2008. Net energy of cellulosic ethanol from switchgrass. *Proceedings of the National Academy of Sciences*, *105*(2), pp.464-469.

Scotti, C. and Brummer, E.C., 2010. Creation of heterotic groups and hybrid varieties. In*Sustainable use of genetic diversity in forage and turf breeding* (pp. 509-518). Springer, Dordrecht.

Serba, D.D., Uppalapati, S.R., Mukherjee, S., Krom, N., Tang, Y., Mysore, K.S. and Saha, M.C., 2015. Transcriptome profiling of rust resistance in switchgrass using RNA Seq analysis. *The Plant Genome*, *8*(2), pp.plantgenome2014-10.

Shen, C., Du, H., Chen, Z., Lu, H., Zhu, F., Chen, H., Meng, X., Liu, Q., Liu, P., Zheng, L. and Li, X., 2020. The chromosome-level genome sequence of the autotetraploid alfalfa and resequencing of core germplasms provide genomic resources for alfalfa research. *Molecular Plant*, *13*(9), pp.1250-1261.

Stewart, A. and Cromey, M., 2011. Identifying disease threats and management practices for bio-energy crops.*Current Opinion in Environmental Sustainability*, *3*(1-2), pp.75-80.

Strange, R.N. and Scott, P.R., 2005. Plant disease: a threat to global food security.*Annu. Rev. Phytopathol.*, *43*, pp.83-116.

Sykes, V.R., Allen, F.L., Mielenz, J.R., Stewart, C.N., Windham, M.T., Hamilton, C.Y., Rodriguez, M. and Yee, K.L., 2016. Reduction of ethanol yield from switchgrass infected with rust caused by Puccinia emaculata. *BioEnergy Research*, *9*(1), pp.239-247.

Thomsen, P.M., Brummer, E.C., Shriver, J. and Munkvold, G.P., 2008. Biomass yield reductions in switchgrass due to smut caused by Tilletia maclaganii. *Plant Health Progress*, *9*(1), p.21.

Tubritt, T., Gilliland, T.J., Delaby, L., McHugh, N. and O'Donovan, M., 2018. Comparison of grass utilisation performance of perennial ryegrass varieties. In Sustainable meat and milk production from grasslands. Proceedings of the 27th General Meeting of the European Grassland Federation, Cork, Ireland, 17-21 June 2018 (pp. 54-59). Teagasc, Animal & Grassland Research and Innovation Centre.

Uppalapati, S.R., Serba, D.D., Ishiga, Y., Szabo, L.J., Mittal, S., Bhandari, H.S., Bouton, J.H., Mysore, K.S. and Saha, M.C., 2013. Characterization of the rust fungus, Puccinia emaculata, and evaluation of genetic variability for rust resistance in switchgrass populations. *Bioenergy research*, *6*(2), pp.458-468.

USDA-NRCS Plant Materials Centers. (2020). Cover Crop Performance and Adaptation Trials at PMCs. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/plantmaterials/technical/publications/?cid=nrcseprd1610414

VanWallendael, A., Bonnette, J., Juenger, T.E., Fritschi, F.B., Fay, P.A., Mitchell, R.B., Lloyd Reilley, J., Rouquette Jr, F.M., Bergstrom, G.C. and Lowry, D.B., 2020. Geographic variation in the genetic basis of resistance to leaf rust between locally adapted ecotypes of the biofuel crop switchgrass (Panicum virgatum). New Phytologist, 227(6), pp.1696-1708.

Volaire, F. and Thomas, H., 1995. Effects of drought on water relations, mineral uptake, water-soluble carbohydrate accumulation and survival of two contrasting populations of cocksfoot (Dactylis glomerata L.). Annals of Botany, 75(5), pp.513-524.

Waghorn, G.C. and McNabb, W.C., 2003. Consequences of plant phenolic compounds for productivity and health of ruminants. *Proceedings of the Nutrition Society*, *62*(2), pp.383-392.

Wayman, S., Kissing Kucek, L., Mirsky, S. B., Ackroyd, V., Cordeau, S., & Ryan, M. R. (2017). Organic and conventional farmers differ in their perspectives on cover crop use and breeding. Renewable Agriculture and Food Systems, 32(4), 376–385. http://dx.doi.org.prox.lib.ncsu.edu/10.1017/S1742170516000338

Zale, J., Freshour, L., Agarwal, S., Sorochan, J., Ownley, B.H., Gwinn, K.D. and Castlebury, L.A., 2008. First report of rust on switchgrass (Panicum virgatum) caused by Puccinia emaculata in Tennessee. *Plant disease*, *92*(12), pp.1710-1710.

Zhu, Q., Bennetzen, J.L. and Smith, S.M., 2013. Isolation and diversity analysis of resistance gene homologues from switchgrass. *G3: Genes, Genomes, Genetics*, *3*(6), pp.1031-1042.

Land Grant Participating States/Institutions

IL,VT,GA,MN,NY,RI

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Bishop-von Wettberg, Eric	Yes	Vermont - University of Vermont	1,2	202	1640	1080	0.10	0.00	0.00	0	0
Brown, Rebecca N	Yes	Rhode Island - University of Rhode Island	1,2	202	1641	1081	0.10	0.00	0.00	0.02	313
Ehlke, Nancy J	Yes	Minnesota - University of Minnesota	1,2	204	1621	1081	0.10	0.00	0.00	0	0
Gan, Susheng		New York -Ithaca : Cornell University	1,2	204	1640	1081	0.10	0.00	0.00	0	0
Missaoui, Ali M	Yes	Georgia - University of Georgia	1,2	201 202 203 212	1621 1631 1640 1649	1080 1081 1081 1081	0.10	0.00	0.00	0	0
Sacks, Erik		Illinois - University of Illinois	1,2	202	1620	1081	0.10	0.00	0.00	0	0
Smith, Shavannor		Georgia - University of Georgia	1,2	201	1621	1080	0.10	0.00	0.00	0	0

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
202-1620-1081	0.1	0	0
202-1640-1080	0.1	0	0
201-1621-1080	0.1	0	0
204-1621-1081	0.1	0	0
204-1640-1081	0.1	0	0
201-1621-1080	0.03	0	0
202-1631-1081	0.03	0	0
203-1640-1081	0.03	0	0
212-1649-1081	0.03	0	0
202-1641-1081	0.1	0	0
Grand Total:	**0.70**	**0.00**	**0.00**

Program/KA	Total FTE
0	0
0	0
0	0
0	0
0	0
0	0
313	0.01
Grand FTE Total:	**0.02**

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2210: Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Nutritive Value

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
Team has a history of collaboration and productivity in the form of new varieties and climate-based plant responses on a wide geographic scale. This proposal would broaden the scope of the project to a legume (birdsfoot trefoil). Project is thorough and involves a number of different scientific disciplines. Project will result in manuscripts on GxE interactions that will be relevant for multiple efforts across the US and other countries.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2210: Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Nutritive Value

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project with revision
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
This multistate project has proven successful under NE-1710 with the networking across Northeast US and Eastern Canada regions and disciplines. The project aligns with four of the NIFA's Priority Science Areas. The improvement of forage and bioenergy crops will positively impact the livestock and biofuel industries in the US. The project highlights a reduction of negative environmental impact in this agricultural system. The group addresses pre-breeding activities in Alfalfa as an essential part of the breeding efforts. The importance of multistate trials is emphasized. The forage and bioenergy are facing a shortage of breeders and extension workers. It is a national problem across different crops in the US.

Milestones are clear. Maybe need to add more milestones. The release of new species and their respective cultivars should be considered milestones.
I didn't see any release in the Literature Cited (Journal of Plant of Registrations).
How do the new species compare with the check cultivars?
List the most threatening diseases and pests to the crops in the project and assess yield reduction and economic impact.
Better agronomic practices and integrated pest management should be considered.
Any plans to conduct some feed studies on animals? The nutritional values of both legumes and grasses should be considered in the proposal.
Is the group considering the use of genomic tools and databases? Many major quantitative trait loci (QTL) related to agronomic traits have been identified in alfalfa. This might benefit the group in taking decisions of what kind of germplasm to use. The genes responsible for key agronomic and disease traits will be discovered with novel diversity panels, genomic tools and databases, and innovative analysis methods. It will accelerate the process of cultivar development.
Your Recommendation:
Approve/continue project with revision

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2210: Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Nutritive Value

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
This proposal was a pleasure to read. The need for the work is strongly justified. I appreciated the focus on understudied forage species that are widely grown (e.g. orchardgrass and trefoil). With a collaborative approach, the team seems primed to accomplish the proposed objectives.

The focus on aggregation and analysis of existing breeding program data is an efficient way to use small funding sources, and will likely yield greater benefits than attempting trendy methods like genomic selection. The organizational structure matches the lean style of the proposal, with lead scientists vested in the research coordinating each objective.

The GxE objectives and specific methods described in 1.2 and 1.4 seem highly related to the continent-wide forage adaptation and resilience research coordinated by Valentin Picasso. What can be learned from that work to make a similar study for these different forage species more successful?

The proposal states that all improved material will be released through the GRIN system. However, the outputs and outreach plan discuss releasing new cultivars with seed companies. Does the release of improved material as germplasm accessions work for those companies?
Your Recommendation:
Approve/continue project

Appendix G: Response to review

We thank the three reviewers for their time and insightful comments. We have responded to them below. There are several useful suggestions that we have incorporated. Our specific responses are set off from the other text in italics to separate them from reviewer comments. We have also updated our proposal. In particular we have updated the methods and measurement of progress and results. We have clarified where and how we will release germplasm. We have also updated our use of checks.

Appendix G: Peer Review (Submitted)
Status: Complete
Project ID/Title: NE_TEMP2210: Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Nutritive Value
Rate the technical merit of the project:
1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent

Comments
Team has a history of collaboration and productivity in the form of new varieties and climate-based plant responses on a wide geographic scale. This proposal would broaden the scope of the project to a legume (birdsfoot trefoil). Project is thorough and involves a number of different scientific disciplines. Project will result in manuscripts on GxE interactions that will be relevant for multiple efforts across the US and other countries.
Your Recommendation:
Approve/continue project

Thank you for the positive comments.

Appendix G: Peer Review (Submitted)
Status: Complete
Project ID/Title: NE_TEMP2210: Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Nutritive Value
Rate the technical merit of the project:
1. Sound Scientific approach:
Approve/continue project with revision
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:

Excellent
Comments
This multistate project has proven successful under NE-1710 with the networking across Northeast US and Eastern Canada regions and disciplines. The project aligns with four of the NIFA's Priority Science Areas. The improvement of forage and bioenergy crops will positively impact the livestock and biofuel industries in the US. The project highlights a reduction of negative environmental impact in this agricultural system. The group addresses pre-breeding activities in Alfalfa as an essential part of the breeding efforts. The importance of multistate trials is emphasized. The forage and bioenergy are facing a shortage of breeders and extension workers. It is a national problem across different crops in the US.

Milestones are clear. Maybe need to add more milestones. The release of new species and their respective cultivars should be considered milestones.
I didn't see any release in the Literature Cited (Journal of Plant of Registrations).

Response:
We have added the release of cultivars and submissions to Journal of Plant of Registrations (JOPR) to our milestones. Several participants mentioned that their recent releases are currently in extended approval queues at State Agricultural Experiment Stations due to COVID delays.

How do the new species compare with the check cultivars?

Response: It is unclear which forage or bioenergy crop the reviewer is focused on with the question. Given the scope of the project, different crops will have different answers. We have however clarified that our experiments will include appropriate checks. The methods are now updated.

List the most threatening diseases and pests to the crops in the project and assess yield reduction and economic impact.

Response: We have listed significant diseases and pests where space allows. We have emphazied them in switchgrass where they are our specific focus. We have not added as much about alfalfa, as different regions face different stresses.

Better agronomic practices and integrated pest management should be considered.

*Response: Yes, this is a good suggestion. In this renewal we have expended the second aim, Genotype*Environment*Management interaction to include a greater focus on three way interactions between genotypes, environment, and agronomic practices. Due to the scope of funding involved however, we do not have the capacity to include significant IPM in the work, except where individual participants have other funding focused on this very important issue.*

Any plans to conduct some feed studies on animals? The nutritional values of both legumes and grasses should be considered in the proposal.

Response: Many participants would love to move in this direction. However, due to funding limitations this is outside the scope of this proposal per se. Several participants have aligned funding to look at this. In birdsfoot trefoil this is particularly important, due to the focus on condensed tannins.

Is the group considering the use of genomic tools and databases? Many major quantitative trait loci (QTL) related to agronomic traits have been identified in alfalfa. This might benefit the group in

taking decisions of what kind of germplasm to use. The genes responsible for key agronomic and disease traits will be discovered with novel diversity panels, genomic tools and databases, and innovative analysis methods. It will accelerate the process of cultivar development.

Response: Yes, we are very interested as a group in genomic tools and databases. The nature of multi-state Hatch support means that this project lacks the funding to specifically develop tools. However, several participants have genomic research programs. Academic Advisor von Wettberg has a program on comparative legume domestication. The Cornell Breeding Insight team is developing tools for alfalfa, in the wake of the shift in focus at the Noble Foundation. Participant Rios in Florida is developing a genomic selection program for alfalfa. Switchgrass has seen a rapid development of genomic tools. However, we have not included these in this proposal, due to the absence of dedicated funding to support these activities, with the exception of places where participant Rios in Florida is funded to do such work.

Your Recommendation:
Approve/continue project with revision
Appendix G: Peer Review (Submitted)
Status: Complete
Project ID/Title: NE_TEMP2210: Improving Forage and Bioenergy Crops for Better Adaptation, Resilience, and Nutritive Value
Rate the technical merit of the project:
1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
This proposal was a pleasure to read. The need for the work is strongly justified. I appreciated the focus on understudied forage species that are widely grown (e.g. orchardgrass and trefoil). With a collaborative approach, the team seems primed to accomplish the proposed objectives.
The focus on aggregation and analysis of existing breeding program data is an efficient way to use small funding sources, and will likely yield greater benefits than attempting trendy methods like genomic selection. The organizational structure matches the lean style of the proposal, with lead scientists vested in the research coordinating each objective.
The GxE objectives and specific methods described in 1.2 and 1.4 seem highly related to the continent-wide forage adaptation and resilience research coordinated by Valentin Picasso. What can be learned from that work to make a similar study for these different forage species more Successful?

Response: We appreciate this suggestion. Although academic advisor von Wettberg has a new Kerza project (NSF Biology Integration Institute New Roots For Restoration) *with the Danforth Center and the Land Institute, and others in the project have interest in intermediate wheatgrass, Prof. Picasso has not yet joined this multistate Hatch, as IWG breeding has occurred in other projects in the past. We have reached out to Prof. Picasso, and expect to have a big tent, open door approach to learn from each other as much as possible.*

The proposal states that all improved material will be released through the GRIN system. However, the outputs and outreach plan discuss releasing new cultivars with seed companies. Does the release of improved material as germplasm accessions work for those companies?

Response: As our project encompasses several crops, the answer varies by activity, crop and by the company. For alfalfa, where we specified release, we are developing pre-breeding populations. These will be released to GRIN. Participant Professor Rios at the University of Florida publicly released a re-selection from Florida 99 in 2021. For work with native bioenergy grasses, they will be exclusively licensed through Roundstone Native Seed Company. Heat tolerant ryegrass and heat tolerant orchardgrass will be licensed to Ampac Seed.

Your Recommendation:
Approve/continue project

Thank you for the positive comments.

NE_TEMP2220: Multi-state Coordinated Evaluation of Grape Cultivars and Clones

Status: Submitted As Final

Duration	10/01/2022 to 09/30/2027
Admin Advisors:	[Margaret E. Smith]
NIFA Reps:	

Statement of Issues and Justification

Established in 2004, the NE1020 (and currently NE1720) projects have been critically important with the objective of evaluation of new and emerging grapevine cultivars (and advanced breeding selections) through a period of extensive growth in vineyards and wineries in the United States. The adoption of regionally adapted wine grape varieties has enabled the production of wine in most U.S. states in the 21st century. Grape production was limited to most non-traditional regions due to climate constraints on *Vitis vinifera*, the European winegrape species that is not suitable to most areas in the U.S. The development of new interspecific hybrids and the testing of rootstocks, cultivars, and clones has expanded wine production.

Winegrape cultivar selection is among the most important components of vineyard and viticulture industry management. Selection of suitable cultivars with cold hardiness and disease resistance, in addition to response to edaphic and climate conditions, is necessary to inform best practices for industry stakeholders [1-4]. Grape breeding programs, clonal selection, and the identification of regionally appropriate materials from germplasm collections and similar global production regions are sources for new cultivars for evaluation. Many of the grape cultivars grown in the emerging regions are only decades old and may not reflect the consumer or market demand, thus creating an impetus for improved grape cultivars for economic success and sustainability. This is especially true in response to climate change, which includes the management of abiotic stress, new and invasive insect pests, and disease. As new winegrape industries experience continued growth, and subsequently the economic impact that comes with it, the winegrape industries are dependent on improving quality and quantity of grapes and wine produced. Continued discovery, development, and evaluation of grape cultivars and clones is critical for maintaining growth within this emerging agricultural sector.

The NE1020 project was structured for coordinated plantings and data collection. The current NE1720 project has focused on a flexible design, allowing researchers opportunities to evaluate materials locally alongside sentinel varieties for direct feedback to state and regional stakeholder audiences. We are writing to renew the NE1720 multistate project for an additional 5 years, with the goal of addressing present and anticipated needs of grape producers in the United States.

1. Needs Identified by Stakeholders

NE1720 (and prior NE1020) project members include research and extension faculty from institutions across the U.S. that regularly solicit stakeholder input for continued development of their programs. In this integrated industry, stakeholders include grape growers (farmers), winemakers, as well as tasting room and event managers. Consistent responses from stakeholders include support not only for continued cultivar development and evaluation, but also for developing best management practices to improve consistency, quantity, and quality of fruit from evaluated winegrape cultivars and clones. For sake of brevity, “cultivars” refers to newly released varieties from breeding programs, varieties introduced from other wine regions, advanced breeding selections (pre-commercial), and clones (bud sports, mutants, etc.) with improved characteristics. There is also a need to evaluate these cultivars across a wide range of environments. For example, in Colorado, intermittent extreme cold temperatures in the past ten years have repeatedly decimated *V. vinifera* cultivars and caused the industry to realize the need for better-adapted cultivars with improved cold hardiness. Recent major weather events such as polar vortices, rapid temperature swings, and early spring growing conditions with frost events have exacerbated the need to identify cold hardy cultivars in many regions. For cold-climate regions, there is a need for cultivars that mitigate high acidity to produce different wine styles, since the *Vitis riparia*-based cultivars presently grown in the region have very high titratable acidity that requires significant winemaking effort to reduce acidity [5-14]. In the Dakotas, Colorado, and northern Minnesota realizing survival under extreme low temperature conditions and sustained productivity is an issue.

A national industry survey conducted in 2017 by the National Grape Research Alliance identified stakeholder priorities for genetic improvement of grapevines. Key findings of this survey included the selection of quality, disease and insect resistant cultivars in order to improve vineyard practices for more sustainable production. In 2018, the American Vineyard Foundation, a national grape and wine industry-funded research organization, conducted a survey of the most important research needs for grape and wine producers. Plant material selection including clonal and cultivar selection was ranked third, with 25.1% of respondents ranking it within their top two goals [15]. However, the two higher-rated research areas, “production efficiency & profitability” and “disease & insect control”, were key objectives in NE1020/1720 as they address sustainability initiatives critical for stakeholder success.

Objectives

1. Comprehensive evaluation of grapevine cultivars and clones for viticultural, pest susceptibility and fruit and juice quality characteristics, including enological characteristics and local adaptation for sustainable production.
2. Conduct initial screening evaluations of promising emerging cultivars and advanced breeding lines (pre-commercial) to determine suitability for regional adaptation of viticulture and wine quality attributes.
3. Explore new germplasm resources including disease resistant cultivars being released in Europe, plant introductions including Asian accessions, and less-known cultivars that may have economic potential for the US grape industry.

2. Importance of the Work and Consequences If It Is Not Done

Testing of new cultivars is typically limited to a few areas. Coordinated, multi-state testing is needed to evaluate adaptation in a variety of environments. With changing climate and increased weather variability, cultivar adaptation, including physiological hardiness and robustness to changes in insect and disease pressure will be an increasing issue. This multi-state project will leverage substantial investments made in breeding programs and help evaluate genotype x environment interactions. Sustaining these efforts over several years is a requirement to fully evaluate fully grape cultivars over the life cycle of a typical vineyard and across multiple years of weather occurrences. This is especially important for inland 'continental climate' regions, which are more subject to extreme swings in temperature than more maritime-influenced climates. Interestingly, growers are often interested in new varieties due to the environmental plasticity of many modern hybrid grapes and potential economic benefits, despite a breeding program not being able to test multiple environments prior to variety release. This multi-state project aims to reduce this knowledge gap and reduce the risk of advancing varieties. The NE1020/1720 projects facilitate the breeding timeline by providing descriptions of environmental response characteristics useful for the breeder and stakeholder. These experiments lead to the removal of underperforming selections from the advancement pipeline or help target specific growing regions. Availability of grapes adapted to these continental climates has greatly increased interest in grape growing, as has growth of farm wineries in most of the U.S. However, planting **a poorly-adapted cultivar in the wrong place is a costly mistake.** Vineyard managers can face expensive replanting and retraining costs after cold injury. Cultivar selection in specialty crops, especially in grapes, focuses on vine health, yield, but also fruit quality and wine quality attributes.

Cold temperatures; short, cool growing seasons, and humidity that are conducive to disease development limits the production of traditional *V. vinifera* cultivars in most emerging winegrape regions, and novel cultivars may be more suitable even in regions where *V. vinifera* cultivars may thrive. Cultivar selection is the primary method for reducing losses from cold injury in vineyards, and the relatively new development of cold-hardy winegrape cultivars suitable for the eastern U.S. and other emerging regions is only beginning to be optimized. Members of the multi-state project routinely conduct cold hardiness, disease resistance and training studies providing the breeders feedback on advanced selections as well as moving our understanding of vine physiology.

Grape breeding programs in NY, MN, and AR, have successfully evaluated and released new cultivars [16-18]. A nascent, yet robust, breeding program in ND expands the northern range for winegrape production. Ongoing research on trait genetics is being adopted rapidly via marker-assisted selection to increase breeding precision in the New York, North Dakota, and Minnesota breeding programs (vitisgen2.org). Hatterman-Valenti, Reisch, and Clark are actively adopting genetic marker technology to facilitate the development of disease-resistant, cold hardy cultivars with high quality wine potential. Information generated through NE1020/1720 and this proposed product trials will feed back into those breeding programs to either field-validate genetic bases for cold hardiness, disease resistance, and other important traits. Private breeders have also been important in developing cold-climate grape cultivars which require evaluation across diverse regions and climates [19,20]. In addition, novel *V. vinifera* and other hybrid cultivars from Europe, in particular those developed for disease resistance, are candidates for evaluation in this program. The number of available selections for a vineyard manager to evaluate is daunting and could result in years of lost revenue unless public, long-term evaluation of cultivars is conducted to reduce evaluation time prior to commercial planting.

University and Agriculture Experiment Station (AES) researchers are uniquely and best suited to conduct this research. Among the participants in this project are numerous experienced researchers with land, staff, equipment, and facilities capable of conducting comprehensive and objective field research. The support of the AES received by each cooperator does not represent simply a plot of land on which to plant their vineyard. The support systems and expertise of University and AES researchers include statistical support, computing hardware and software, basic and field laboratories with modern equipment, field research stations with suitable land, equipment, technical staff, and faculty colleagues who may provide ad hoc support and review of projects. Research and intellectual properties protections in-place within the University and AES systems ensures that all parties including breeders, nurseries, growers, wineries, and researchers themselves will be adequately protected, and ensure that research is conducted in a thorough and objective manner.

3. Technical Feasibility

The NE1020/1720 project has developed a network of sustained collaboration of viticulture and enology specialists across multiple states since 2005. Presently, participants from 15 states have active plantings, and partners from several other states who do not have formal plantings contribute expertise to the project. The existing team has the expertise to plan new plantings, apply appropriate viticultural practices, and collect data to evaluate new cultivars and clones. Objectives from the initial phase of this project were intentionally limited so as to develop a trial with maximum applicability across multiple regions with robust statistical design. However, the limitations built into the original methodology, including establishment of a single NE1020 planting design implemented in 2008 on specific rootstocks, training systems, and management programs, was deemed too restrictive by several participants, who dropped out of the project as a result. In NE1720 researchers adopted a more flexible model, allowing for more rapid evaluation and testing and continued planting of new and reducing limitations on individual collaborators to conduct cultivar evaluations that do not fit into a single national model. Successful collaboration over past years provides the foundation for this new model and continued success. While the robust, multi-site evaluation of cultivars within specifically defined climatic regions has not been conducted to date due to unforeseen differences in data sets, loss of collaborators, and vine loss in certain regions due to weather or management-related events, several plantings have resulted in published cultivar comparisons that are establishing performance benchmarks in the literature.

4. Advantages of a Multi-state Effort

Multi-state efforts capitalize on university faculty expertise for cultivar and breeding line evaluation where infrastructure exists for grape management. Evaluating cultivars in multiple growing environments in a coordinated and collaborative fashion makes data collection, analysis, and reporting more efficient and useful. Coordinated effort shortens the time to evaluate cold-hardiness and environmental adaptations by having many locations experiencing diverse weather events. The shared professional comradery among NE1020/1720 participants has allowed programs in each state to optimize their effectiveness by identifying gaps in knowledge, infrastructure, and experience, and has facilitated collaborations that address those shortcomings within a particular institution or program. The current project has allowed sharing of winemaking expertise for processing grapes from several plantings (e.g., multiple states have contributed grapes to Cornell and MN winemaking projects conducted through related, leveraged SCRI projects; several states have “outsourced” differential thermal analysis for cold hardiness at other participating universities). This leverages the winemaking and other expertise in states that do not have University winemaking or other specialized facilities.

Project participants cover diverse disciplines and areas of expertise. Faculty associated with the project hold appointments across the land-grant spectrum including teaching, research, and extension, and most faculty hold split appointments among those foci. Expertise includes viticultural management, which is the most common thread among participants, but also: plant pathology and entomology; Integrated Pest Management systems; Clean Plant certification; enology; plant breeding; genetics; enology, plant physiology; biostatistics; and interdisciplinary plant science. A unique attribute to NE1720 was the addition of community college representation from KS.

Collaboration among participants in the NE1020/1720 project has provided an opportunity and tools for securing funding for four Multi-state SCRI projects, Hatch funds available from participating AESs, funds from private foundations, state block grant programs, etc. Other collaborations with team members include Specialty Crop Block Grant (Clark and Hatterman-Valenti), the Vitisgen projects (SCRI), among other pending or non-funded projects and collaborations.

5. Likely Impacts

Notable Impacts of the Current project:

A key outcome of the current project (NE1720) has been local observation of plant response to extreme environments. The genotype x environment interactions that can be tested by a plant breeder are generally limited and the current project allows for multiple environments for testing. A “test winter” or other extreme weather event is increasingly uncommon (at any one site) but is much more likely to occur across the range of project participant plantings. Extreme weather events within the realm of “cold hardiness” include rapid and devastating temperature swings; extreme cold weather temperatures especially with polar vortex events in midwinter, and late spring frosts. Single weather events with major impacts at one site can inform varietal selection at multiple sites. The NE region offers a range of USDA Hardiness zones (3-7) for testing grapevines and represents typical growing conditions for cool and cold climate grape growing in the eastern US.

Cold hardy cultivars now account for nearly 20 % of Colorado’s vineyard area compared to about 1 % ten years ago, and approximately one third of that area was planted with cultivars tested in NE1020/1720. Limited comprehensive production statistics are available for the current planting and economic impact of vineyards and wineries since 2017. However, anecdotal evidence supports continued growth of the industries with the addition of new wineries and expanded acreage throughout the participating states. A maturation of the market has become obvious with indicators such as transitions of business to new ownership, slower adaptation of new cultivars, and simultaneously demands for replacement cultivars for a 3rd generation of varieties more suitable to consumer acceptance and production sustainability.

Expected Future Impacts:

Under our new model, we expect to be able to screen and test more candidate cultivars over a shorter amount of time by conducting efficient evaluations, and by continual establishment of plantings over the course of the project. Continued release of new, 3rd generation cultivars (e.g. Itasca, Verona, and Crimson Pearl released from cold-climate breeding programs in 2016 alone) and pre-release trialing of promising ones. Successful testing and education will result in more informed growers who make better planting decisions and suffer fewer losses from planting a poorly-adapted cultivar in the wrong site. Multi-state, interdisciplinary evaluation will allow for assessment of other attributes (e.g. insect, fungal disease, phytotoxicity of agrochemicals, unique juice characteristics) to maximize potential productivity and quality of this new germplasm. Under this project, we expect that wine industries in our regions will continue to grow, with an average increase in acreage in production and wine value during the project period.

Related, Current and Previous Work

The original experimental design for NE1020 evaluation vineyards was based on a coordinated planting to be completed in 2008 in all states, with an exception for one vineyard that required installation in 2007 to comply with funding availability. During annual meetings of project cooperators from 2005-2007, specific objectives, experimental design, and data collection procedures were developed. Because collaborators had differing land, staffing, and facility resources, plantings were designed to be flexible in terms of the number of cultivars or clones evaluated. However, each planting had the following common characteristics: common two ‘sentinel cultivars’ that would remain consistent in each planting in a similar climate zone; randomized complete block replication; six replicates of each cultivar; cordon and spur pruning; low-wire cordon (VSP) trained vines for *V.vinifera*, high-wire cordon training for hybrids; grafted to 101-14 rootstock for *V. vinifera* and tender hybrids unless local conditions prohibited, own-rooted for cold-hardy hybrids; required guard vines or rows; all vines planted within one year of one another (e.g. dead vines were allowed to be replanted in year two only); and consistent targets established for yield and vine growth. Vine orders were centrally coordinated and funded by grants from the (now defunct) CREES Viticulture Consortium or from individual investigator’s own research funds. In total, NE1020 trial vineyards were established in 19 states in 2007 or 2008, however several cooperators left the project prior to completion and two established their plantings after 2008. Twenty plantings in 13 states were active in the project. Additional states joined the group but were not able to install ‘official’ NE1020 vineyards because protocols for inter-state comparisons required all vineyards to be planted at the same time, and those states have maintained membership in a collaborative capacity.

Restrictive protocols for the original NE1020 plantings, while designed to allow for robust comparisons between sites and cultivars, actually discouraged interstate comparisons because allowances were not made for vineyards that failed completely due to weather or other crop damage. In several states, severe winter cold decimated plantings early in the project; in others, herbicide drift or phytotoxic pest management sprays damaged plantings beyond their ability to provide consistent data. Retirements and other personnel changes among participants, and withdrawal from the program by some AES directors further reduced participation in the formal trials.

In the NE1720 project, we made a strategic change to allow plant materials (breeding lines, new cultivars, etc.) to be submitted at any time as they became available. This new direction provided more flexibility in the experimental design, and ultimate was aimed at getting timely information returned to aid in variety advancement. Our experience in NE1020 demonstrated a key need to describe grapevine survival at test sites as a base line. This is now embedded as part of Obj 1 in the current proposal. Vines that were not cold-hardy or otherwise suitable (at an individual site) became a burden on the labor needs and negatively impacted the experimental design. Advancement into Obj 2 of the current study builds on earlier iterations of this project by advancing selections that have passed the initial survival screening.

Collaborators within the NE1020 project have consistently attended annual meetings where results from project plantings have been discussed. Separate from, but complementary to the initial NE1020, trials have been substantial multi-state collaborations between NE1020 members and other participants that would not have been possible without collaboration and networking derived through this project. Collaborative projects derived from NE1020 participation and often including NE1020 vineyards as primary data sources and educational sites include:

- Improved grape and wine quality in a challenging environment: An Eastern US model for sustainability and economic vitality. USDA SCRI 2010-51181-21599. PD A. Wolf, Virginia Polytechnic Inst. $3,796,693. http://cris.nifa.usda.gov/cgi-bin/starfinder/0?path=fastlink1.txt&id=anon&pass=&search=R=39155&format=WEBLINK(see resulting publications & outputs, Appendix 1).
- Northern Grapes: Integrating viticulture, winemaking, and marketing of new cold-hardy cultivars supporting new and growing rural wineries. USDA SCRI 2011-51181-30850. PD T. Martinson, Cornell Univ. $5,139,193. http://cris.nifa.usda.gov/cgi-bin/starfinder/0?path=fastlink1.txt&id=anon&pass=&search=R=47150&format=WEBLINK(see resulting publications & outputs, Appendix 1).
- Midwest grape production guide. Dami, I., Bordelon, B., Ferree, D., Brown, M., Ellis, M., Williams, R., Doohan, D. The Midwest Grape Production Guide was compiled by Extension specialists at Ohio State University and Purdue University. Its comprehensive topics include: planning your vineyard; grapevine anatomy and propagation; integrated pest management; pruning, training, and canopy management; vineyard maintenance; and harvest and marketing. http://articles.extension.org/pages/63522/midwest-grape-production-guide
- Accelerating grape cultivar improvement via phenotyping centers and next generation markers. USDA NIFA SCRI 2011-51181-30635. PD B.I. Reisch $4.5 million, 2011-2016. <http://cris.nifa.usda.gov/cgi-bin/starfinder/16023/crisassist.txt>
- *Vitis*Gen2: Application of next generation technologies to accelerate grapevine cultivar development. USDA NIFA SCRI (award pending). PD B.I. Reisch $6.5 million 2017-2021.

The outputs associated with the above projects, in addition to citations listed with the NE1720 annual reports (and subset in Appendix 1) highlight significant effort and progress toward addressing the goals. Continued effort under the NE2220 project will build upon the successful collaborations fostered under the current project and will address shortcomings discovered in the original protocols. The focus of the project will continue to be on the evaluation of new or emerging grape germplasm with the intention of identifying superior cultivars that meet the needs of regional sites and production systems. The following objectives will support these efforts to improve sustainability of the U.S. winegrape industry:

Objectives

1. I. Conduct initial screening evaluations of promising emerging cultivars and advanced breeding lines (pre-commercial) to determine suitability for regional adaptation of viticulture and wine quality attributes.
2. II. Comprehensive grapevine cultivars and clone evaluation for viticultural, pest susceptibility, fruit and juice quality characteristics, including enological characteristics, and local adaptation for sustainable production.
3. III. Explore new germplasm resources including disease resistant cultivars being released in Europe, plant introductions including Asian accessions, and less-known cultivars that may have economic potential for the US grape industry.

Methods

This multi-state project capitalizes on the varying growing conditions throughout the Eastern United States and the expertise at multiple participating universities and colleges. Grape germplasm evaluation across multiple sites is meant to capture the stability of genotypes across the region and characterize potential genotype x environment interactions. The key aim of this project is to test plant materials so that recommendations can be made to breeders on which lines to advance for cultivar release and to inform stakeholders at the state and regional levels as to which cultivars are suitable for planting. The replicated experiments outlined are designed to gather production, yield, pest tolerance, and fruit and wine quality (where available) to inform recommendations for growers and wineries. Our experience in NE1020 and NE1720 has shown a need for flexibility in entering materials for planting across the project period, identification of appropriate test sites, and quality data collection and reporting. Further, this project aims to catalog and characterize germplasm improvement (including clones) using a global lens to facilitate exchange of plant materials for testing in this project or by individual researchers or stakeholders. Trial vineyards may include Objective 1 and 2 plantings so long as the experimental design is maintained as outlined below. All sites will collect weather data. Phenotypic data will be collected using Field Book, a digital platform that will allow for uniform data acquisition and centralized database management with support from USDA initiative Breeding Insight at Cornell University. Commitments have been made by PIs from Cornell Agritech to host Objectives 1 and Objectives 2 at Geneva, NY and/or the Cornell Lake Erie Research Extension Laboratory in Portland, NY. Additional sites will be based on enrollment by other states in the project, specific environments being targeted by genotype, and plant availability. Consistent participation, planning, and leadership in this project from Vermont, New Jersey, Massachusetts, and Maryland demonstrate additional commitment to establish and maintain plantings in the NE region.

Objective 1 Conduct initial screening evaluations of promising emerging cultivars and advanced breeding lines (pre-commercial) to determine suitability for regional adaptation of viticulture and wine quality attributes.

Breeders will submit advanced breeding lines (near commercialization) for entry into limited multi-state screening at a minimum of 4 states including one in the NE region. Each site will evaluate a minimum of 4 vines for each entry. Vines can be part of a randomized design or together in one planting (one panel). This design reduces the burden on the breeder in producing propagules for all locations and can focus on preliminary data collection for submission into Objective 2. This preliminary screening will include up to 3 years of data collection during the establishment period typical for grapes. The evaluation may continue after year three to include fruit evaluation as outlined in Objective 2. Ideally, after the initial screening sufficient data will inform advancement into a new planting with additional replication within sites and more sites added. This project is described for 5 years, but with renewal, the plantings and evaluations can continue as in previous NE1020/1720 project iterations.

Vines will be planted and established using standard training systems and management protocols for the region. New entries may be submitted each year as materials become available from breeders or other sources under testing agreements. Initial evaluations will include plant survival (living/dead) as this has been a critical barrier in NE1020/1720 because of extreme weather events (such as the polar vortex). Data will be collected on cold hardiness (primary and secondary bud survival); disease resistance (powdery mildew, downy mildew, black rot, phomopsis), insect resistance (foliar phylloxera, Japanese beetle), and budbreak phenology (EL5). Data will be collected using Field Book. Results will be reported annually to NE2220 members to guide future replicated trials and data will be reported to the associated breeder.

Objective 2 Comprehensive grapevine cultivar (and elite breeding selections) and clone evaluation for viticultural, pest susceptibility, fruit and juice quality characteristics, including enological characteristics, and local adaptation for sustainable production.

Replicated cultivar evaluation vineyards using promising materials identified in Objective 1 or from researchers' current trial plots will be established at a minimum of 4 locations with plantings based on plant material availability and proposed market suitability. Each genotype will be planted into a replicated experiment with 4 vine panels and 6 panels (replications). These 24 vines per site will provide sufficient data points for capturing within vineyard variation, estimating yield components, and providing enough juice for enological evaluation (where expertise is available). Vines will be planted and trained onto trellis systems using standard protocols for the region. It is expected that entries may be submitted in different years at different locations to test materials in additional environmental locations after the first four locations are established. Ideally, vines will be evaluated for 5-10 years. Genotypes will be evaluated yearly as in Objective 1, with additional traits collected during fruiting including yield (g per 30.48 cm linear spacing), 10 cluster weight, 50 berry weight), fruit quality (soluble solids content, total titratable acidity*, pH, yeast assimilable nitrogen*), wine quality* (percent alcohol, total titratable acidity, pH, residual sugars, organic acids, volatile acidity, phenolic compounds, color), phenology (harvest and budbreak), disease and insect resistance, and hardiness.

Results will be reported annually to NE2220 members and published in peer-reviewed journals at completion. Data will be provided to breeders or entities entering germplasm into testing with recommendations for variety release.

*When enology and chemical analysis resources available at the site or in collaboration. Standard wine processes will be followed (red and white wines with separate protocols) across sites.

Objective 3 Explore new germplasm resources including disease resistant cultivars being released in Europe, plant introductions including Asian accessions, and less-known cultivars that may have economic potential for the US grape industry.

A NE2220 subcommittee will be formed to solicit information from grapevine breeding and evaluation programs around the globe in order to identify germplasm resources that could be valued by stakeholders in the NE region and participating states. Public and private grape breeding programs in the U.S. will be invited to submit new introductions for testing (Objectives 1 and 2) and their contact information will be compiled. Additionally, germplasm repositories and other current variety testing projects will be cataloged. Disease resistance breeding programs across Europe have recently released new varieties. A list of foreign cultivars and advanced selections will be curated and made available to stakeholders. Importation of protected plant materials into the U.S. is difficult, requires sponsorship (financial and otherwise), and is on a timeline that is outside the scope of this project. However, this objective aims to work with Clean Plant Centers (Foundation Plant Services, M. Fuchs Lab, Cornell University among others) to develop a protocol for best practices that can be implemented by interested stakeholders who wish to import materials.

Measurement of Progress and Results

Outputs

- New varieties released
- Comprehensive data provided to breeders and germplasm evaluators
- Importation of novel cultivars or germplasm
- Cataloged inventory of new variety development and known importation or distribution plans into the United States.

Outcomes or Projected Impacts

- NE2220 project recommendations and educational programs will guide the planting or replanting of 1000+ acres of winegrapes in the next 5 years in participants' states,
- Grape growers in emerging regions will see increased net income per acre, and more consistent income and yield as a result of adopting regionally-adapted cultivars.
- NE2220 will help to reduce the time from initial cross to released cultivars.
- NE2220 collaborations will result in federal research investment through block grant, multi-state, CAP, or other grant programs.

Milestones

(**2021**):Project team designed objectives and associated methods to allow new breeding lines or other germplasm to be propagated and planted in Spring/Summer 2022 for Obj 1 and Obj 2. Continued evaluation of germplasm from earlier NE1020/1720 plantings.

(**2022**):Continue to establish plantings on a rolling basis in accordance with a specific guidelines for Obj 1 and Obj 2.

(**2025**):Propagate and distribute selections that will move from Obj 1 to Obj 2 at each site to increase the replication.

(**2024**):First fruit evaluations for new entries as part of Obj 2.

Outreach Plan

NE2220 data will be compiled and provided to the associated breeders and germpalsm evaluators. Some genetic materials and breeding lines may be entered under Material Transfer Agreements that include restrictions on the disclosure of some data related to intellectual property prior to germplasm release. Such data will be handled on a case-by-case basis as to how it is shared with stakeholders. It is expected that the majority of the data collected, especially for Objective 2 on novel varieties, new accessions, and advanced breeding lines will be shared with stakeholders. The website has been established by Dr. Matthew Fidelibus at UC-Davis and could be utilized directly or as a model for sharing NE2220 results. State reports are often shared with individual state stakeholders at annual conferences or annual reports. The aim of this project is to provide information for the recommendation of the advancedment of breeding materials and planting of novel cultivars to stakeholders. Field days hosted by each project participant are another key venue to demonstrate germplasm to stakeholders. The information collected at each site is important for variety adoption within a state or region due to the quality of observations made by the investigators on this project.

Organization/Governance

The NE22020 project will be governed by a rotating executive committee voted annually at the project meeting by meeting attendees. Although all offices will be elected in each year, it is expected that an officeholder will begin in the Secretary position and rotate through to the chair position. Offices thus include: Secretary, Vice-Chair, and Chair (host). Thus, the Secretary is expected to host the meeting two years following, and the vice-chair the following year. After an annual meeting, the secretary shall submit meeting minutes to the meeting chair within 30 days, and the chair will submit the annual report within 60 days of the annual meeting. Reports will be submitted to the NERA Administrative Advisor who will submit reports to NIMMS. Following the annual meeting, the vice-chair (now chair of the following year's meeting) will begin preparation for the following meeting and will assist the secretary and chair in compiling the annual report if needed.

Literature Cited

1.Bradshaw, T.L., A. Hazelrigg, and L.P. Berkett, *Characteristics of the cold-climate winegrape industry in Vermont, U.S.A.* Acta Hort, 2016. ***Accepted Nov 2016***.

2. Berkett, L.P., et al. *2008 Grape Bud Survival on Eight Winegrape Cultivars in Vermont* in *Proceedings of the 2nd Annual National Viticulture Research Conference• July*. 2008.
3. Berkett, L.P., et al. *Disease evaluation of selected cold climate wine grape cultivars in Vermont, USA* in *IOBC-WPRS Working Group Meeting on "Integrated Protection and Production in Viticulture."*. 2013. Ascona, Switzerland: IOBC/WPRS Bulletin 24(5):393-400.
4. Berkett, L., et al., *Disease evaluation of selected cold climate wine grape cultivars in Vermont, USA*.IOBC-WPRS Bulletin, 2014. **105**: p. 29-33.
5. Bradshaw, T.L., L.P. Berkett, and S.L. Kingsley-Richards, *Horticultural Assessment of Eight Cold-Hardy Table Grape Cultivars in Vermont, 2009-2012 (Abstr).* HortScience, 2013. **48**(9): p. 2-3.
6. Del Bel, E., et al. *Sensory Characterization of Frontenac and Marquette Berries and Wines by Descriptive Analysis* 2013; Available from: http://northerngrapesproject.org/wp-content/uploads/2014/02/SensoryCharFrontMarqBerriesWines.pdf.
7. Domoto, P., et al., *Wine grape cultivar trial performance in 2007.* Ann. Prog. Rept.–2007 for Hort. Res. Sta., ISRF07-36, 2008: p. 39-45.
8. Eddy, M., *Performance of Cold Hardy Wine Grape Cultivars at Four Commercial Vineyards in the Champlain Valley of Vermont: Yield, Fruit Quality, and Bud Survival*, in *Plant and Soil Science*. 2006, University of Vermont.
9. Hemstad, P. and J. Luby, *La Crescent, a New Cold Hardy, High Quality, White Wine Variety*.Acta Hort, 2003. **603**: p. 719-722.
10. Luby, J., *Breeding cold-hardy fruit crops in Minnesota.* HortScience, 1991. **26**(5): p. 507-512.
11. Nonnecke, G., P. Domoto, and D. Cochran, *NE-1020 Cold Hardy Wine Grape Cultivar Trial.* Iowa State Research Farm Progress Reports, 2015. **2173. http://lib.dr.iastate.edu/farms_reports/2173**.
12. Pedneault, K., et al., *Flavor of Hybrid Grapes for Winemaking: A Survey of the Main Varieties Grown in Quebec fr Red Wine Production (abstr.).* American Journal of Enology and Viticulture, 2013. **64**(3): p. 421A-422A.
13. Tarko, T., et al., *Chemical composition of cool-climate grapes and enological parameters of cool-climate wines.* Fruits, 2014. **69**(1): p. 75-86.
14. University of Minnesota. *Frontenac Enology*. 2012 [cited 2013 1 May]; Available from: http://www.grapes.umn.edu/Frontenac/FrontenacEnology/index.htm.
15. American Vineyard Foundation. *2015 Viticultural Survey Results*. 2015; Available from: http://www.avf.org/assets/files/surveyresults/2015SurveyResultsViticulture.pdf.
16. Hemstad, P. and J. Luby, *Utilization of Vitis riparia for the development of new wine varieties with resistance to disease and extreme cold.* Acta Hort, 2000. **528**(VII International Symposium on Grapevine Genetics and Breeding 528): p. 487-496.
17. Reisch, B.I., C.L. Owens, and P.S. Cousins, *Grape*, in *Fruit breeding*. 2012, Springer. p. 225-262.
18. Clark, J., *Grape breeding at the University of Arkansas: Approaching forty years of progress*.Acta Hort, 2002. **603**: p. 357-360.
19. Hemstad, P. *Grapevine breeding in the Midwest.* Grapevine breeding programs for the wine industry: Traditional and molecular techniques. Ed. A Reynolds, 2015: p. 411.
20. Swenson, E.P., *Wild Vitis Riparia from northern US and Canada--breeding source for winter hardiness in cultivated grapes--a background of the Swenson hybrids.* Fruit Varieties Journal, 1985. **39**: p. 28-31.

Land Grant Participating States/Institutions

NJ,ND

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Hatterman-Valenti, Harlene	Yes	North Dakota - North Dakota State University	1,2,3	205	1131	1060	0.10	0.00	0.01	0	0
Ward, Daniel L	Yes	New Jersey - Rutgers University	1,2,3	205 204	1131 1139	1020 1020	0.30	0.00	0.20	0	0

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
204-1139-1020	0.15	0	0.2
205-1131-1020	0.15	0	0.2
205-1131-1060	0.1	0	0.01
Grand Total:	**0.40**	**0.00**	**0.21**

Program/KA	Total FTE
0	0
0	0
Grand FTE Total:	**0**

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2220: Multi-state Coordinated Evaluation of Grape Cultivars and Clones

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
The proposal “Multi-state Coordinated Evaluation of Grape Cultivars and Clones” addresses a critical need for research in non-traditional wine grape and wine producing areas. Coordinated cultivar testing is a much more efficient and cost-effective approach than independent activities, and this proposal does a nice job at providing examples of why and how that is the case. The objective and goals are clearly defined and reasonable for the amount of time proposed. The potential impact of developing or identifying well-adapted cultivars may be very long lived and could have significant economic impacts for many years. I believe the history of this coordinated research and successes support continued funding.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2220: Multi-state Coordinated Evaluation of Grape Cultivars and Clones

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
A cooperative effort such as this to evaluate grape cultivars is much preferred over a piecemeal model of everyone doing their own thing. While there will have to be differences between locations I think this model will help to standardize efforts while still being flexible enough to meet individual goals. I particularly like the idea of trying vines on a smaller scale at first in case they immediately wash out.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2220: Multi-state Coordinated Evaluation of Grape Cultivars and Clones

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Good
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
I appreciate the flexible model of this proposal to help alleviate limitations in participation in this type of model. Large projects, especially multistate projects, can be challenging and unwieldy to manage. Having a flexible model allows the project to move forward and adjust to varying conditions in the various locations. If approved, good luck with your efforts.
Your Recommendation:
Approve/continue project

UNIVERSITY OF MINNESOTA

Crookston • Duluth • Morris • Rochester • ***Twin Cities***

~~Department of Horticultural Science~~
College of Food, Agricultural and Natural Resource Sciences

~~305 Alderman Hall~~
1970 Folwell Avenue
St. Paul, MN 55108

612-624-4742
Fax: 612-624-4941
http://horticulture.umn.edu

4/22/2022

Dr. Margaret Smith
343 Roberts Hall
215 Garden Avenue
Ithaca, NY 14853

RE: NE2220 Proposal: Multi-state Coordinated Evaluation of Grape Cultivars and Clones

Dr. Smith,

We would like to thank you for your guidance on the development of the multi-state proposal and especially thank the reviewers of the project for their careful critique. The reviews were favorable, and did not warrant any changes to the document previously submitted. We will attach the unedited version for final review in NIMSS and look forward to the MAC review and NERA approval.

Sincerely,

Matthew Clark

NE_TEMP2227: Contribution of Ovarian Function, Uterine Receptivity, and Embryo Quality to Pregnancy Success in Ruminants

Status: Submitted As Final

Duration	10/01/2022 to 09/30/2027
Admin Advisors:	[David Townson]
NIFA Reps:	

Statement of Issues and Justification

The need as indicated by stakeholders. Reproductive performance in beef and dairy cattle is often suboptimal resulting in increased intervals to conception and/or rebreeding failures which collectively reduces farm revenue due to the effect on milk and calf production efficiencies. Causes of pregnancy failure in cows include lack of resumption of cyclicity (anovulation/ anestrus), fertilization failure, poor oocyte quality, suboptimal hormonal environment due to abnormalities in follicle or corpus luteum, abnormalities in embryonic development and uterine-conceptus interactions, and pregnancy loss. Any of these reproductive problems can lead to reproductive inefficiency, increased culling, and substantial economic losses. Reduced fertility in ruminants also affects the consumer because reduced farm efficiencies (i.e., fewer calves per year and reduced milk production efficiency) reduce product supply and/or increase cost.

Importance of work and consequences if it is not done. Improving fertility in ruminants requires fundamental knowledge about endogenous and exogenous influences on 1) follicle activation and development and oocyte growth and maturation; 2) corpus luteum development, steroidogenesis, and regression; 3) fertilization and pre-attachment embryo development; 4) conceptus-uterine-ovarian interactions; and 5) placental development and function. Understanding ovarian, uterine, and embryo attributes is critical to identify the underlying causes of anovulation, fertilization failure, luteal insufficiency, and pregnancy loss in ruminants, each of which are the critical causes of reduced fertility. This will lead to development of novel and innovative management strategies designed to optimize fertility, and therefore, may lead to management strategies and therapeutics that improve reproductive efficiency and are economical, user and consumer friendly, while still ensuring food quality and safety.

Advantages of performing this work as a multistate effort.We are the first multi-state group established and from its initial inception (NE-1) has been one of the most productive, cohesive, diverse, and collaborative multistate research groups nationwide. Complementary expertise of the group has led to collaborative experimental efforts and incorporation of new technologies that were integrated and directed toward exponential progress of previous objectives. The group uses a variety of animal models that are best suited to address specific molecular and cellular questions associated with tissue function. Additionally, members at different stations have developed best practices for animal and cell culture protocols, conducted collaborative experiments, and exchanged samples to take advantage of unique validated procedures. The commitment of participants to the multistate project is exemplified by numerous collaborative publications, including one in which it was decided to list the project itself as the author, rather than individuals [1]. In addition to research collaborations, the group developed a multi-institution graduate course in reproductive biology. Furthermore, senior members of the group serve as mentors for junior investigators as they develop internationally recognized research programs and become leaders in the field of reproductive biology.

The technical feasibility of the work. The technical members of NE-1727 (molecular biologists, cell physiologists, reproductive physiologists, animal scientists, and veterinarians) are a diverse group of scientists with broad and complementary expertise in follicle growth, corpus luteum function, oocyte development, conceptus-uterus-ovary interactions, reproductive immunology, and reproductive management of ruminants. Recent additions of members with expertise in ovarian reserve and activation of follicle growth, follicle deviation, genomics of oocyte development and maturation, and pre-implantation embryo development will fill important gaps in knowledge to understand pregnancy loss more effectively in cattle. This shared expertise and wide array of technical capabilities has previously fostered more rigorous and impactful research. Moreover, the combination of basic biological research and innovative applied research more effectively support outreach programs and engagement, the goal of which is to improve reproductive performance in livestock more rapidly. This experimental paradigm will be continued in the newly proposed objectives.

Impacts from successfully completing the work. Specific impactful discoveries from the group include, but are not limited to:

- Roles of pro-inflammatory cytokines and environmental stressors on follicle growth, steroidogenesis, luteal regression, and conceptus-uterine-immune interactions.
- Use of trace mineral supplementation to improve oocyte quality and increase systemic progesterone concentrations.
- Impact of beef cow body condition score and weight on the metabolome of follicular fluid and serum
- Genetic determinants of ovulation and implantation rates
- Endocrine and molecular mechanisms controlling ovulation rate in cows
- Mechanisms associated with preimplantation embryo development and quality.
- Reproductive management strategies that improve reproductive performance in dairy and beef herd management practices.
- Factors that cause pregnancy loss in cattle and development of methods to reduce losses.

Data were generated using intramural and extramural competitive grant funds garnered by the members (more than $23 million). From 2017-2021, the group individually and collaboratively published refereed research papers (225), conference papers (70) theses and dissertations (50), book chapters and invited reviews (19), technical/extension publications (9), and deposited sequences into the Gene Expression Omnibus (14). Workshops and lectures (128) were also presented to producers, veterinarians, and consultants locally and internationally. In addition, to dissemination of the data, the findings were used to develop programs to improve synchronization of ovulation and to overcome environmental and nutritional factors that can disrupt reproduction. The group developed new management strategies and technologies to effectively use artificial insemination (AI) and embryo transfer (ET) technologies to improve reproductive performance and thereby immediately impact producers. Indeed, veterinarians, consultants, pharmaceutical companies, breed organizations, and companies serving animal industries benefitted from the work from this project. For example, collaborators have presented annual reports on the project to cattle AI organizations (Select Sires, Genex CRI, Alta Genetics), the National Association of Animal Breeders (NAAB), the American Association of Bovine Practitioners (AABP), dairy industry associations (Minnesota Dairy Association, Vermont Dairy Association), pharmaceutical and technology companies (Zoetis, Merck, Allflex), and extension education organization (PRO-Dairy). In turn, those groups spread the technology to farm families/producers for implementation which benefits the on-farm profitability and sustains agricultural production systems that are highly competitive in the global economy.

Student training is another important impact of this project. The members of NE1727 (NY, IA, MS, OH, OR, PA, KY, MA, NE, TN, VT, WI, VA, and WV) developed a course on Contemporary Topics in Reproductive Biology to improve student understanding of the breadth of reproductive physiology including topics outside their primary area of research. Ninety-seven graduate students enrolled in this course during the last project period. This accounted for a majority of the 103 students who completed or are currently pursuing a M.S. or PhD across the experiment stations. Numerous undergraduate students with an interest in reproductive physiology were also introduced to investigative research. These activities represent an important contribution of the project to the education of the next generation of scientists, consultants, and other workers in animal agriculture industries.

Related, Current and Previous Work

Folliculogenesis: Ovarian follicles progress through dynamic and functionally distinct stages; from growth, selection and dominance to ovulation or atresia. The follicle consists of an oocyte and surrounding granulosa and theca cells that support development and maturation of the oocyte, and their functions are under endocrine and paracrine regulation. Members of the transforming growth factor β (TGFβ) superfamily of ligands activate transcription factors (SMADs) which regulate follicle development and ovulation rate. A novel high fecundity genotype (Trio) was identified in cattle and evaluated extensively as part of this project[2–4]. We found that the presence of the Trio allele results in increased abundance of SMAD6 mRNA, which is an intracellular negative modulator of BMP15, in the granulosa cells of antral follicles resulting in decreased granulosa proliferation, reduced follicle growth rate, and selection of three to four dominant/ovulatory follicles instead of just one. How the Trio allele alters follicle growth during the preantral stages of folliculogenesis has not been documented.

Within human follicles, oxygen concentration is reportedly 1.3% to 5.5%[5]. Thus, follicle growth and granulosa proliferation occur under hypoxic conditions. In cancer cells, high levels of O-GlcNAcylation (i.e., hyper-O-GlcNAcylation) promote a metabolic shift from aerobic, oxidative phosphorylation to anaerobic glycolysis (i.e., Warburg effect), which enables cells to continue undergoing rapid proliferation. Despite the knowledge that O-GlcNAcylation occurs in granulosa cells of follicles of different sizes, and it influences granulosa cell proliferation, the existence of and mechanisms responsible for such a switch in granulosa cell metabolism have not been extensively examined.

Steroidogenesis: Theca and granulosa cells produce steroids that coordinate with neuroendocrine hormones to regulate follicle selection and ovulation. Steroid hormones are derived from cholesterol and their synthesis is primarily regulated by pituitary hormones. However, growth factors, cytokines, and androgens/estrogens also modify steroidogenesis via paracrine and autocrine mechanisms. In addition to expression and activity of the steroidogenic enzymes, cholesterol uptake and *de novo* synthesis by theca cells is also an important determinant of steroid production. This is emphasized by recent studies demonstrating a regulatory loop between LH signaling and sterol regulatory-element binding protein (SREBP-2) which increases cholesterol synthesis, is necessary for optimal steroid production [7,8]. Despite these studies, there are gaps in understanding the paracrine regulation of steroidogenesis.

Oocyte Maturation and Fertilization: Oocyte quality, defined as its ability to be fertilized and support embryonic development, is a pivotal factor in female fertility. Divalent cation homeostasis is required for oocyte, influencing maturation, fertilization, and early embryonic development. For example, zinc (Zn^{2+}) is essential for resumption of meiosis and meiosis exit after fertilization. The Ca^{2+} oscillations of fertilization induce egg activation and initiate development. Finally, Mg^{2+} impacts Ca^{2+} oscillations, and Mg^{2+} is essential for embryo development. However, the channel(s) underlying influx of these cations remains unknown in all large domestic species. The TRPM7 locus, which mediates intracellular cation influx, is positively associated with conception rates in heifers [9]. We hypothesize that TRPM7 not only contributes to bovine fertility, but potentially allows extracellular ion supplementation to affect intracellular ion levels that will positively impact the cellular processes under their regulation and, ultimately, improve fertility.

Corpus Luteum Function and Angiogenesis: After ovulation, the somatic cells of the follicle differentiate into luteal cells, forming a corpus luteum (CL). This structure, which produces progesterone, is essential for establishment and maintenance of pregnancy. In the absence of pregnancy, it undergoes regression and the concomitant loss of progesterone leads to initiation of a new estrous cycle. In addition to differentiation of luteal cells, formation of the CL is accompanied by extensive blood vessel growth or angiogenesis. The building of a rich vascular network requires the coordinated actions of a variety of angiogenesis-related factors. Toward this end, a functional angiotensin system is present in the bovine ovary [10] and the physiologically active angiotensin II (AngII) regulates the expression of VEGF [11] in the bovine CL. In turn, VEGF and basic fibroblast growth factor upregulate Ang II and steroidogenesis in the bovine CL [10]. In HEK293T cells, Ang II also increases the expression of cellular communication network factor 1 (CCN1) [12]. However, Ang II mechanisms regulating CCN1 expression in steroidogenic bovine luteal cells have not been established.

Environmental Factors Impacting Folliculogenesis and Oocyte Quality: Inherent to the efficiency of reproduction in domestic production systems is the need to overcome an array of management-based, nutritional and/or environmental stressors. Heat-stressed cows have lower circulating glucose concentrations coupled with higher concentrations of insulin [13] leading to poor oocyte quality. Chromium propionate improves insulin/glucose dynamics in heat-stressed cows, but it is unclear if and how it will improve oocyte quality.

The environmental contaminant, perfluoro alkylated substances (PFAS) have *in vivo* half-lives of several years. In humans, PFAS exposures are associated with abnormal ovarian cyclicity, decreased steroidogenesis, increased follicular atresia, and decreased corpora lutea [14]. However, it is unclear how PFAS affects folliculogenesis in ruminants. Additionally, since heat stress alters circulating insulin and insulin regulates chemical metabolizing enzymes in the ovary, a potential for a synergistic effect on PFAS and heat stress exists to hamper fertility.

Sperm capacitation: Efficient fertilization is dependent not only on oocyte quality but also sperm capacitation within the female reproductive tract. Preliminary data indicates that a short incubation with the Ca^{2+} ionophore A_{23187} can induce *in vitro* fertilizing capacity in sperm from mice sterile knock-out (KO) genetic models and improve embryo development rates in sperm from wild type mice [16]. Consistently, when used with bovine sperm, a temporary increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) induces significant improvement in *in vitro* fertilization (IVF) rates and embryo development.

Embryo Development: Genes that are expressed in the oocyte play a key role in mediating initial stages of embryonic development. Most of our knowledge related to maternal genes required for early embryonic development is derived from gene targeting studies in mice. Our understanding of the role of specific oocyte-expressed genes in regulation of early embryogenesis is far from complete, particularly in species such as cattle where the number of cell cycles from fertilization until completion of the maternal-to-embryonic transition (embryonic genome activation) is greater than in mice [17]. Through analysis of the bovine oocyte transcriptome, we have identified several novel oocyte-specific genes (both protein coding and non-coding).

Maternal Recognition of Pregnancy: During the second week of pregnancy, embryonic and extraembryonic tissues, which are essential for producing a conceptus that can signal its presence to the maternal system, are formed. If the pregnancy recognition signal is not sufficiently robust or if the uterus cannot respond correctly to this signal, pregnancy loss will occur. Differentiating between conceptus and uterine receptivity components of fertility remains a major challenge. In the event of failure of adequate signaling and response, the uterus is primed to regress the CL, and reestablish cyclicity for another opportunity to establish a pregnancy.

Uterine Environment and Conceptus-Uterine Interactions: A significant amount of pregnancy failure also occurs during the second and third weeks of gestation in cattle primarily due to insufficiencies within the uterine environment or poor paracrine communication between the conceptus and endometrium. These interactions also involve regulating immune cell function. The early embryo alters the proportions and function of resident uterine immune cells as well as affecting immune cell function in the peripheral circulation [18,19]. In other species these changes were shown to be essential for establishment of pregnancy and to support development of the placenta. However, relatively little is known about the changes in uterine immune cells and immune mediating molecules during early pregnancy in cattle.

Selection of High Fertility Cattle: Techniques in molecular genetics and genomics offer excellent possibilities for selection of high fertility cattle through identification of key genes/processes involved in optimizing fertility. Reduced genetic merit for fertility has been linked with unfavorable metabolic status, delayed resumption of ovulation, and inadequate hormone levels [20,21]. Moreover, reproductive traits have been linked with genotypes of tumor necrosis factor α [22], leptin promoter [23], IGF-I [24], growth hormone receptor [25–27], Coenzyme Q9 (COQ9) [28,29] and paraoxonase-1 (PON1) [30]. Genetic and genomic studies provide opportunities for selection of higher fertility cattle and to unravel physiological mechanisms related to genotypes and fertility.

Targeted Reproductive Management is a novel approach that consists of managing subgroups of cows that share biological features or expected performance with programs specifically designed to optimize cow performance, herd profitability, or other outcomes of interest. This approach to management leverages recent advances in high throughput genotyping and phenotyping tools such as genomic testing and automated systems to characterize predictors of cow reproductive and performance outcomes. Multiple sources of biological, management, and performance data are then combined with environmental and economic data to identify subgroups of cows that benefit by tailored reproductive programs.

AI and ET: Improved understanding of the response of cattle to hormonal interventions used in synchronization of ovulation protocols for timed AI and timed ET was accomplished under project NE-1727 and others. This has led to major gains in reproductive and farm labor efficiencies through improved service rates and fertility to AI and ET services. Despite these gains, the response to specific hormonal treatments as part of synchronization of ovulation protocols remains suboptimal [31,32]. Biological conditions observed in cows undergoing synchronization and management conditions of commercial farms which constrain use of more complex interventions hamper responses to some programs. Therefore, research is needed to develop practical mechanisms to circumvent these biological and management limitations for achieving optimal responses and efficiencies.

Project NE-1727, and its predecessors, NE-1227, NE-1027, NE-1007, NE-161, NE-72, NE-41 and NE-1, has a long track record of defining core physiological, cellular, and molecular processes that control ovarian function, oocyte quality, fertilization, embryo development, and conceptus-uterine interactions with the goal of identifying what controls pregnancy maintenance or failure in ruminants. These discoveries have then been used to develop innovative management practices to improve fertility of female ruminants. The current proposal will build upon the outcomes of NE-1727 to further our basic understanding of the physiological mechanisms contributing to ovarian function, embryo development, and conceptus-uterine interactions. It will also continue to develop innovative management strategies based on findings from the basic and applied research. Proposed studies will address the following three objectives.

Objectives

1. Identify Mechanisms that Regulate Ovarian Function and Oocyte Quality during the Estrous Cycle
2. Determine Factors Associated with Fertilization, Embryo Development, and Conceptus-Endometrial Interactions that Dictate Pregnancy Success
3. Develop and Evaluate Novel Reproductive Management Strategies and Technologies to Improve Reproductive Performance of Ruminant

Methods

Obj 1A: Identify basic mechanisms that coordinate oocyte, follicle, and CL function

1A.1 Oocyte Quality and Fertilization: The TRPM7 locus was positively associated with conception rates in heifers. The hypothesis is that the TRPM7 channel mediates intracellular cation influx that regulates oocyte growth, maturation, and competence. Bovine oocytes will be in vitro matured and Ca^{2+} content, free-Zn^{2+} levels, Zn^{2+} sparks, and free Mg^{2+} levels will be monitored during different stages of development using different dyes (Fura2-Am, FluoZin3 AM and Acid, and Mag-Fura). Expression of TRPM7 in GV and MII oocytes and each stage of pre-implantation embryo development will be determined using a monoclonal antibody [33,34].

1A.2: Folliculogenesis and Steroidogenesis: Pro-inflammatory signaling alters LH-dependent regulation of cholesterol homeostasis in theca cells resulting in increased estradiol (E2) and androstenedione (A4) levels We hypothesize that E2 and A4 regulate cholesterol homeostasis. Theca cell cultures will be treated with LH, E2 and/or A4, inflammatory factors, pathway inhibitors, and/or estrogen or androgen receptor agonists or antagonists. The importance of lipid uptake receptors, cholesterol synthesis enzymes, and lipid droplet associated proteins will be determined using siRNA transfection, qPCR, and Western blot. Secreted A4 and E2 will be measured in conditioned media. Changes in cholesterol trafficking will be determined using a combination of organelle dependent dyes and fluorescent-tagged cholesterol.

High levels of androgen activate a novel androgen receptor (ZIP9) leading to impaired granulosa cell differentiation and function [35]. Thus, granulosa cells will be cultured and expression and localization of ZIP9 will be determined using Western blot and IHC. Gene ablation approaches will determine the ZIP19 dependent effects of androgen on granulosa cell survival, proliferation, and differentiation. Finally, the downstream signaling molecules activated by ZIP9 in bovine granulosa cells will be identified using a combination of protein biochemistry, spectroscopy, and proteomic approaches. These experiments will determine a novel mechanism by which androgen contributes to development of anovulation and cyst formation.

The effect of the nutrient sensor O-GlcNAcylation, on granulosa cell responsiveness to hormone stimulation, metabolism and steroidogenic capacity will be examined, Granulosa cell cultures will be treated with Thiamet-G and OSMI-1 to induce O-GlcNAcylation. Estradiol (E2) and progesterone (P4) will be measured in conditioned media and RNA-seq and proteomic analysis performed using cell lysates.

To determine how hypoxic conditions augment granulosa cell growth, cells will be cultured under low oxygen tension or with a hypoxia mimetic. Changes in protein expression and cell proliferation will be determined using Western blot and MTS assays/ Ki-67 staining, respectively.

The Trio allele is a novel marker of high fecundity. We hypothesize that reduced follicle growth in cattle with the Trio allele is due to increased abundance of SMAD6 and reduced granulosa cell proliferation but not preantral follicle numbers. Ovarian tissue from Trio carrier and non-carrier heifers will be fixed and paraffin embedded. Sections will be stained with hematoxylin and eosin and follicle numbers and dimensions will be determined. Relative abundance and localization of SMAD6 and the cell proliferation marker Ki67 will be determined using immunohistochemistry.

The hypothesis is that a compensatory mechanism exists after unilateral ovariectomy (ULO) and would be evidenced by 1) increased number of antral follicles present in the retained ovary, and 2) an increase in circulating AMH. In addition, we hypothesize that the length required for the intact ovary to undergo compensation in follicular development will be slower in cows carrying the Trio allele. Antral follicle counts, serum AMH, and ovulation rate will be determined before ULO and at multiple times up to 230 days after ULO in each cow.

1A.3 Corpus Luteum (CL) Function: The hypothesis is that angiotensin II (Ang II) regulates the expression of cellular communication network factor 1 (CCN1) and progesterone biosynthesis in steroidogenic luteal cells. The CL will be collected from dairy cows on day 4 of the estrous cycle, cells dissociated, and then treated with 10^{-6} to 10^{-9} M Ang I or Ang II for 0.5, 2, 6, 24 and 48 hours. Quantitative PCR will measure expression of CCN1, vascular endothelial growth factor, basic fibroblast growth factor, and the angiotensin receptors, AT1 and AT2. Immunohistochemistry will be used to localize AT1 and AT2, while P4 concentrations will be determined by radioimmunoassay.

Obj 1B: Determine production stressor effects on follicle and oocyte development.

1B.1 Heat Stress: The hypothesis is that hormonal programs used in advanced reproductive techniques (ART), extreme body condition score (BCS), and extreme temperature impact the dominant follicle which subsequently alters oocyte developmental competence. We will model these conditions and collect follicular fluid, granulosa cells, cumulus cells, and the oocyte from pre-ovulatory follicles. Steroids, transcriptome and/or metabolome profiles of mural granulosa cells, cumulus cells, or oocytes will be compared. Matured oocytes will undergo *in vitro* fertilization and embryo cleavage rates, blastocyst rates, blastocyst transcriptome profiles, and blastocyst metabolome profiles. We expect to unveil reduced metabolic capacity of oocytes or embryos from small pre-ovulatory follicles or low BCS, altered meiotic maturation of oocytes from high temperatures, and that all treatments will result in hormone and metabolic differences in the follicular fluid.

The hypothesis is that tempering hyperinsulinemia/hypoglycemia observed during heat stress[13,38] with chromium propionate will improve reproductive performance. Dairy cows with clinically normal periparturient periods will be assigned to receive control ration or chromium propionate-supplemented ration. Ovarian structures will be monitored for benchmarks of follicle and CL development. Plasma concentrations of insulin, glucose, E2 and P4 will be determined. We expect that cows receiving chromium propionate will have greater circulating glucose and lower insulin concentrations during the summer months, and that this improved glycemic status will be associated with outcomes indicative of improved fertility, especially hastened postpartum resumption of cyclicity.

1B.2 Micronutrients: Pilot data suggest constant exposure to vitamin A and moderate levels of vitamin D reduce the number of unfertilized cattle oocytes during in vitro fertilization. We will determine if long-term supplementation with vitamin A and D will improve follicular function in cattle prior to breeding and shorten the window of resumption of ovarian activity post-calving. Gestating beef cattle will be injected with either vehicle control or a 45kIU vitamin D and 300 KIU vitamin A. Females will calve in late winter/early spring followed by artificial insemination 45 days later. At the same time, they will receive another vitamin premix injection at the same dosage. Pregnancy will be determined by ultrasound on GD30. During the treatment interval and breeding protocols serum progesterone, antimullerian hormone (AMH) and vitamin D will be monitored by validated ELISAs.

Supplemental selenium (Se) is provided to cows which affects both luteal phase and gestational concentrations of P4[15]. We hypothesize that supplementation with an equimolar 1:1 blend of organic and inorganic forms of Se increases systemic production of P4 via the stimulation of cholesterol uptake by luteal cells and the developing placental interface. CL and placentomes will be retrieved on Day 45 from pregnant heifers and in vitro assays (e.g., qPCR) used to determine mechanisms by which systemic concentrations of P4 are affected by the form of Se provided. By understanding how the form of Se affects the synthesis and secretion of P4 during the establishment and maintenance of pregnancy, recommendations can be made to the producer that will improve fertility outcomes.

1B.3 Toxins: Perfluorooctanoic acid (PFOA; a PFAS member), is detected in human follicular fluid[36] and phenotypically impairs fertility in exposed rats [37]. However, mechanisms by which PFAS impairs female fecundity remains ill-defined in livestock animals. Pre- and post-pubertal pigs will experience either thermal neutral or heat stress conditions in a diurnal pattern, with or without PFOA. After 14 days, liver, ovaries and uteri will be collected and weighed, follicular fluid retrieved, and the ovaries fixed in 4% paraformaldehyde or flash frozen. Ovarian protein will be isolated, and LC-MS/MS performed to identify global proteomic changes. Serum and follicular fluid will be analyzed by GC-MS to identify PFOA-dependent effects. The contralateral ovary will be sectioned and stained with hematoxylin and eosin for follicle counting. Statistical analysis to determine an additive impact of heat stress on PFOA ovarian endpoints will be conducted.

Obj 2: Identify factors that improve early pregnancy and develop technologies and management practices to limit conceptus mortality

2.1 Gamete Contribution to Embryos: Successful embryogenesis is dependent on gamete quality. In vitro matured oocytes will be microinjected with small interfering RNA (siRNA) or in vitro transcribed mRNAs. The effect of increased and decreased levels of specific maternal mRNAs on early embryonic development will be determined using IVF and embryo culture. Specific effects on embryonic genome activation, maternal mRNA degradation, and expression of specific embryonic genes will be determined using fluorescent in situ hybridization. Epigenetic effect on the embryonic genome will be measured by immunofluorescence and differential expression of specific embryo genes will be identified using transcriptomics which will be used to identify changes in gene families and developmental pathways.

The effect of changes in $[Ca^{2+}]_i$ and metabolism on bull sperm function will be determined. Transient calcium elevation, starvation or a combination of these methods will be used to develop new assisted reproductive technologies ART in the bovine model.

2.2 Conceptus Development and Uterine Interactions: Mechanisms that sustain luteal health during the second month of gestation are critical to maintain pregnancy. We will test the hypothesis that CL maintenance during the second month of gestation is accomplished through an increase in ipsilateral utero-ovarian blood flow resulting in reduced transfer of endometrial $PGF_{2\alpha}$ to the ovarian artery, thus preventing regression of the CL

To understand key milestones of conceptus development and its interaction with the endometrium, we will: 1) investigate mechanisms of placenta trophectoderm growth and function during conceptus elongation and production of the maternal recognition of pregnancy factor interferon-tau (IFNT), 2) perform functional tests of newly discovered conceptus and/or endometrial secretory factors that likely have important functions during early pregnancy, 3) study physical interactions between the trophectoderm and uterine mucosa that lead to placentation, 4) study the yolk sac to better understand the importance of this extraembryonic tissue during early pregnancy, and 5) identify differences in endometrial gene expression between the ipsilateral compared to the contralateral uterine horn during early pregnancy as it relates to regression of the contralateral accessory CL.

We propose that the ~50% reduction in conception rates between heifers and mature lactating dairy cattle is due in part to immune dysregulation at the fetal-maternal interface. The hypothesis is that conceptus secretory proteins alter immune cell proportions and functions to promote immune tolerance and to facilitate endometrial remodeling and angiogenesis necessary for the formation of a placenta. Changes in immune cells and expression of immune mediators during early pregnancy will be measured and compared between fertile heifers and sub-fertile lactating dairy cows. Chronic uterine catheterization will be used to infuse IFNT, and pregnancy associated glycoproteins, two major conceptus secretory proteins during early pregnancy, alone and in combination to determine their role in regulating immune cell function.

2.3 Artificial Reproductive Technology (ART) In vitro embryo production (IVP) systems, somatic cell nuclear transfer (SCNT) and gene editing technologies will be used to 1) identify oviduct and uterine factors that improve IVP and SCNT embryo development, 2) discover inherent embryonic mechanisms controlling embryonic lineage specification, 3) uncover regulatory mechanisms that are critical for normal embryo development, and 4) examine how interventions occurring during IVP affect post-transfer survival of pregnancies and calf health. Correlations between pregnancy loss and circulating hormones related to IVP and SCNT cattle embryos will be made to better understand early embryo/conceptus mortality related to these ART methods.

2.4 Uterine Contribution to Conceptus Development: Reproduction in ruminants involves complex cellular processes between reproductive cells or tissues which may change over time and biological context. The development of new -omics technologies allow for the identification of physiologically relevant molecules during early reproductive processes that may cause pregnancy failure. Transcriptomic, proteomic and metabolomic approaches will be used to characterize conceptus induced uterine histotroph necessary to support development of the early cow conceptus. The same techniques will test the hypothesis that IVP bovine embryos alter the endometrial transcriptome and uterine histotroph, resulting in a suboptimal uterine environment for pregnancy.

Obj 3: Identify and develop novel hormonal, genomic, and biomarker-based reproductive management strategies and technologies that maximize reproductive performance and farm profitability through improved fertility, service rates, and labor efficiencies of AI and ET programs for cattle.

3.1 Synchronization Protocols: The first objective is to optimize the response to synchronization of ovulation protocols will increase pregnancy per AI (P/AI) and ET (P/ET). We will develop and test different strategies to improve ovulatory response to initial GnRH treatment during ovulation synchronization protocols for timed artificial insemination (TAI). We will determine the effect of GnRH dose and pre-synchronization using PGF2α (PGF) or PGF and P4 before initiation of a CO-Synch protocol on estrus expression and fertility to TAI. Ultrasonography and hormone profiles will be used to evaluate ovulation, follicular wave dynamics and the hormonal environment before and after AI.

The second objective is to design reproductive management programs that optimize the response to TAI and embryo transfer (TET) protocols to reduce the interbreeding interval. The impetus is to reduce interbreeding intervals and develop methods for earlier pregnancy diagnosis. First, we will evaluate the effect of oxytocin and low dose hCG treatment of non-bred cows on timing of luteolysis. We will subsequently use cows that have previously received TAI. A third experiment will test the overall fertility and reproductive efficiency of dairy cows upon use of ReBreed21. This approach will be tested at multiple experiment stations.

A third objective is to reduce interbreeding interval and develop methods for an earlier pregnancy diagnosis in ET recipients using ReBreed21. This program was previously developed and tested in beef heifers [39]. Heifers will be synchronized to receive ET by the traditional method (ET/35 d) or with the ReET21 method (ET/21 d). Fertility and reproductive efficiency from using ReET21 and a traditional Resynch program will be compared. If promising, this ReET21 will be tested at other stations.

A fourth objective is to optimize hormonal responses during a 5-day fixed time ET ovulation synchronization protocol and determine risk factors affecting fertility in dairy cows. We will determine: 1) the effect of GnRH doses on ovulatory response, estrus expression and fertility to ET; 2) the effect of PGF dose and treatment number on luteolysis, estrus expression and fertility to ET; and 3) identify recipient management factors associated with greater fertility and reduced pregnancy loss. We will use ultrasonography and P4 and E2 profiles to evaluate ovarian response.

3.2 Genetic Determinants of Fertility: We will test the hypothesis that high *vs* low fertility in dairy cows is associated with individual or combined SNPs in genes linked to reproductive activity. Thus far, blood samples for DNA genotyping and fertility phenotype data have been collected from 1,060 lactating dairy cows across 7 research stations. Genotypes for GHR, TNFα, IGF-1, COQ9, and PON1, and other related genes will be determined as well as their association with fertility to first AI and pregnancy rates.

Identification of protein biomarkers is hampered due to a lack of tools to deplete high abundant proteins in biological fluids to improve discovery of low abundant proteins, we will develop species-specific reagents to efficiently deplete high abundance proteins in bovine plasma/serum. This strategy will be deployed concurrent with data-independent acquisition proteomics to remove bias for high abundant proteins. This method will enable identification of biomarkers, including low abundant proteins, that predict pregnancy status and detect early embryonic mortality earlier after AI.

3.3 Nutritional Effects on Fertility: Perinatal nutritional environment modulates developmental organization of hypothalamic neurocircuits, which alters programming of the reproductive neuroendocrine axis [40,41]. Pregnant cows will be assigned to low, moderate, or high energy diets to achieve different target BCS. After calving, heifer offspring will be weaned and assigned to a high or low-concentrate diets. At 8 mo. of age all heifers will transition to a common diet until 24 mo. of age and estrus will be synchronized. Heifers detected in estrus will be examined with ultrasonography to characterize the follicular wave dynamics, count antral follicles, and determine timing of ovulation. The effects of peri- and post-natal nutrition on E2/P4 profiles, estrous cyclicity, and pregnancy per AI will be determined. Ultimately, nutritional management recommendations for commercial beef operations will be developed.

3.4 Targeted Reproductive Management (TRM): The objective is to identify predictors for use in targeted reproductive management (TRM) of dairy cattle. Using data collected from cows (n=5,000+) we will determine associations between reproductive and herd outcomes and a) genomic traits; b) behavioral and physiological parameters collected by sensors (e.g., activity, rumination, body temperature, BW, BCS); and c) cow features and historical data (e.g., reproductive and health history). Measures with the greatest predictive value for outcomes of interest will be used to create algorithms with traditional statistical techniques and machine learning to create subgroups of cows for TRM.

Once predictive values have been identified, we will develop a suite of reproductive management strategies for dairy heifers and cows to evaluate the benefits of using TRM. Randomized controlled experiments will be conducted and reproductive performance, herd management practices, and farm profitability measured. The goal is to design TRM programs to optimize AI and targeted hormonal therapy that will increase fertility, optimize the value of offspring (e.g., use of sexed semen), and optimize timing of pregnancy during lactation.

Measurement of Progress and Results

Outputs

- Novel data will be generated and new techniques will be shared amongst experiment stations and with colleagues outside of the multi-state project. Comments: Large datasets from -omics-focused studies will be shared on open access servers. Outcomes of basic research will be used to develop applied studies. Through the latter venues, stakeholder input for further work and reactions to work in progress will be obtained.
- An additional output is the continued training of undergraduate and graduate students. Comments: Faculty at the extension stations will continue team-teaching the previously developed Contemporary Topics in Reproductive Biology. Graduate student training and introduction of undergraduates to research will continue with opportunities to work collaboratively in the group.

Outcomes or Projected Impacts

- Increased technologies and management strategies to mitigate pregnancy loss and improve animal fertility in the face of climate change. This proposal aligns with Strategic Goals 2 and 7 of the USDA Strategic Plan (2018-2022) which are to “maximize the ability of American agricultural producers to prosper by feeding and clothing the world” and “to provide all Americans access to a safe, nutritious, and secure food supply”. Collectively, outcomes from these studies will close important gaps in knowledge regarding follicle growth and estrous cyclicity, oocyte quality, early embryonic development, conceptus-maternal signaling, conceptus implantation and maintenance of pregnancy in cattle. The complex molecular environments that govern these processes will also be characterized using new omics and single cell technologies. Importantly, the impact of assisted reproductive technologies such as IVP and SCNT on embryo quality will also be elucidated with the goal of bettering these technologies for advancement in food production. Collectively, these outcomes will lead to technologies and/or management strategies that mitigate embryonic/conceptus mortality, and increase pregnancy success. The work proposed also has the potential to lead to housing, management, and nutritional approaches to improve fertility in the face of climate change. In the long-term, continued research by this multi-state group will result in the generation of important new information to enhance the efficiency of reproductive performance in ruminant species which will contribute to meet the growing world-wide demands for animal protein.

Milestones

(**2022**):All studies will be initiated including tissue collections and design of cross-station animal studies. Raw data will be collected from -omics studies. New techniques will be piloted.

(**2024**):Analyze and interpret on omics data. New techniques will be shared. Outcomes of studies in 2022-2023 will be disseminated as described in the Outreach Plan.

(**2027**):Validate -omics data to establish reliable biomarkers of reproductive traits. Interpret the output of basic studies in order to develop new application-based hypotheses. Determine the efficacy of new management programs. Continue to disseminate data as described in the Outreach plan.

Outreach Plan

The basic research described herein will be disseminated in annual reports, peer-reviewed journals, at scientific meetings by faculty and students, and on websites of the experiment stations involved.

Outcomes from applied studies will be presented to each experiment station advisory board and local extension personnel and commodity groups in short courses and at field days. Some extension specialists and agents will aid in collecting applied data and selecting cooperating farms. Research workers will prepare news releases or participate in interviews.

Members of the group will continue private-public partnerships with cattle AI organizations (Select Sires, Genex CRI, Alta Genetics), the National Association of Animal Breeders (NAAB), the American Association of Bovine Practitioners (AABP), dairy industry associations (Minnesota Dairy Association, Vermont Dairy Association), pharmaceutical and technology companies (Zoetis, Merck, Allflex), and extension education organization (PRO-Dairy). In turn, those groups spread the technology to farm families/producers for implementation which benefits the on-farm profitability and sustains agricultural production systems that are highly competitive in the global economy.

Organization/Governance

A Technical Committee will be organized with voting membership including at least one representative from each cooperating Agricultural Experiment Station as appointed by the respective Director. Non-voting members shall consist of the Administrative Advisor and a USDA consulting member. All voting members of the Technical Committee are eligible for office. A chairperson, a secretary, and a director will be elected for 2-year terms to compose an Executive Committee. The Technical Committee will meet at least annually. The chairperson, in consultation with the administrative advisor, will notify members of the time and place of meetings. The chairperson is responsible for the preparation of the annual report. The secretary records the minutes and other duties as assigned by the Technical Committee. The Executive Committee may be delegated to conduct the business of the Technical Committee between meetings. Other subcommittees may be named by the chairperson as required.

Literature Cited

1. Cooperative Regional Research Project, N.-161. Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. *Journal of Animal Science* **74**, 1943–1952 (1996).
2. Kamalludin, M. H., Garcia-Guerra, A., Wiltbank, M. C. & Kirkpatrick, B. W. Trio, a novel high fecundity allele: I. Transcriptome analysis of granulosa cells from carriers and noncarriers of a major gene for bovine ovulation rate. *Biol Reprod* **98**, 323–334 (2018).
3. Garcia-Guerra, A., Kamalludin, M. H., Kirkpatrick, B. W. & Wiltbank, M. C. Trio a novel bovine high-fecundity allele: II. Hormonal profile and follicular dynamics underlying the high ovulation rate. *Biol Reprod* **98**, 335–349 (2018).
4. García-Guerra, A. *et al.* Trio, a novel bovine high fecundity allele: III. Acquisition of dominance and ovulatory capacity at a smaller follicle size. *Biol Reprod* **98**, 350–365 (2018).
5. Van Blerkom, J., Antczak, M. & Schrader, R. The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. *Hum Reprod* **12**, 1047–1055 (1997).
6. Wood, J. R. & Strauss, J. F. 3rd. Multiple signal transduction pathways regulate ovarian steroidogenesis.*Rev Endocr Metab Disord* **3**, 33–46 (2002).
7. Shimizu-Albergine, M. *et al.* SCAP/SREBP pathway is required for the full steroidogenic response to cyclic AMP.*Proc Natl Acad Sci U S A* **113**, E5685-5693 (2016).
8. Nakanishi, T. *et al.* LH Induces De Novo Cholesterol Biosynthesis via SREBP Activation in Granulosa Cells During Ovulation in Female Mice. *Endocrinology* **162**, (2021).
9. Kiser, J. N. *et al.* Identification of loci associated with conception rate in primiparous Holstein cows.*BMC Genomics* **20**, 840 (2019).
10. Kobayashi, S., Berisha, B., Amselgruber, W. M., Schams, D. & Miyamoto, A. Production and localisation of angiotensin II in the bovine early corpus luteum: a possible interaction with luteal angiogenic factors and prostaglandin F2 alpha. *J Endocrinol* **170**, 369–380 (2001).
11. Carbajo-Lozoya, J. *et al.* Angiotensin II modulates VEGF-driven angiogenesis by opposing effects of type 1 and type 2 receptor stimulation in the microvascular endothelium. *Cell Signal* **24**, 1261–1269 (2012).
12. Wang, J., Jiang, Y., Soulixay, S., Fu, D. & You, Y. [Angiotensin induces apoptosis of HEK293T cells by up-regulating Cyr61 expression]. *Nan Fang Yi Ke Da Xue Xue Bao* **39**, 810–815 (2019).
13. Wheelock, J. B., Rhoads, R. P., Vanbaale, M. J., Sanders, S. R. & Baumgard, L. H. Effects of heat stress on energetic metabolism in lactating Holstein cows. *J Dairy Sci* **93**, 644–655 (2010).
14. Ding, N., Harlow, S. D., Randolph, J. F. J., Loch-Caruso, R. & Park, S. K. Perfluoroalkyl and polyfluoroalkyl substances (PFAS) and their effects on the ovary. *Hum Reprod Update* **26**, 724–752 (2020).
15. Carr, S. *et al.* Form of Supplemental Selenium in Vitamin-Mineral Premixes Differentially Affects Early Luteal and Gestational Concentrations of Progesterone, and Postpartum Concentrations of Prolactin in Beef Cows. *Animals (Basel)* **10**, (2020).
16. Navarrete, F. A. *et al.* Transient exposure to calcium ionophore enables in vitro fertilization in sterile mouse models.*Sci Rep* **6**, 33589 (2016).
17. Telford, N. A., Watson, A. J. & Schultz, G. A. Transition from maternal to embryonic control in early mammalian development: a comparison of several species. *Mol Reprod Dev* **26**, 90–100 (1990).
18. Ott, T. L. Symposium review: Immunological detection of the bovine conceptus during early pregnancy.*J Dairy Sci* **102**, 3766–3777 (2019).
19. Ott, T. L. Immunological detection of pregnancy: Evidence for systemic immune modulation during early pregnancy in ruminants. *Theriogenology* **150**, 498–503 (2020).
20. Cummins, S. B., Lonergan, P., Evans, A. C. O. & Butler, S. T. Genetic merit for fertility traits in Holstein cows: II. Ovarian follicular and corpus luteum dynamics, reproductive hormones, and estrus behavior. *J Dairy Sci* **95**, 3698–3710 (2012).
21. Moore, S. G., Scully, S., Browne, J. A., Fair, T. & Butler, S. T. Genetic merit for fertility traits in Holstein cows: V. Factors affecting circulating progesterone concentrations. *J Dairy Sci* **97**, 5543–5557 (2014).

22. Shirasuna, K. *et al.* Relationships between the first ovulation postpartum and polymorphism in genes relating to function of immunity, metabolism and reproduction in high-producing dairy cows. *J Reprod Dev* **57**, 135–142 (2011).
23. Liefers, S. C. *et al.* Leptin promoter mutations affect leptin levels and performance traits in dairy cows. *Anim Genet* **36**, 111–118 (2005).
24. Nicolini, P., Carriquiry, M. & Meikle, A. A polymorphism in the insulin-like growth factor 1 gene is associated with postpartum resumption of ovarian cyclicity in Holstein-Friesian cows under grazing conditions. *Acta Vet Scand* **55**, 11 (2013).
25. Khatib, H. *et al.* Single gene and gene interaction effects on fertilization and embryonic survival rates in cattle. *J Dairy Sci* **92**, 2238–2247 (2009).
26. Waters, S. M. *et al.* Associations between newly discovered polymorphisms in the Bos taurus growth hormone receptor gene and performance traits in Holstein-Friesian dairy cattle. *Anim Genet* **42**, 39–49 (2011).
27. Schneider, A., Corrêa, M. N. & Butler, W. R. Association between growth hormone receptor AluI polymorphism and fertility of Holstein cows. *Theriogenology* **80**, 1061–1066 (2013).
28. Ortega, M. S. *et al.* A single nucleotide polymorphism in COQ9 affects mitochondrial and ovarian function and fertility in Holstein cows. *Biol Reprod* **96**, 652–663 (2017).
29. Michel-Regalado, N. G. *et al.* Effect of COQ9 and STAT5A polymorphisms on reproductive performance in a Holstein cow herd in Mexico. *Anim Reprod* **17**, e20200039 (2020).
30. Silveira, P. A. S. *et al.* Polymorphisms in the anti-oxidant paraoxonase-1 (PON1) gene associated with fertility of postpartum dairy cows. *Theriogenology* **125**, 302–309 (2019).
31. Carvalho, P. D. *et al.* Relationships between fertility and postpartum changes in body condition and body weight in lactating dairy cows. *J Dairy Sci* **97**, 3666–3683 (2014).
32. Cardoso Consentini, C. E., Wiltbank, M. C. & Sartori, R. Factors That Optimize Reproductive Efficiency in Dairy Herds with an Emphasis on Timed Artificial Insemination Programs. *Animals (Basel)* **11**, (2021).
33. Wakai, T., Zhang, N., Vangheluwe, P. & Fissore, R. A. Regulation of endoplasmic reticulum Ca(2+) oscillations in mammalian eggs. *J Cell Sci* **126**, 5714–5724 (2013).
34. Que, E. L. *et al.* Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation. *Theriogenology* **127**, 41–48 (2019).
35. Thomas, P., Pang, Y., Dong, J. & Berg, A. H. Identification and characterization of membrane androgen receptors in the ZIP9 zinc transporter subfamily: II. Role of human ZIP9 in testosterone-induced prostate and breast cancer cell apoptosis. *Endocrinology* **155**, 4250–4265 (2014).
36. Heffernan, A. L. *et al.* Perfluorinated alkyl acids in the serum and follicular fluid of UK women with and without polycystic ovarian syndrome undergoing fertility treatment and associations with hormonal and metabolic parameters. *Int J Hyg Environ Health* **221**, 1068–1075 (2018).
37. Du, G. *et al.* Neonatal and juvenile exposure to perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS): Advance puberty onset and kisspeptin system disturbance in female rats. *Ecotoxicol Environ Saf* **167**, 412–421 (2019).
38. Rhoads, M. L. *et al.* Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. *J Dairy Sci* **92**, 1986–1997 (2009).
39. Andrade, J. P. N. *et al.* Development of a novel 21-day reinsemination program, ReBreed21, in Bos indicus heifers. *Theriogenology* **155**, 125–131 (2020).
40. Martin, J. L., Vonnahme, K. A., Adams, D. C., Lardy, G. P. & Funston, R. N. Effects of dam nutrition on growth and reproductive performance of heifer calves. *J Anim Sci* **85**, 841–847 (2007).
41. Cardoso, R. C., West, S. M., Maia, T. S., Alves, B. R. C. & Williams, G. L. Nutritional control of puberty in the bovine female: prenatal and early postnatal regulation of the neuroendocrine system. *Domest Anim Endocrinol* **73**, 106434 (2020).

Land Grant Participating States/Institutions

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
Grand Total:	**0**	**0**	**0**

Program/KA	Total FTE
Grand Total:	**0**

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2227: Contribution of Ovarian Function, Uterine Receptivity, and Embryo Quality to Pregnancy Success in Ruminants

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Good
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Good
5. Overall technical merit:
Good
Comments
Due to the limited descriptions of the technical approaches it is difficulty to accurately rate the technical approach, but in general, all the methods discussed should be feasible and provide valued information. The global approach that can be achieved through the collaborative efforts will provide information on all levels - cow and genetic management and cellular/molecular mechanisms at the tissue/cell/embryo level, which should help improve reproductive efficiencies.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2227: Contribution of Ovarian Function, Uterine Receptivity, and Embryo Quality to Pregnancy Success in Ruminants

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project with revision
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Good
5. Overall technical merit:
Excellent
Comments
Well written proposal from a productive group. Projects planned should meet goals and enable excellent research to be conducted and published. Most multistate groups are developing symposia at regional or national meetings where extension educators will be present to aid outreach efforts. Plans for at least one symposia and potential for extension educators or industry representatives should be mentioned in outreach efforts. While field days and workshops are mentioned --no specific ones are named that this group participates in. At least naming where a symposia or field day/workshop, etc would be helpful to determine who might be the participants and would enhance the outreach goals..
Your Recommendation:
Approve/continue project with revision

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2227: Contribution of Ovarian Function, Uterine Receptivity, and Embryo Quality to Pregnancy Success in Ruminants

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
This is a very well written multi-state project proposal. My only real concern is that under each objective there is a very detailed list of sub-objectives that could lead the new member to think that there is not a place for their work. I would recommend a statement before each list indicating that these are some of the anticipated activities to accomplish the objective, while other approaches would be welcome (considered).
Your Recommendation:
Approve/continue project

Response to Reviewers

Reviewer 1
Due to the limited descriptions of the technical approaches, it is difficulty to accurately rate the technical approach, but in general, all the methods discussed should be feasible and provide valued information. The global approach that can be achieved through the collaborative efforts will provide information on all levels - cow and genetic management and cellular/molecular mechanisms at the tissue/cell/embryo level, which should help improve reproductive efficiencies.

No revisions requested. We did group experiments based on topic to better explain technical approaches

Reviewer 2
Well written proposal from a productive group. Projects planned should meet goals and enable excellent research to be conducted and published. Most multistate groups are developing symposia at regional or national meetings where extension educators will be present to aid outreach efforts. Plans for at least one symposia and potential for extension educators or industry representatives should be mentioned in outreach efforts. While field days and workshops are mentioned --no specific ones are named that this group participates in. At least naming where a symposia or field day/workshop, etc would be helpful to determine who might be the participants and would enhance the outreach goals.

Specific information was added to the outreach section regarding specific interactions with stakeholders. This includes the existing extension symposia led by members of the group. There is also a goal to increase private-public partnerships to reach more extension educators and producers across the multiple experiment stations.

Although it does not directly impact stakeholders, the collaborative course is an important component of student training with the expectation that after graduation these students will be able to effectively communicate to extension educators and industry representatives.

Reviewer 3
This is a very well written multi-state project proposal. My only real concern is that under each objective there is a very detailed list of sub-objectives that could lead the new member to think that there is not a place for their work. I would recommend a statement before each list indicating that these are some of the anticipated activities to accomplish the objective, while other approaches would be welcome (considered).

This point is well taken. Revisions were made in the methods to make it easier for potential members to see how their work will fit into the group. Instead of the list of experiments, they were grouped under broad categories (e.g., gamete quality, uterine-conceptus interactions, and targeted management). We feel that this identifies areas of interest in the group with examples of current projects listed below each sub-objective.

NE_TEMP2248: Mastitis Resistance to Enhance Dairy Food Safety, Milk Quality, and Animal Welfare

Status: Submitted As Final

Duration	10/01/2022 to 09/30/2027
Admin Advisors:	
NIFA Reps:	

Statement of Issues and Justification

The United States dairy industry continues to experience significant monetary drain via losses associated with common diseases. Bovine mastitis is the costliest infectious disease currently affecting dairy cattle. While significant advances have been made in controlling some types of mastitis, the complex etiology of the disease and ongoing changes in dairy practices dictate that new and more effective methods for control and treatment be developed over time. Single site studies are often limited in terms of expertise and cattle numbers. A multi-state project provides advantages in terms of increased numbers of herds and cattle as well as multiple levels of expertise and contributions that are not present at single institutions.

Mastitis is defined as inflammation of the mammary gland and is almost always associated with bacterial infection within the cow's mammary gland. Mastitis affects every dairy farm and approximately 38% of dairy cows in the United States experience clinical signs of disease. The National Mastitis Council estimates that this devastating disease costs the dairy industry more than 2 billion dollars per year or approximately $180.00 per cow. These losses are primarily due to subpar milk production and diminished milk quality, increased veterinary costs and antibiotic usage, increased cow mortality, and discarded of abnormal and antibiotic laden milk.

In the United States (US), cash receipts from marketing of milk during 2020 totaled $40.5 billion (NASS, 2021). In the US, the dairy industry contributes >$140 billion per year to the national economy and provides > 900,000 jobs making it a vital part of our nation's economy and food system (Adcock et al., 2015; ERS, 2015).

Currently, intramammary antimicrobial therapy is the most widely used and most effective management strategy to eliminate intramammary infections and alleviate pain and suffering. Bovine mastitis is a unique disease wherein multiple infectious agents can trigger an inflammatory response. When considering treatment of an individual cow for mastitis, the causative infectious agent is usually unknown. Therefore, producers commonly treat with different combinations of antimicrobials and routes of treatments. This common management strategy can lead to overuse of antimicrobials, thus increasing the risk of residues in milk and the selection for antimicrobial resistant pathogens. Growing consumer concerns regarding antimicrobial use, the risk of antimicrobial residues in milk and meat and the potential for antimicrobial resistance have led to the examination of alternative strategies for controlling mastitis while reducing the use of antimicrobials on-farm, which is one of the primary goals of this multi-state group.

The primary goal of NE-1748 has been to coordinate and enhance multidisciplinary research efforts on mastitis that are being conducted at various laboratories throughout the United States and internationally, e.g., Canada, Australia, and Europe. The magnitude and scope of attempting to solve the problems arising from mastitis extend far beyond the ability of any one institution. The ability to cooperate on a regional, national, and international basis allows the integration of resources and knowledge to address these problems. Recognition of the need for a coordinated effort to study resistance of the pathogen and the need for non-antibiotic alternative therapeutics for the dairy cow for the control of mastitis resulted in the design and initiation of multi-State Project NE-1748. The NE-1748 project has provided a forum for new and established researchers to develop collaborative relationships, and to share resources and expertise. NE-1748 meetings are well attended, and 20-40 presentations are typically made by participants each year. International visitors and collaborators are often included in these presentations. We are proposing to continue these efforts with NE-2248 in a multistate and international setting to better control mastitis, reduce antimicrobial usage for the treatment of mastitis, and reduce the negative economic consequences of this prevalent and burdensome disease.

The mastitis research workers group has met in conjunction with the NE-1748 annual meeting for many years. International visitors and collaborators are often included in these presentations. In addition to the mastitis research workers conference, the NE-1748 members provide new management strategies to reduce antibiotic usage and technology transfer to the scientific community and industry stakeholders. In the last 4 years, members of the project have collectively published multiple book chapters, peer-reviewed journal articles, abstracts and proceedings, and presented numerous oral and poster presentations related to mastitis, milk quality, and food safety. Venues for oral and poster presentations have included the National Mastitis Council regional and annual meetings (attendees include researchers, veterinarians, dairy producers, and representatives from industry), Conference for Research Workers in Animal Diseases, American Association of Bovine Practitioners annual meetings, International Dairy Federation meetings, American Dairy Science Association meetings, World Buiatrics Congress meetings, American Society of Microbiology meetings, Conference on Production Diseases in Farm Animals, Plant and Animal Genome Conference, Agriculture and Agri-Food Canada - Food Safety meetings, American College of Veterinary Internal Medicine annual forum meetings, and several regional extension and veterinary continuing education meetings.

The continuation of the NE-1748 multistate project by means of the proposed NE-2248 multistate project is of utmost importance to foster impactful mastitis research leading to the provision of science-based information to dairy producers and the dairy industry. The impact of the European Union’s strict enforcement of import regulations on milk quality highlights the need to continue efforts to reduce the incidence of mastitis and antibiotic use. These regulations require milk export companies to certify that any farm contributing milk must show a bulk milk cell count below 400,000 cells/mL. This regulation has been supported by the National Mastitis Council as a goal for all US dairies. Mechanisms leading to improvement in milk quality, dairy animal welfare, and appropriate use of antimicrobial therapeutics form the basis of research conducted in the NE-1748 multistate project. It is clear that continued mastitis research and education are required to maintain the global competitiveness of the US dairy industry. Furthermore, the animal agriculture industry in general is under closer scrutiny than ever before by various interest groups. The work of NE-1748 is clearly focused on reducing mastitis, reducing antibiotic use and improving economic outcome and animal welfare. Mastitis is the most significant animal health issue in the dairy industry. In summary, continuation of the NE-1748 project is a productive group of collaborators that has provided new and meaningful information to all levels of the dairy industry from the bench scientist to the dairy producer with regard to bovine mastitis control, treatment and prevention. In the next 5 years we will continue to pursue collaborative projects under our 3 stated objectives which will lead to new information of value to the management of dairy cattle mastitis. Mastitis is an evolving disease syndrome, as is the science that studies mastitis; therefore, continued research efforts are needed.

Related, Current and Previous Work

The Multi-State Mastitis Research Project (MMRP) has a strong productive history in applied and basic mastitis research. The project was begun in 1977 as NE-112, then renewed in 1982, 1987, 1992, 1997, in 2002 as NE-1009, in 2007 as NE-1028, NE-1048 in 2012, and NE-1748 in 2017. A substantial percentage of international mastitis research is conducted by MMRP members and affiliates. Members of MMRP collaborate extensively within the project and with other national and international research groups that have interests in bovine mastitis. The 2017-2022 iteration of the MMRP had 3 main objectives pertaining to the host, the pathogen, and the use of new technology. In the current proposal we intend to continue work and begin new studies using these objectives but also incorporate the importance of reducing the use of antibiotics and improving animal welfare in the dairy industry. The following are brief reviews, listed by objective, of current and previous work conducted during the last 5 years by the MMRP. In this summary, we focus on some of the most recent and topical accomplishments. Multiple stations have contributed to the various objectives and are listed following each sub-objective.

Objective 1: Characterize host mechanisms associated with mastitis susceptibility, and resistance to improve economic outcomes and animal welfare.

(i) Environment, nutrition, and management related host factors associated with intramammary infections (NJ, OH, MD, MN, MI, NY, OR, ID).

The risk of mastitis increases from late-pregnancy to early lactation. During this period, cows are under the hormonal influence of pregnancy, and are most likely in negative energy balance during the early part of lactation. Research has, therefore, focused on developing dietary strategies aimed at improving the immune response during this time, as well as better understanding the relationship between negative energy balance, other nutritional factors, versus immunity. Idaho (ID), collaborating with Oregon (OR), reported that 2,4-thiazolidinedione (TZD) administration altered the abundance of long-chain fatty acids in milk, which is expected to influence the immune system's response to intramammary infection (Tsai et al., 2020). Researchers at Ohio State University (OH) reported that mammary blood vessels respond to the needs of the mammary epithelium which highlights the importance of capillaries and their role in intramammary infection susceptibility and resolution (Hardy et al., 2021); Ohio State also continues to investigate how mastitis affects mammary growth and development in the non-lactating mammary gland (Enger et al., 2020) in collaboration with researchers in Virginia. Scientists at Maryland determined that chromium propionate supplementation does not affect the metabolic status of mid lactation cows but influences monocyte responses to cytokines (Garcia et al., 2017). Researchers at Michigan State University continue to identify factors that increase mastitis risk (Moore-Foster et al., 2019; Erskine et al., 2019) and antibiotic usage in commercial dairy systems (Leite de Campos et al., 2021). A primary focus of researchers at University of Minnesota (MN) has been to delineate how, and to what degree, different bedding sources (Rowe et al., 2019) and udder hygiene practices (Rowe et al., 2019) affect mastitis risk and mastitis prevalence by using multiple farms, while also identifying how the teat microbiome and the cow's genetics influence a cow's risk to intramammary infections by collaborating with scientists at USDA Ames National Animal Disease Laboratory. Researchers at Cornell (NY) found the role of *Lactococcus lactis* and *garvieae* important for clinical mastitis but also chronic animals specially during the first phase of lactation (Scillieri et.al 2020).

(ii) Candidate Genes of Mastitis Susceptibility (TN and UT).

Projects within the MMRP have focused on studying genes related to the immune response during mastitis. Their work may allow consideration of selective breeding for mastitis resistance, which may prove valuable to the dairy industry as a whole. For instance, MMRP members at the University of Tennessee (TN) identified 16 key single-nucleotide polymorphisms that were linked to a cow's response to *Streptococcus uberis* challenge and highlighted that the most pronounced single nucleotide polymorphisms were involved with cell signaling, migration, and apoptosis. These cell processes are implicated in immune cell infiltration into diseased tissues as well as resolution to disease (Siebert et al., 2018). This is complementary to the efforts at Utah State University (UT) where fellow MMRP members classified 15 mastitis resistant, and 28 mastitis susceptible, phenotypes to evaluate single nucleotide polymorphisms related to mastitis resistance and susceptibility in a commercial herd (Kurz et al., 2019). Ten novel, and 17 previously identified, quantitative trait loci were identified; 4 of which indicated that teat length affects mastitis resistance. Additionally, single nucleotide polymorphisms of RAS guanyl-releasing protein 1 gene are noted to be of importance and warrant future investigations as to how this protein affects mastitis resistance.

Objective 2: Characterize agents associated with intramammary infections and assess their impact on milk quality and animal welfare (NJ, MN, OH, MO, VT).

Bacterial infections are the primary cause of intramammary infections and mastitis. Understanding the bacterial species and virulence factors will help understand their impact on milk quality and help to determine where prevention measures are needed. Many MMRP members have worked to characterize important pathogens associated with bovine intramammary infections. For example, members at Rutgers University have examined the mechanisms of metal toxicity to *Staphylococcus aureus* and evaluated novel molecules that are bactericidal against *S. aureus.* Members at the University of Minnesota have worked to apply precision dairy farming and diagnostic technologies to detect mastitis at the time of and following dry off in dairy cows in a field study. They have also worked to investigate if recycled manure solids (RMS) processing methods are associated with udder health, milk production, and with bedding bacteria counts (BBC) in ready-to-use RMS bedding samples. Results showed that herds using mechanically dried RMS or drum composted RMS processing systems generally had improved udder health and, for dried RMS, improved milk production, as compared to herds using digested or green solids. At Ohio State, members worked to determine if a commercially available teat sealant was effective in preventing new cases of mastitis from occurring in dairy heifers before they calve and begin producing milk. This work was done in collaboration with members from the University of Missouri. At the University of Missouri, members also worked to evaluate the association between staphylococcal species intramammary infection, milk somatic cell count, and persistence of infection during lactation and over the dry period were found in dairy goats. They reported an association existed between the use of intramammary pirlimycin and short-term changes in the fecal microbiome of dairy cattle being treated for staphylococcal intramammary infections. These members also demonstrated an association between teat end preparation techniques and contamination of milk samples collected for milk culture with more contaminants being present when teats were not scrubbed with alcohol prior to sample collection. Finally, they also evaluated 16S rRNA gene amplicon sequencing of milk samples and results supported the use of higher PCR cycle numbers to evaluate these low microbial biomass samples. The Barlow lab at the University of Vermont has completed a study of the mammary microbiome of lactating organic dairy cattle. The Barlow lab has completed a study applying machine learning to identify key predictors of pathogen strain type from multilocus sequence typing databases for *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus uberis.* The Barlow lab has initiated a study comparing milk quality and mastitis prevalence on organic dairy herds utilizing different bedding management practices. NE-1748 members from Minnesota (Godden), Missouri (Adkins) will collaborate in this research.

Objective 3: Assess and apply new technologies and preventative strategies that advance mastitis control, milk quality and/or reduce antimicrobial usage (LA, MI, MN, VT, UT, OR).

New technologies and preventative strategies are vital to help control mastitis on the dairy farm. Several MMRP members have contributed work in this area. For example, members at Louisiana State University continue to evaluate botanical formulations from plants for antimicrobial activity against mastitis pathogens. At Michigan State, members have conducted a randomized clinical trial that included evaluation of differences among selected *Streptococcus* like organism causing mastitis in response to differing durations of antimicrobial therapy. They have also characterized usage of antimicrobials on 40 large dairy farms and identified the proportion of antimicrobial usage that is associated with prevention and treatment of mastitis. These members are developing novel applications of vacuum analysis to determine milking efficiency in dairy herds. This will provide an on-farm education platform for producers and/or employees regarding milking protocols. At the University of Minnesota, members have conducted randomized controlled non-inferiority trial investigating the effect of two selective dry cow therapy protocols on antibiotic use and udder health and quarter-level outcomes. An economic analysis showed an positive economic return, on average, with either SDCT program. They conclude that SDCT can be used in appropriate U.S. dairy herds to reduce antibiotic use while maintaining udder health. At the University of Vermont, The Barlow lab completed collecting data from a field study exploring *Staphylococcus aureus* strain variation among dairy cattle and farm workers on 21 dairy farms producing farmstead of artisan cheeses. Strain typing and characterization of antimicrobial susceptibility phenotypes and genotypes has been completed for approximately 160 isolates and cross species (zoonotic) transmission dynamics of *S. aureus* will be explored using these data. Members at Utah State University compared 4 dry treatment groups including casein hydrolysate (CH) intramammary infusion alone or in combinations, and control (dry cow antibiotic plus teat sealant) in a split udder design. Microscopic morphometry measured changes in alveolar epithelial cell height, alveolar luminal diameter, and interstitial stromal thickness during the first 7 days dry. CH alone or combined with antibiotic and/or teat sealant was associated with some histological indications of increased mammary involution compared to controls at d 2 and d 7 dry. Casein hydrolysate may be a useful adjunct or replacement for dry cow antibiotic treatment. At Oregon State University, members carried out an experiment with the objective to determine if the combination of feeding chicory and supplementing selenium would improve the response to intramammary infection with *Strep. uberis*.

The following are just some of the key efforts and products resulting from collaborative participation of members in this group:

- USDA NIFA grant (Milk Quality Alliance) with collaborations among MI, PA, MS and FL
- USDA grant (Southeast Quality Milk Initiative) with collaboration among TN, VA, KY, FL, MS, GA
- Godden (MN) led a multi-state bedding analysis study with multiple MMRW members.
- Godden (MN) led a multi-state dry cow therapy study with multiple MMRW members.
- USDA NIFA grant awarded to Adkins (MO) and Barlow (VT) to determine the pathogenicity and impacts of *Staphylococcus chromogenes* intramammary infections in heifers.
- Collaboration between Enger (OH) and Adkins (MO) identifying the presence and distribution of causative intramammary infection agents in bred heifers (Larsen et. al., 2021) using formula funds.
- Seminal invited reviews by MMRP members at MO and MN concerning responsible antibiotic usage in the treatment of mastitis.
- Development of an invited review regarding how intramammary infections in heifers' impact mammary gland growth and development
- Collaboration between University of Utah and University of Missouri to determine agreement between 3 key methods for identifying microbial species in bovine milk using formula funds.
- A collaboration with the University of Maryland has been established to evaluate big-data genomics to improve dairy cattle health, including investigating mastitis resistance.
- University of Missouri collaborated with University of Vermont to investigate the local and system factors that reduce milk production resulting from lipopolysaccharide challenge.
- Collaboration between Utah and Missouri assessed bacterial biochemical testing, Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF), and 16S rRNA partial genome sequencing for microbial identification from bovine milk. Agreement among all 3 methods ranged from 97% to 100%, depending on the mastitis pathogen. Any of the 3 methods is a useful tool for identification of bacteria isolated from dairy cow milk.
- Continued collaboration with the Canadian Bovine Mastitis and Milk Quality Research Network **ÇBMQRN**) and other international entities include:
    - The University of Missouri has ongoing collaborations with University of Montreal and University of Calgary to better define the role of non-aureus staphylococci in bovine mastitis and further define their role in affecting udder health and milk yield losses using USDA formula funds.
    -

In summary, the work conducted within the framework of the MMRP has resulted in over 150 refereed publications and over 300 presentations at various scientific and stake-holder forums. We are continuing to build on our past findings to reduce the incidence and impacts of mastitis through additional research and extension activities. Mastitis is clearly a multi-faceted disease that will require continued efforts to not only ensure the production of safe, high-quality food, but to do so in a sustainable fashion and with continued improvements in dairy animal welfare and reductions the use of antimicrobial drugs.

Objectives

1. 1) Characterize host mechanisms associated with mastitis progression, susceptibility, and resistance to improve economic outcomes and animal welfare.
2. 2) Characterize agents associated with intramammary infections and assess their impact on milk quality and animal welfare.
3. 3) Assess and apply new technologies and preventative strategies that advance mastitis control, milk quality and/or reduce antimicrobial usage.

Methods

Five-year plans, including collaborations among experimental stations, are listed below.

Objective 1: Characterize host mechanisms associated with mastitis progression, susceptibility, and resistance to improve economic outcomes and animal welfare (LA, VT, OH, MN, MO, Canada).

The University of Missouri will continue to work in collaboration with the University of Maryland to evaluate big-data genomics to improve dairy cattle health, including investigating mastitis resistance. This work will be done using US dairy genomic databases.

Scientists at Louisiana State University (LA) will identify mastitis pathogens from cows and goats for antimicrobial susceptibility testing. Resistance patterns for mastitis pathogens will be compared to resistance patterns for the same bacterial species isolated from humans to determine possible impacts of agricultural practices on resistance patterns.

Scientists at Ohio State University will investigate how mastitis affects heifer mammary gland growth and development in non-lactating mammary glands using various chemical and immunohistochemical staining approaches and also investigate how mastitis alters milk synthesis in lactating cattle.

Researchers at University of Minnesota (B. A. Crooker, S. M. Godden, L. Caixeta, A. Seykora, M. Schutz, and B. Rosen) will continue the research program investigating the opportunity to reduce mastitis in the dairy cow by increasing the prevalence of beneficial polymorphisms in genes associated with mastitis resistance while working with researchers at USDA Ames National Animal Disease Laboratory (J. D. Lippolis). This will involve assessing the impact of Holstein genotype on immune response to gram negative and gram-positive bacteria that commonly cause mastitis. Other researchers (L. Caixeta, S. Dow, N. Noyes, B. Crooker, S. Godden, D. Nydam, B. Walcheck) will continue investigating techniques to enhance or modulate immune function (e.g. mucosal immune stimulation, microbiome, mechanical defenses) to enhance mastitis resistance during the dry or lactating periods.

Scientists at Vermont will continue to investigate how LPS and cytokines affect milk synthesis in mammary epithelial cells (MECs). An emphasis will be placed on how LPS and cytokines affect glucose uptake by the MECs.

Objective 2: Characterize agents associated with intramammary infections and assess their impact on milk quality and animal welfare (OH, MN, TN, VT, Canada, Australia, Europe).

The University of Missouri will work in collaboration with the University of Vermont to determine predictable molecular patterns of *Staphylococcus chromogenes* isolates deemed to be chronic high somatic cell count associated, chronic low somatic cell count associated, or teat skin associated. The comparison will be done using bacterial whole genome sequencing, MLST strain typing, and MALDI-TOF fingerprinting.

Researchers at MN (Godden, S., S. Wells, E. Royster, B. Crooker) will continue to investigate how environmental management, including bedding management, can be improved to reduce exposure to environmental mastitis pathogens, resulting in enhanced udder health and milk quality.

University of Tennessee will identify virulence factors and pathogenesis mechanisms of*Mycoplasma bovis* mastitis in dairy cattle and evaluate the molecular epidemiology of extended-spectrum β-lactamase producing *E. coli* in eastern Tennessee dairy farms.

The Barlow lab at Vermont will collaborate with Dr. Pamela Adkins at the University of Missouri to explore the epidemiology and pathogenesis of *Staphylococcus chromogenes* intramammary infections. The Barlow lab will complete whole genome sequencing of Staphylococcus species isolates to identify potential virulence factors and antimicrobial resistance genes, and explore the phylogeny of staphylococcus species and mobile genetic elements among these species. The Barlow lab will continue to explore the epidemiology of non-aureus staphylococci in small to medium sized dairy farms.

Objective 3: Assess and apply new technologies and preventative strategies that advance mastitis control, milk quality and/or reduce antimicrobial usage. (MN, OR, MO, TN, UT, VT, Canada, Australia, Europe).

The University of Missouri will be working to determine when heifer IMIs occur to better focus implementation of prevention strategies. This will be done using FNA/cisternal sampling of heifers and gland secretions to determine if results differ between these two collection methods. Additionally, we will be determining if phenotypic behaviors of *S. chromogenes* IMIs are reproducible, using an in vivo challenge model. Heifers will be challenged with *S. chromogenes* and then *S. aureus* to determine if *S. chromogenes* IMIs or teat colonization will result in fewer S. aureus infections.

Scientists at University of Minnesota and Cornell University (Godden, S. and E. Royster, Nydam) will continue to study if we can refine or improve approaches to selective dry cow therapy (SDCT), encourage their adoption on commercial dairies (outreach/extension), and monitor the impacts of adopting SDCT programs on commercial dairies.

Future works at the University of Tennessee will focus on the evaluation of efficacy of staphylococcal and streptococcal surface proteins vaccine to control mastitis in dairy cows and assess the immunogenicity of an enterobactin conjugate vaccine for the control of *E. coli* mastitis in dairy cows.

Works at Oregon State University will continue to investigate novel secondary compounds that may be fed and administered to lactating ruminants to improve immune function, preventing and reducing mastitis incidence.

In Vermont, members of the Zhao lab will target specific cytokines to control mammary inflammation. The Barlow lab will continue to explore the relationship between housing and bedding management practices and mastitis risk and continue to explore the potential role of endogenous inhibitor bacteria and bacteriocins in the epidemiology of Staphylococcus mastitis. We will use in vitro co-culture systems and metagenomic methods to describe bacterial factors influencing colonization and infection of mammary glands.

Measurement of Progress and Results

Outputs

- Membership in NE-1748 has allowed researchers to 1) build a network of collaborators, 2) receive meaningful feedback on project design and execution, 3) provide formal means of idea exchange and collaboration; 4) expand ideas beyond station researchers, 5) opened opportunities for collaborative funding, 6) allowed access to resources such as mastitis bacterial isolates from across a broad geographic distribution, 7) provided a forum for trainees to meet seasoned investigators and fellow trainees to discuss and present their work and receive constructive feedback. Our efforts have resulted in presentations at national and local meetings, non-peer reviewed publications, Extension publications and meeting presentations, bacterial isolate collections with epidemiological data, and joint projects/collaborations.

Outcomes or Projected Impacts

- Curate and summarized the risks and benefits of antibiotic use in lactating dairy animals. Developed and evaluated experimental intramammary treatments, both conventional and organic. Examined alternative therapeutics for the prevention or treatment of mastitis to reduce antibiotic usage. Identified candidate genes relating to mastitis susceptibility and resistance. Described host cytokine and other genetic predictors for mastitis susceptibility, milk production, reproductive performance, and survival. On-farm culture and other methods of reducing antibiotic use on dairies evaluated. Effectiveness of teat dips, teat sealants, bedding types and treatments and dry cow treatments in deterring mastitis evaluated. Identified virulence factors of mastitis pathogens. New diagnostic tests for select organisms, including high-risk human pathogens and non-bacterial causes of mastitis, developed. Developed multiple decision support tools aimed at improving milk quality, reducing mastitis and economics. Nutritional effects, including enhancing the host immune system, on mastitis evaluated. Strategies developed to improve immune responses during the dry and transition period, using molecular analyses of host responses. Use positional/behavioral patterns to predict intramammary infections in dairy cattle. Describe the molecular epidemiology of mastitis pathogens. Describe selected mastitis pathogen’s gene distribution and genetic diversity in milk. Develop potential mastitis vaccine candidates.

Milestones

(**2022**):Submission for publication of findings in studies of the immune and metabolic dynamics and their relationship to host and/or pathogenic response.

(**2023**):Submission for publication of findings in studies regarding improving animal welfare.

(**2024**):Submission for publication of findings in studies regarding new technologies to advance mastitis control, milk quality and/or dairy food safety.

(**2025**):Submission for publication of findings in studies regarding antibiotic use and alternative therapeutics for mastitis control.

(**2026**):Completion and submission for publication of the remaining studies on focused research objectives, as well as summaries of surveillance studies.

Outreach Plan

Multiple centers have described outreach projects in their plans for the upcoming period. A number of stations are involved in Extension-based proposals to improve milk quality. These projects, and thus the MMRP, will be characterized by their emphasis on producer communications, and on including experts in communication, sociology, economics, dairy management, mastitis, and milk quality programs. The MMRP has, and continues to, involve numerous Cooperative Extension members. This factor, and the willingness of the dairy industry to seek new tools for improvement, will enhance the current and future effectiveness of the MMRP. In the words of Dr. William Owens of Louisiana, "A major impact of the multistate projects is the credence or impact that a prestigious organization gives to data it generates. The reputation and long history of this project and the many years of scientific expertise that it represents greatly increased the weight of its recommendations. Many of the scientists participating in this project have been continuously involved with this project since the 1980s. This long history allows a continuity of purpose that provides valuable leadership and helps maintain the focus of the group. This in turn makes the outputs of the projects more focused and more valuable."

Organization/Governance

Regional System Administrator: David Leibovitz, david_leibovitz@uri.edu

Northeastern Regional Association of State Agricultural Experiment Station Directors- NERA

14 East Farm Road, Room 111

University of Rhode Island, Kingston, RI, 02881

Phone: 401-874-4801

Mastitis Research Workers Conference Coordinator: Pamela Adkins and John Middletonadkinsp@missouri.edu

Middletonjr@missouri.edu

University of Missouri

College of Veterinary Medicine

900 E Campus Drive

Columbia MO 65211

Phone: 573-882-6857

The organization of this project will be in accordance with that set forth in the Manual for Cooperative Multistate Research. A technical committee that includes the project participants from each of the participating stations will administer the project. An executive committee will consist of the Chairman, Vice-Chairman, Secretary, and the Administrative Advisor. The officers will serve one year after which the Vice-Chairman automatically becomes Chairman and the Secretary becomes Vice-Chairman. This executive committee will conduct business between meetings. An annual meeting will be called by the Administrative Advisor and will be held in conjunction with the Mastitis Research Worker's Conference. At these meetings, research accomplishments will be reviewed, updates and summaries of joint projects will be presented, and new projects will be planned and a project coordinator selected. The Vice-Chairman will call for annual reports of research data from each station. These reports will be compiled and sent to each participant prior to the annual meeting. The responsibility for multi-State summaries and publications will be assigned at the meetings.

If no annual report is submitted for two consecutive years from a participating station, or if no one from a station is present at the MMRP meeting for two years out of five, then this station will be eliminated from the project. The Administrative Advisor will contact the member station before the member is formally eliminated from the roster to ensure that extenuating circumstances do not exist. International members will be expected to submit an annual report and to have representation at annual meetings, as per stations in the United States. However, these participants will not be required to submit Appendix E forms detailing formal FTE commitments.

Minute/Annual Report Filing within 60 days of Annual Business Meeting

1. File minutes onto NIMSS Site – Secretary –
2. Obtain completed worksheets from all stations and compile into annual report – NIMSS (Co-Chair/Chair)

    1. The annual report is due within 60 days of the business meeting. You will use the annual reports (worksheets) received from the various participants to file the annual report in NIMSS. Circulate a draft of the annual report via e-mail list for comments then submit final copy through NIMSS.

        1. On the NIMSS website, log in, go to ‘projects; then ‘my active projects’, click on ‘NE2248’ and go to the reports/meetings tab to the left then click on ‘reports’ and ‘draft/edit report’. Click on new report. On the left of the screen you will see a menu – fill in each of the tabs. For example, “Participants” – you will get a box to fill in each person who attended the business meeting. You can attach the minutes as a WORD file. Send the minutes out for review on the listserv before submitting in NIMSS. The accomplishments section will be filed using the annual report summaries boiled down to one document. The impact statements can be taken from the project description on the project website.
        2. The past annual reports are available in NIMSS and can be found by using the reports/meetings tab and then search for report.

Secretary	Vice-Chair	Chair	Past-Chair
Presides over MRW meeting **Record number of attendees**	**Collect worksheet from each station prior to annual meeting (set deadline at least 2 wks prior to meeting date)**	Main contact for Administrative Advisor	**Support Exec Board as needed.**
Takes minutes/attendance at business meeting	Annual reports: compile worksheet in annual report for distribution prior to annual business meeting and comment collection at meeting.	Monitor/initiate collaborative efforts among Multi-state regional members	Use excel spreadsheet and minutes to track station attendance. Removal of non-active stations (via David Leibovitz) according to bylaws.
Send minutes out to group for review	File annual report into NIMSS site within 60 day of annual meeting (as Chair).	Prepares agenda and runs yearly business meeting	
File minutes onto NIMSS site		Complete annual report (started as vice-Chair). Oversee/lead report completion: Rewrite (2021/22 request), Midterm (2019/2020), Termination (2021/22).	
		2-3 months prior to scheduled business meeting request that the Administrative Advisor obtain meeting authorization through NIMSS	

Land Grant Participating States/Institutions

ID, LA, MD, ME, MI, MN, MO, MS, NJ, NY, OH, OR, PA, TN, UT

Non-Land Grant Participating States/Institutions

University of Sydney, University of Montreal, University of Saskatchewan, Ghent University

Literature Cited

Peer-Reviewed Literature

Michigan State University

Fuenzalida, M.J. and P.L. Ruegg. 2020. Molecular epidemiology of non-severe clinical mastitis caused by*Klebsiella pneumoniae* occurring in cows on 2 Wisconsin dairy farms. J. Dairy Sci. 103:3779-3792. https://doi.org/10.3168/jds.2019-17464

Leite de Campos, J., A. Kates, A. Steinberger, A. Sethi, G. Suen, J. Shutske, N. Safdar, T. Goldberg, and P.L. Ruegg. 2021. Quantification of antimicrobial usage in adult cows and preweaned calves on 40 large Wisconsin dairy farms using dose-based and mass-based metrics. Accepted J Dairy Sci, Nov 2020

Ohio State University

Enger, B.D., S.N. Lehner, C.L.M. Parsons, R.M. Akers, and N.R. Hardy. 2020. Short Communication: Effect of intramammary infection and parenchymal region on collagen abundance in non-lactating bovine mammary glands. Appl. Anim. Sci. 36:688-693. doi:10.15232/aas.2020-02003.

Enger, B.D., S.C. Nickerson, R.M. Akers, L.E. Moraes, and C.E. Crutchfield. 2020. Use of commercial somatic cell counters to quantify somatic cells in non-lactating bovine mammary gland secretions. Vet. Prev. Med. 174:104775. doi:10.1016/j.prevetmed.2019.104775.

Rutgers University

Ferrer-Gonzalez E., Fujita J., Yoshizawa T., Nelson J., Pilch A., Hillman E., Ozawa M., Kuroda N., Al-Tameemi H., Boyd J.M., LaVoie E., Matsumura H., and Pilch D. Structure-Guided Design of a Fluorescent Probe for the Visualization of FtsZ in Clinically Important Gram-Positive and Gram-Negative Bacterial Pathogens. Nature Scientific Reports, 2019 PMID: 31882782

Norambuena J., Miller M., Boyd J.M.*, Barkay T.*, Expression and regulation of the mer operon in Thermus thermophilus. Environmental Microbiology, 2020 PMID: 32090420

Rudra, P., Boyd J.M. Metabolic control of virulence factor production in*Staphylococcus aureus*. Current Opinion in Microbiology, 2020 PMID: 32388086.

Tiwari N., López-Redondo M., Miguel-Romero L., Kulhankova K., Cahill M.P., Al-Tameemi H., Herfst C.A., Kirby J.R., Boyd J.M., McCormick J.K., Salgado-Pabón W., Marina A., Schlievert P.M., Fuentes E.J., The SrrAB two-component system regulates *Staphylococcus aureus* pathogenicity through redox sensitive cysteines. Proceedings of the National Academy of Sciences, 2020 PMID: 32354997.

Price E.E., Boyd J.M., Genetic control of metal ion homeostasis in*Staphylococcus aureus*. Trends in Microbiology, 2020 PMID: 32381454.

Dubovoy V., Nawrocki S., Verma G., Wojtas L., Desi P., Al-Tameemi H., Brinzari T.V., Stranick M., Chen D., Xu S., Ma S., Boyd J.M., Asefa T., Pan L., Synthesis, characterization, and investigation of the antimicrobial activity of cetylpyridinium tetrachlorozincate. ACS Omega, 2020 PMID: 32426592.

Dubovoy V., Desai P., Hao Z., Cheng C., Verma G., Wojtas L., Brinzari T.V., Boyd J.M., Ma S., Asefa T., Pan L., Synthesis, Characterization, and Antimicrobial Investigation of a Novel Chlorhexidine Cyclamate Complex. ACS Crystal Design and Growth, 2020 doi.10.1021/acs.cgd.0c00107

Carabetta, V.J., Esquilin-Lebron K., Zelzion E., Boyd J.M., Genetic approaches to uncover gene products involved in iron-sulfur protein maturation: High throughput genomic screening using transposon-sequencing. Methods in Molecular Biology, 2020 accepted.

Al-Tameemi H., Beavers W.N., Norambuena-Morales J., Skaar E., Boyd J.M. *Staphylococcus aureus* lacking a functional MntABC manganese import system have increased resistance to copper. Molecular Microbiology. 2020 PMID: 33034093.

Juttukonda L.J., Beavers W.N., Horning K.J., Unsihuay D., Horvath D.J., Kim K., Weiss A., Pishchany G., Al-Tameemi H., Boyd J.M., Sulikowski G., Bowman E.B., and Skaar E.P. A small molecule modulator of metal homeostasis is toxic to Gram-positive pathogens. mBio. 2020 PMID: 33109764.

University of Minnesota

Rowe, S. , S. Godden, D. Nydam, P. Gorden, A. Lago, A. Vasquez, E. Royster, J. Timmerman, M. Thomas. 2020. Randomized controlled non-inferiority trial investigating the effect of two selective dry cow therapy protocols on antibiotic use at dry-off and dry period intramammary infection dynamics. J. Dairy Sci. Vol. 103:6473-6492

Rowe, S. , S. Godden, D. Nydam, P. Gorden, A. Lago, A. Vasquez, E. Royster, J. Timmerman, M. Thomas. 2020. Randomized controlled trial investigating the effect of two selective dry cow therapy protocols on udder health and performance in the subsequent lactation. J. Dairy Sci. Vol. 103:6493-6503

Rowe, S. , S. Godden, D. Nydam, A. Lago, A. Vasquez, E. Royster, J. Timmerman. 2020. Randomized Equivalence Study Comparing the Efficacy of Two Commercial Internal Teat Sealants in Dairy Cows. J. Dairy Sci. 103:5398-5413

Rowe, S. , S. Godden, D. Nydam, P. Gorden, A. Lago, A. Vasquez, E. Royster, J. Timmerman, M. Thomas. 2020. Evaluation of rapid culture, a predictive algorithm, esterase somatic cell count and lactate dehydrogenase to detect intramammary infection in quarters of dairy cows at dry-off. Prev. Vet. Med. 179:1-11

Rowe, S., S. Godden, E. Royster, J. Timmerman, B. Crooker and M. Boyle. 2019. Cross-sectional study of the relationship between bedding materials, bedding bacteria counts and intramammary infection in late lactation dairy cows. J. Dairy Sci. 102:11384–11400

Rowe, S., S. Godden, E. Royster, J. Timmerman, B. Crooker and M. Boyle. 2019. Cross-sectional study of the relationship between cloth udder towel management, towel bacteria counts and intramammary infection in late lactation dairy cows. J. Dairy Sci. 102:11401–11413

Dean, C.J., Slizovskiy, I.B., Crone, K.K., Pfennig, A.X., Heins, B.J., Caixeta, L.S., and Noyes, N.R. 2020. Investigating the skin and teat canal microbiomes of the bovine udder using differing sampling and sequencing approaches. J Dairy Sci. 2020 Oct 29;S0022-0302(20)30860-2. doi: 10.3168/jds.2020-18277.

University of Missouri

Bernier Gosselin V, Dufour S, Middleton JR. 2020. Association between species-specific staphylococcal intramammary infection and milk somatic cell score over time in dairy goats. Prev Vet Med. Jan 174:104815

Adkins PRF, Ericsson A, Middleton JR, Witzke M. 2020. The effect of intramammary pirlimycin hydrochloride on the fecal microbiome of early lactation heifers. J Dairy Sci. 103(4):3459-3469

Wattenburger K, Schmidt R, Placheta L, Middleton JR, Adkins PRF. 2020. Evaluation of four different teat disinfection methods prior to collection of milk samples for bacterial culture in dairy cattle. J Dairy Sci. 103(5):4579-4587

Shwani A, Adkins PRF, Ekesi NS, Alrubaye A, Calcutt MJ, Middleton JR, Rhoads DD. 2020. Whole genome comparisons of *Staphylococcus agnetis* isolates from cattle and chickens. Appl Environ Microbiol. Jun 2;86(12):e00484-20

Witzke MC, Gullic A, Yang P, Bivens NJ, Adkins PRF, Ericsson AC. Influence of PCR cycle number on 16S rRNA gene amplicon sequencing of low biomass samples. J Microbiol Methods. Sep 176: 106033.

University of Vermont

Alexander Jonathan Spitzer, Qing Tian, Ratan Choudhary, and Feng-Qi Zhao. (2020). Bacterial endotoxin induces oxidative stress and reduces milk protein expression and hypoxia in the mammary gland. Oxidative Medicine and Cellular Longevity, Volume 2020, Article ID 3894309, 16 pages. DOI: 10.1155/2020/3894309

Oregon State University

Tsai, C., Rosa, F., Bionaz, M., & Rezamand, P. (2020). Effects of 2,4-thiazolidinedione (TZD) on milk fatty acid profile and serum vitamins in dairy goats challenged with intramammary infusion of *Streptococcus uberis*. Journal of Dairy Research, 1-8

Jaaf, S.; Batty, B., Krueger A., Estill, C.T.; Bionaz, M. (2020). Selenium biofortified alfalfa hay fed in low quantities improves selenium status and glutathione peroxidase activity in transition dairy cows and their calves. Journal of Dairy Research, May;87(2):184-190

Cornell University

Monistero V, Barberio A, Biscarini F, Cremonesi P, Castiglioni B, Graber HU, Bottini E, Ceballos-Marquez A, Kroemker V, Petzer IM, Pollera C, Santisteban C, Veiga Dos Santos M, Bronzo V, Piccinini R, Re G, Cocchi M, Moroni P. Different distribution of antimicrobial resistance genes and virulence profiles of *Staphylococcus aureus* strains isolated from clinical mastitis in six countries. J Dairy Sci. 2020 Apr;103(4):3431-3446. doi: 10.3168/jds.2019-17141. Epub 2020 Jan 31. PMID: 32008788.

Bronzo V, Lopreiato V, Riva F, Amadori M, Curone G, Addis MF, Cremonesi P, Moroni P, Trevisi E, Castiglioni B. The Role of Innate Immune Response and Microbiome in Resilience of Dairy Cattle to Disease: The Mastitis Model. Animals (Basel). 2020 Aug 11;10(8):1397. doi: 10.3390/ani10081397. PMID: 32796642; PMCID: PMC7459693.

Hoekstra J, Zomer AL, Rutten VPMG, Benedictus L, Stegeman A, Spaninks MP, Bennedsgaard TW, Biggs A, De Vliegher S, Mateo DH, Huber-Schlenstedt R, Katholm J, Kovács P, Krömker V, Lequeux G, Moroni P, Pinho L, Smulski S, Supré K, Swinkels JM, Holmes MA, Lam TJGM, Koop G. Genomic analysis of European bovine *Staphylococcus aureus* from clinical versus subclinical mastitis. Sci Rep. 2020 Oct 23;10(1):18172. doi: 10.1038/s41598-020-75179-2. PMID: 33097797; PMCID: PMC7584570.

Wemette M, Safi AG, Beauvais W, Ceres K, Shapiro M, Moroni P, Welcome FL, Ivanek R. New York State dairy farmers' perceptions of antibiotic use and resistance: A qualitative interview study. PLoS One. 2020 May 27;15(5):e0232937. doi: 10.1371/journal.pone.0232937. PMID: 32459799; PMCID: PMC7252592.

Kumar R, Register K, Christopher-Hennings J, Moroni P, Gioia G, Garcia- Fernandez N, Nelson J, Jelinski MD, Lysnyansky I, Bayles D, Alt D, Scaria J. Population Genomic Analysis of Mycoplasma bovis Elucidates Geographical Variations and Genes associated with Host-Types. Microorganisms. 2020 Oct 10;8(10):1561. doi: 10.3390/microorganisms8101561. PMID: 33050495; PMCID: PMC7650767.

Calonzi D, Romano A, Monistero V, Moroni P, Luini MV, Biscarini F, Castiglioni B, Cremonesi P. Technical note: Development of multiplex PCR assays for the molecular characterization of *Streptococcus uberis* strains isolated from bovine mastitis. J Dairy Sci. 2020 Jan;103(1):915-921. doi: 10.3168/jds.2019-16823. Epub 2019 Nov 6. PMID: 31704014.

Scillieri Smith JC, Moroni P, Santisteban CG, Rauch BJ, Ospina PA, Nydam DV. Distribution of*Lactococcus* spp. in New York State dairy farms and the association of somatic cell count resolution and bacteriological cure in clinical mastitis samples. J Dairy Sci. 2020 Feb;103(2):1785-1794. doi: 10.3168/jds.2018-16199. Epub 2019 Dec 19. PMID: 31864733.

Biscarini F, Cremonesi P, Castiglioni B, Stella A, Bronzo V, Locatelli C, Moroni P. A Randomized Controlled Trial of Teat-Sealant and Antibiotic Dry-Cow Treatments for Mastitis Prevention Shows Similar Effect on the Healthy Milk Microbiome. Front Vet Sci. 2020 Sep 2;7:581. doi: 10.3389/fvets.2020.00581. PMID: 32984415; PMCID: PMC7492605.

Mann S, Curone G, Chandler TL, Moroni P, Cha J, Bhawal R, Zhang S. Heat treatment of bovine colostrum: I. Effects on bacterial and somatic cell counts, immunoglobulin, insulin, and IGF-I concentrations, as well as the colostrum proteome. J Dairy Sci. 2020 Oct;103(10):9368-9383. doi: 10.3168/jds.2020-18618. Epub 2020 Aug 20. PMID: 32828510.

Wemette M, Greiner Safi A, Wolverton AK, Beauvais W, Shapiro M, Moroni P, Welcome FL, Ivanek R. Public perceptions of antibiotic use on dairy farms in the United States. J Dairy Sci. 2021 Jan 14:S0022-0302(21)00003-5. doi: 10.3168/jds.2019-17673. Epub ahead of print. PMID: 33455793.

University of Idaho

Tsai, C. Y. R. Hassan, H. C. Hung, T. Weber, W. J. Price, P. Rezamand*, and Q. Huo. 2020. A Rapid Blood Test to Monitor the Immune Status Change of Dairy Cows and to Evaluate their Disease Risk during the Periparturient Period. Sensors International. https://doi.org/10.1016/j.sintl.2020.100078

University of Tennessee

Amenu K, Agga GE, Kumbe A, Shibiru A, Desta H, Tiki W, Kerro Dego O, Wieland B, Grace D, S. A. 2020. Community-tailored training to improve the knowledge, attitudes, and practices of women regarding hygienic milk production and handling in the Borana pastoral area of southern Ethiopia. Journal of Dairy Science 103:9748 – 9757. DOI:https://doi.org/10.3168/jds.2020-18292.

Kerro Dego O. Bovine mastitis part I, IntechOpen, DOI: 10.5772/intechopen.93483, Published online (September 2, 2020), Available from, https://www.intechopen.com/online-first/bovine-mastitis-part-i.

Kerro Dego O. Control and Prevention of Mastitis: Part Two. IntechOpen, DOI: 10.5772/intechopen.93484, Published online (August 27, 2020). Available from, https://www.intechopen.com/online-first/control-and-prevention-of-mastitis-part-two.

Kerro Dego O. Current Status of Antimicrobial Resistance and Prospect for New Vaccines against Major Bacterial Bovine Mastitis Pathogens.IntechOpen, *DOI: http://dx.doi.org/10.5772/intechopen.94227*, Published online (October 19, 2020), Available from: https://www.intechopen.com/online-first/current-status-of-antimicrobial-resistance-and-prospect-for-new-vaccines-against-major-bacterial-bov

Kerro Dego O, Pacha PA, Gillespie BE, Pighetti GM. Experimental*Staphylococcus aureus* Mastitis Infection Model by Teat Dipping in Bacterial Culture Suspension in Dairy Cows. MDPI Animals 2020, 10(5), 751. https://doi.org/10.3390/ani10050751.

Lee, A, S Schexnayder, L Schneider, S Oliver, G Pighetti, C Petersson-Wolfe, J Bewley, S Ward, P Krawczel. 2019. Dairy producers in the Southeast United States are concerned with cow care and welfare. J Dairy Res. 1-4. DOI: https://doi.org/10.1017/S0022029919000943.

Vaughn JM, Abdi RD, Gillespie BE, Kerro Dego O. 2020. Genetic diversity and virulence characteristics of*Staphylococcus aureus* isolates from cases of bovine mastitis. Microb Pathog 144:104171. https://doi.org/10.1016/j.micpath.2020.104171.

Abstracts

Oregon State University

Busato S. and M Bionaz. 2020. Selenium-biofortified alfalfa hay supplementation modulates liver and macrophage gene expression in periparturient dairy cows. J. Dairy Sci. 103 (Suppl. 1):124

Ohio State University

Enger, K.M., N.R. Hardy, and B.D. Enger. 2020. Effect of intramammary infection on colostrum antibody concentrations. Pages 209-210 in Natl. Mastitis Counc. Annu. Mtg. Proc., Orlando, FL, Natl. Mastitis Council Inc., New Prague, MN.

Hardy, N.R., K.M. Enger, and B.D. Enger. 2020. Quantifying mammary growth and proliferative effects of estradiol in Holstein heifer calves. J. Dairy Sci. 102 (Suppl. 2):XXX. Accepted.

Hardy, N.R., K.M. Enger, and B.D. Enger. 2020. Mammary blood vessel development in response to estradiol administration in heifer calves. J. Dairy Sci. 103. (Suppl. 1):168.

University of Minnesota

Lippolis, J. D., E. J. Putz, T. A. Reinhardt, E. Casas, W. J. Weber, B. A. Crooker. 2020. The effect of 50 years of breeding on the ability of Holsteins to fight mastitis. International Milk Genomics Consortium. 17th International Symposium (Virtual). October 13-16. Hosted by UC Davis.

Godden, S., F. Peña Mosca, E. Royster, B. Crooker, J. Hadrich, P. Raynor, R. Singer, and K. Janni. Investigation of the Relationship between Method of Processing Recycled Manure Solids Bedding and Udder Health and Production on Midwest Dairy Farms. Proc. Annu. Mtg. Am Assoc. Bovine Pract. Sept. 24-26, 2020. (virtual).

Godden, S., F. Peña Mosca, E. Royster, B. Crooker, J. Hadrich, P. Raynor, R. Singer, and K. Janni. Investigation of the Relationship between Method of Processing and Bacteria Counts in Ready-to-Use Recycled Manure Solids Bedding on Midwest Dairy Farms Proc. Annu. Mtg. Am Assoc. Bovine Pract. Sept. 24-26, 2020. (virtual).

Godden, S., E. Royster, J. Timmerman and N. O'Sell. Efficacy of a Stabilized Liquid Potassium Sorbate Preservative to Reduce Bacterial Proliferation in Colostrum or Tank Milk Stored by Refrigeration or at Room Temperature. Proc. Annu. Mtg. Am Assoc. Bovine Pract. Sept. 24-26, 2020. (virtual).

Rowe, S.M*., Nydam, D.V., Godden, S.M., Gorden, P., Lago, A., Royster, E., Vasquez, A., Thomas, M. 2020. Partial budget analysis of selective dry cow therapy strategies. American Dairy Science Assoc. Annual Meeting, USA. June 22-24, 2020 (virtual)

Rowe, S.M.*, Godden, S.M., Nydam, D.V., Lago, A., Thomas, M., Royster, E., Vasquez, A., Timmerman. J., Thomas. M.. 2020. Randomized controlled trial investigating the effect of two selective dry cow therapy protocols on antibiotic use and udder health, Proc. 59th Annual meeting of the National Mastitis Council. Jan 28-30, 2020. Orlando, FL.

Rowe, S.M.*, Godden, S.M., Nydam, D.V., Lago, A., Royster, E., Vasquez, A., 2020. Randomized controlled trial evaluating the efficacy of two commercial internal teat sealants in dairy cows. Proc. 59th Annual meeting of the National Mastitis Council. Jan. 28-30, 2020. Orlando, FL

Rowe, S.*, S. Godden, E. Royster, J. Timmerman, D.V. Nydam, A.K. Vasquez, P. Gorden, A. Lago, and M. Thomas. Selective Dry Cow Therapy on US Dairy Farms: Impact on Udder Health and Productivity. 2019 Conference of Research Workers in Animal Diseases. Nov. 2-5, 2019. Chicago, IL.

Rowe, S.*, S. Godden, E. Royster, J. Timmerman, D.V. Nydam, A.K. Vasquez, A. Lago. 2019. Randomized controlled trial evaluating the efficacy of two commercial internal teat sealants in dairy cows. 2019 Conference of Research Workers in Animal Diseases. Nov. 2-5, 2019. Chicago, IL

Dean, C., Fernandes, L., Sharpe, K., Junior, A., Ray, T., Baumann, C., Wehri, T., Heins, B., Pinedo, P., Machado, V., Caixeta, L.S., Noyes, N. “Community research and education program to use the microbiome for the advancement of organic livestock production”. Proceedings of the 100th Annual Conference of Research Workers in Animals Diseases, Chicago, IL, 2019.

Baumann, C., Dean, C., Fernandes, L., Sharpe, K., Antunes Junior, A. M., Ray, T., Wehri, T., Heins, B., Pinedo, P., Formiga, A., Machado, V., Caixeta, L.S., Noyes, N. “Incidence rates of mastitis in heifers on organic dairy farms’. Proceedings of the 100th Annual Conference of Research Workers in Animals Diseases, Abstract 041. Poster presentation. Chicago, IL, 2019.

Fernandes, L., Guimaraes, I., Noyes, N.R., Caixeta, L.S., Machado, V.S. “Impact of subclinical mastitis during the first month of lactation on milk yield, fertility, and culling of dairy cows on USDA-certified organic herds”. ADSA Conference. Virtual Conference, 2020.

University of Missouri

Kurban D, Bolduc E, Roy JP, DeVries T, Gervais R, Adkins P, Middleton JR, Keefe G, France A, Dufour S. 2020. An observational transversal study on the prevalence of staphylococcal intramammary infections after calving in primirparous vs multiparous cows milked using an automatic milking system. Journée de la recherche FMV - Research day at FVM. University of Montreal, Quebec, Canada. 19 March 2020.

University of Vermont

F.-Q. Zhao, T.B. McFadden, R.K. Choudhary, E.M. Shangraw, R.O. Rodrigues, A.J. Spitzer. Intramammary endotoxin challenge elicits time-dependent local and systemic effects on lactating bovine mammary glands. CRWAD 2019, Chicago. Oral

Choudhary RK, Spitzer A, McFadden TB, Shangraw EM, Rodrigues RO, Linder HF, Zhao F-Q (2019) Quantitative histological changes in lactating bovine mammary gland after endotoxin challenge. ADSA Annual Meeting 2019, Cincinnati, Ohio. Oral

Conference Proceedings

Michigan State University

Erskine, R.J. and A. J. Swan. The Veterinarian’s/Consultant’s Role in Milk Quality. Short course presented at the 60th Annual Meeting of the National Mastitis Council, Orlando, FL, January 30th, 2020.

Erskine, R.J. Three ways to lose money on a farm: A view from the udder. Seminar presented at the Western Canadian Dairy Seminar, Red Deer, Alberta, March 11th, 2020.

Erskine R.J. Dry cow management for udder health. Webinar presented for University of Wisconsin webinar series-Dry cow management for a successful lactation. June 11th, 2020.

Erskine R.J. and R.S. Thomson. Podcast presented for the A.A.B.P. Why udder prep matters. November 19th, 2020.

Ruegg, P.L. Understanding the economic impact of mastitis. The role of duration and drug selection. 2020. Pp 84-91 in Proc. 3rd Am. Assoc. Bov. Pract. Recent Graduate Conf. Columbus OH, Feb 21-22, 2020. Available online: www.aabp.org

University of Minnesota

Bedding management and udder health – Getting better results from recycled manure solids and sand bedding. Godden, S., F. Pena Mosca, E. Royster, J. Timmeran, B. Crooker. 2020. Proc. Annu. Mtg. MN Dairy Health Conference. Aug. 6, 2020. (virtual).

Using Rapid Culture Systems to Guide Selective Treatment of Clinical Mastitis and at Dry-off. S. Godden, E. Royster and J. Timmerman. 3 hrs Short Course #1 at 59th Annu Meeting of the National Mastitis Council. Jan 28-30, 2020. Orlando, FL.

University of Missouri

Kurban D, Bolduc E, Roy JP, DeVries T, Gervais R, Adkins P, Middleton JR, Keefe G, France A, Dufour S. 2020. An observational transversal study on the prevalence of staphylococcal intramammary infections after calving in primirparous vs multiparous cows milked using an automatic milking system. Proceedings of the 59th Annual Meeting of the National Mastitis Council, Orlando, FL.

Ankney SR, Rivero LA, Witzke MC, Naclerio A5, Adkins PRF, Middleton JR. 2020. Comparison of two techniques for collecting milk samples to diagnose intramammary infection in dairy cattle. Proceedings of the 59th Annual Meeting of the National Mastitis Council, Orlando, FL

Witzke MC, Rodrigues RO, Shangraw EM, Ericsson AC, McFadden TB, Adkins PRF. 2020 Feed restriction and heat stress alters the fecal microbiome. Proceedings of the 59th Annual Meeting of the National Mastitis Council, Orlando, FL

University of Vermont

F.-Q. Zhao, T.B. McFadden, R.K. Choudhary, E.M. Shangraw, R.O. Rodrigues, A.J. Spitzer. Intramammary endotoxin challenge elicits time-dependent local and systemic effects on lactating bovine mammary glands. CRWAD 2019, Chicago. Oral

Choudhary RK, Spitzer A, McFadden TB, Shangraw EM, Rodrigues RO, Linder HF, Zhao F-Q (2019) Quantitative histological changes in lactating bovine mammary gland after endotoxin challenge. ADSA Annual Meeting 2019, Cincinnati, Ohio. Oral

Utah State University

Wilson DJ, Britten JE, Clancy CS, Rood KA: Bovine mammary gland involution histology and morphometry following casein hydrolysate and other dry treatments. Proc 63rd Ann Conf Am Assoc Vet Lab Diag: 2020.

Poster Presentations

Michigan State University

Leite de Campos, J., A. Steinberger, T. Goldberg, N. Safdar, A. Kates, J. Sutske, A. Sethi, G. Suen and P. Ruegg. 2020. Distribution of antimicrobial usage for mastitis using two metrics. Pp 223-224 in Proceedings 59th Ann. Meeting NMC. Jan 28-31, 2020. Available www.NMConline.org

Kolar, Q. K., S. M. Godden, and P.L. Ruegg. 2020. Duration of subclincial mastitis prior to occurrence of non-severe clinical mastitis – results of a preliminary analysis. Proceedings 59th Ann. Meeting NMC. Jan 28-31, 2020. Available www.NMConline.org

Rutgers University

Schaeffer L.M., Begum-Gafur R., Zaidel L., Crane S., Norabuena-Morales J., Boyd J.M., The effect of Zinc and tin of the growth and oxidative stress response of key oral bacteria. International Association for Dental Research Meeting. Washington D.C. 3/2020.

Norambuena J., Boyd J.M., Aeration Influences Copper Toxicity in*Staphylococcus aureus*. Rutgers Microbiology Symposium. New Brunswick, NJ. 2/2020.

Esquilin-Lebron K., Foley M., Carabetta V., Beavers W., Skaar E., Boyd J.M., Iron-Sulfur Protein Assembly in Gram Positive Bacteria. Rutgers Microbiology Symposium. New Brunswick, NJ. 2/2020.

Almeda-Ahmadi A., McGinley C.M., Boyd J.M., The Effects of Varying Concentrations of Cetylpyridinium Tetrachloride with Tin on the Growth of Common Oral Cavity Bacteria. Rutgers Microbiology Symposium. New Brunswick, NJ. 2/2020.

Norambuena J., Al-Tameemi H., Boyd J.M. *Staphylococcus aureus* lacking a functional MntABC manganese import system have increased resistance to copper. American Society National Meeting. Virtual 7/2020.

Price, E.E. Mashruwala A.A., Boyd J.M. Examining Activators of SaeRS in S. aureus Fermentative Biofilm Formation American Society National Meeting. Virtual 7/2020.

Norambuena J., Al-Tameemi H., Boyd J.M. *Staphylococcus aureus* lacking a functional MntABC manganese import system have increased resistance to copper. Boston Bacteriology Meeting. Virtual 7/2020.

Esquilín-Lebrón K., Foley M., Carabetta V., Beavers W., Skaar E.P., Boyd J.M. Investigating the role of YlaN in iron homeostasis in Staphylococcus aureus. Boston Bacteriology Meeting. Virtual 7/2020.

University of Minnesota

Rowe, S.M.*, Godden, S.M., Nydam, D.V., Lago, A., Royster, E., Vasquez, A. 2020. Randomized controlled trial evaluating the efficacy of two commercial internal teat sealants in dairy cows. Poster presentation. National Mastitis Council Annual Meeting. Jan. 28-30, 2020. Orlando, FL, USA

Rowe, S.M.*, S.M. Godden, E. Royster, J. Timmerman and M. Boyle. Cross-sectional study of the relationship between cloth udder towel management, towel bacteria counts and intramammary infection in late lactation dairy cows. Poster Presentation. 2019 Conference of Research Workers in Animal Diseases. Nov. 2-5, 2019. Chicago, IL.

Rowe, S.M.*, S.M. Godden, E. Royster, J. Timmerman and M. Boyle. Cross-sectional study of bedding bacteria counts and intramammary infection in late lactation dairy cows. Poster presentation. 2019 Conference of Research Workers in Animal Diseases. Nov. 2-5, 2019. Chicago, IL.

University of Vermont

Shangraw E, Rodrigues R, Witzke M, Linder HF, Choudhary R, Spitzer A, Zhao, F-Q, McFadden TB (2019) Intramammary lipopolysaccharide infusion elicits local or systemic effects depending on milk component. ADSA Annual Meeting 2019, Cincinnati, Ohio. Poster

Other Publications

Ohio State University

Enger, B.D. 2020. Blanket, selective, or no dry cow therapy: Which should I choose? OARDC Extension. Dairy in brief (DIB) # 22-20.

Enger, B.D. 2020. Therapy of Clinical Mastitis in Tough Economic Times. OARDC Extension. Dairy in brief (DIB) # 41-20.

University of Tennessee

Eckelkamp, E., K. Hash, and G. Pighetti. 2020. On-Farm Culture Setup. UT Extension Publication, W 946.

Hash, K., E. Eckelkamp, and G. Pighetti. 2020. Aseptic Milk Sampling Guide: Taking Clean Samples. UT Extension Publication, W 945.

Cox, M., E. Eckelkamp, G. Pighetti, P. Krawczel, and R. Trout Fryxell. 2019. Dairy parlor management: Fly control. UT Extension Publication, W 817.

Couture, V. G. Pighetti, P. Krawczel, and E. Eckelkamp. 2019. Managing mastitis in heifers and dry cows. UT Extension Publication, W 813.

Land Grant Participating States/Institutions

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
Grand Total:	**0**	**0**	**0**

Program/KA	Total FTE
Grand FTE Total:	**0**

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2248: Mastitis Resistance to Enhance Dairy Food Safety, Milk Quality, and Animal Welfare

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Good
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Good
5. Overall technical merit:
Excellent
Comments
NE_TEMP2248: Mastitis Resistance to Enhance Dairy Food Safety has existed since 1977. The multi-state project addresses mastitis, a complex disease in the dairy industry, which hampers animal health and hurts the economic success of the industry. The project addresses factors from the farm setting to mechanisms of immune response to genetics to therapeutics. This range of examination provides a tremendous breadth and integration of factors on risk on the disease. Approaches are varied and supported by previous work by members. Specifics are limited but based upon productivity of the past project will be acceptable by the scientific community. The majority of the work proposed though has little to do with food safety so a possible change in title should be considered.

The goals are the same as the previous project. Based upon the significant amount of literature published from the project, gains in knowledge and management practices toward a reduction in mastitis, improvement in animal health, and reduction in use of antibiotics is likely. An update to the objectives might be in order to focus advancement in fewer areas.

The previous project addressed the same set of objectives with activity toward each objective noted. Several members participate in activities toward each objective such that the proposed scope will be covered sufficiently.

For Objective 3, a study at University of Utah in dairy calves is noted. This does not fit with the project goals and objectives so should be dropped.

Members have generated a number of collaborative products in the past and are set to continue working together. The group engages well with outreach and extension to deliver information to producers for significant impact. The potential is great for outcomes which benefit the dairy industry.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2248: Mastitis Resistance to Enhance Dairy Food Safety, Milk Quality, and Animal Welfare

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Good
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Good
5. Overall technical merit:
Excellent
Comments
Mastitis Resistance to Enhance Dairy Food Safety is a long-term project that benefits the dairy industry as a whole. The project is a multi-state, multi-collaboration effort that holistically addresses mastitis and milk quality issues that plague the dairy industry worldwide. The breadth and variety of these collaborations is commendable. The proposal focuses on dairy herd health, milk production, and milk quality rather than dairy food safety. The University of Utah study in dairy calves is an odd fit. The study is interesting and worthy of support, considering the impact the early growth period has on future production. At a minimum, the effort could and should, tie in with impact on future milk production and milk quality. The proposal is lacking in detail, assumingly due to the scope and complexity of the project; continued success of the program is likely with renewed funding based on past performance.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2248: Mastitis Resistance to Enhance Dairy Food Safety, Milk Quality, and Animal Welfare

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Good
4. Potential for significant outputs(products) and outcomes and/or impacts:
Fair
5. Overall technical merit:
Excellent
Comments
Your Recommendation:

The work of NE-1748 Project brings together a productive group of collaborators. Moreover, single site studies are often limited in terms of expertise and cattle numbers. This multi-state project provides advantages in terms of enrollment of increased numbers of herds and cattle as experimental units. Additionally, research endeavors are amplified by taking advantage of multiple levels of expertise and contributions from a wide variety of researchers that are not present at single institutions.

2. Achievable goals/objectives:
The Project has 3 major goals:
1) Characterize host mechanisms associated with mastitis progression, susceptibility, and resistance to improve economic outcomes and animal welfare.
2) Characterize agents associated with intramammary infections and assess their impact on milk quality and animal welfare.
3) Assess and apply new technologies and preventative strategies that advance mastitis control, milk quality and/or reduce antimicrobial usage.
These are all achievable goals in as much as they are ongoing and long term. The field of bovine immunology is advancing in parallel with advances in mammalian immunology. The institutions and research groups involved in achieving this objective and the degree of their collaboration suggests that they should make good strides in fulfilling Objective 1 needs. Mastitis pathogens are constantly evolving and there are some new and emerging pathogens. Two prime examples are the development of antimicrobial resistance by pathogens and the emergence of Mycoplasma bovis mastitis in areas that were deemed to be free of this disease. It should be noted that several collaborative efforts are directed at the latter example and the former is a major thrust in research effort at the University of Tennessee. Thus, good progress should be achieved under Objective 2. The last objective, 3, targets the development of new strategies that will be designed to prevent the disease and reduce antibiotic use. Most of the research in this section has targeted reduced antimicrobial use. Some research groups are looking for alternative, non-antibiotic strategies to treat mastitis. Whereas such endeavors are always warranted, history has suggested that alternative to antibiotic compound treatments have not been very fruitful. Casein hydrolysate has been studied extensively by an Israeli group 10-20 years ago and the results of their efforts has not encouraged the development of a meaningful test treatment product. Characterization of

antimicrobial resistance of streptococcal organisms at Michigan State and S. aureus in Vermont could lead to future studies that decrease routine antibiotic use. The study by the Minnesota team to examine efficacy of selective dry cow therapy clearly has potential to reduce antibiotic use on a dairy. The Minnesota group is also studying the milking machine function, vacuum analysis, presumably in an effort to reduce the impact of the milking units' function as a risk factor for mastitis. This appears to be the only effort to develop on farm strategies to prevent mastitis. Thus, the aggregate efforts by the research teams working to accomplish progress to the goal of on farm mastitis prevention is limited.

3. Appropriate scope of activity to accomplish objectives:

The Project constitutes as diverse group of research interests with those of expertise in many fields of study closely related, and ancillary, to the mastitis syndrome. Thus, the Project has a wide scope of endeavors. Historically the Project participants have interacted very well and this proposed new project, or update, demonstrates that history will go forward. In short, the scope of activity is narrow in terms of the individual participating projects but broad enough in aggregate to make significant progress to the stated goals and objectives.

4. Potential for significant outputs(products) and outcomes and/or impacts:

Some research efforts have good potential for having near-term and long-term impact on mastitis abatement and the improvement of milk quality. Such impact would be in the form of techniques or products that could be applied at the dairy. In Objective 1, several research groups will be studying the effect of environment, nutrition, and management related host factors that increase or decrease the risk of developing intramammary infection. The ability to understand how the bovine immune system can be manipulated to improve resistance to mastitis, whether that understanding be applied to manipulation of the environment, ration, or other management factors, could have direct and indirect impacts on the development of products and strategies to control the disease. There is a good focus of two research groups to identify genes related to mastitis susceptibility. Such identification would improve our understanding of host resistance to mastitis pathogens and how these factors could be manipulated, and/or selected for, in an effort to improve control against specific pathogens. But it is difficult to envision how the applicability of this research would be broad enough to cover a wide range of resistance to many mastitis pathogens.

In Objective 2, the efforts of the research group are largely directed to characterization of some agents of mastitis. Mastitis abatement in general will improve cattle welfare as cows can suffer from this disease. Additionally, such abatement will improve milk quality. Characterization of agents and their environment can be used to develop new methods of more rapid and more targeted diagnoses, although none of the planned research efforts appear to be focused to that end point. Characterization of pathogens may lead to new methods treatment and indeed the Rutgers efforts could lead to such development in reducing the impact of S.aureus intramammary infection.

In Objective 3, efforts to develop product as well as control strategies are more evident. UT and LA stations are examining new antimicrobial treatment strategies. However, based on previous experience, this reviewer agrees to the potential such products will have impact as future mastitis therapies, but does not envision a strong likelihood. However, other purported projects, eg., the research on milking equipment analysis in real time and the studies examining selective dry cow therapy protocols, have good potential to be translated directly into some use on dairy operations.

5. Overall technical merit:

The research groups in aggregate bring together a wide array of expertise, using classic as well as cutting edge laboratory methods. The technical merit of the project as a whole is strong given the melding of such techniques and experience of the researchers. There is good balance in this project.

Overall recommendation:

I believe the Project evaluation should be considered in light of both previous and future success.

The Project has a long history of successful developing new strategies to lead to mastitis abatement. Some efforts were successful, some efforts did not result in new product or development. For example, some 30 years ago some in the Project actively pursued the development a S. aureus vaccine by way of attempting to characterize the pathogen and the host immune response to the pathogen. These efforts were unsuccessful and the efforts were eventually abandoned. Yet the same approach at the same time were made with the development of a coliform vaccine in mind. These efforts were successful and hastened the development of a commercial vaccine. Some efforts lead to success, many do not. But the project has had a major impact on control of mastitis.

Current Project efforts are likely to “enjoy” the same fate. Mastitis is a multifactorial disease with several predominant agents, with many vectors of transmission, modes of action, reservoirs of existence. Thus, it is difficult to make progress against this disease syndrome in a short period of time such as 5 years. But the Project efforts over the longer term have been very successful. Streptococcus agalactiae, once clearly the most predominant pathogen is now but non-existent. Staphylococcus aureus seemed to replace it in terms of prevalence, and it to is much more rare. There are emerging pathogens. But the nationwide herd milk somatic cell count has dropped considerably over the history of the project and continues to drop today. This dramatic decrease in the milk somatic cell count has resulted in improved milk quality and productivity.

Thus, the project has a good likelihood that it will contribute overtime to the success in the control of mastitis. It follows the path that researchers who were successful have followed. That path includes excellent collaboration between research groups. The result is experiments done using more experimental units and broader expertise of scientific methods. Moreover the research group has a good understanding of the disease syndrome as outlined and most are dedicated researchers that have focused their careers achieving ways to improve mastitis control.

Your Recommendation:

Approve/continue project

Dear Reviewers,

We sincerely thank you for your time and appreciate your input on our Project: NE_TEMP2248: Mastitis Resistance to Enhance Dairy Food Safety. Please see below for comments specific to each of your concerns.

Reviewer #1:

The majority of the work proposed though has little to do with food safety so a possible change in title should be considered.

AU: Thank you for the comment. This title is one that has been used for years, but we agree that food safety is not the major focus. We have changed it to be more inclusively of all aspects of our work. The new title is: 'Mastitis Resistance to Enhance Food Safety, Milk Quality and Animal Welfare'. We kept food safety as part of the title, as many researchers within our project focus on reduced antimicrobial usage. Reduced mastitis and reduced antimicrobial usage are associated with lower risk of antimicrobial residues in the milk, which is a food safety concern.

The goals are the same as the previous project. Based upon the significant amount of literature published from the project, gains in knowledge and management practices toward a reduction in mastitis, improvement in animal health, and reduction in use of antibiotics is likely. An update to the objectives might be in order to focus advancement in fewer areas.

AU: Thank you for the comment. While we agree that our focus is broad, we also must highlight the fact that our scientific team has a broad range of specialties covering many aspects of bovine mastitis. Therefore, we believe our goals are well suited for our group to continue to work and make further advances in the areas stated without our listed objectives.

For Objective 3, a study at University of Utah in dairy calves is noted. This does not fit with the project goals and objectives so should be dropped.

AU: Thank you for the comment, we have removed that study from the project.

Reviewer #2:

The proposal focuses on dairy herd health, milk production, and milk quality rather than dairy food safety.

AU: Thank you for the comment. Based on your suggestion and that of other reviewers, we have updated the title. We have changed it to 'Mastitis Resistance to Enhance Food Safety, Milk Quality and Animal Welfare'. We kept food safety as part of the title, as many researchers within our project focus on reduced antimicrobial usage. Reduced mastitis and reduced antimicrobial usage are associated with lower risk of antimicrobial residues in the milk, which is a food safety concern.

The University of Utah study in dairy calves is an odd fit.

AU: We agree. We have removed that study from the overall project.

The proposal is lacking in detail, assumingly due to the scope and complexity of the project; continued success of the program is likely with renewed funding based on past performance.

AU: Thank you for the comment. It is challenging to add details with the large scope and varying subsets of the project and therefore we elected to keep it less detailed at this time to allow coverage of all areas.

Reviewer #3

Thus, the aggregate efforts by the research teams working to accomplish progress to the goal of on farm mastitis prevention is limited.

AU: This comment was in reference to objective 3. We agree that assessment of new prevention strategies is difficult and not always successful. The focus of this specific objective is not only to evaluate mastitis prevention but also to identify ways to reduce antimicrobial usage. With that, we do think several aspects of our work that are addressing this specific objective. For example, upcoming work on understanding heifer mastitis will help lead to prevention programs, improvement of selective dry cow therapy will reduce antimicrobial usage, and early-stage vaccine work may lead to new prevention strategies.

2022 Experiment Station Section Award for Excellence in Multistate Research

Region: Northeast **Nominator**: Matthew Wilson **Email**:matt.wilson@mail.wvu.edu
Project or Committee Number and Title: NE1962: Outdoor Recreation, Parks and Other Green Environments: Understanding Human Community Benefits and Mechanisms
Technical Committee Chair: Sohyun Park_ **Email**: __sohyun.park@uconn.edu_____
Administrative Advisor: _Matthew Wilson_**Email**:matt.wilson@mail.wvu.edu____

Project Summary:

Issue, problem, or situation addressed (5%): For decades, research has shown that nature-based recreation can positively impact human health and well-being in various ways. This research, however, is scattered across a wide array of disciplines; it is rarely accessible to practitioners or integrated in a way that coherently informs policy and decision-making across multiple scales. In 2012, the NE1962 Multistate Research Group was developed to improve understanding of the complex links between outdoor recreation, parks and green spaces, environmental literacy, health, and community vitality. The group's name, NE1962, honors the 1962 Outdoor Recreation Resources Review Commission report that examined the increasingly prominent role of outdoor recreation in American life - a report that has not been revisited in over 50 years! Consequently, the NE1962 group investigates the dynamics that motivate, constrain, and sustain outdoor recreation activity among various population groups, examining these activities in the context of the socio-ecological systems in which they are embedded.

The group explores parks and outdoor recreation strategies to promote active and healthy lifestyles that combat chronic disease, cultivate connections to nature, and fuel positive social and cognitive development. Importantly, the group works to ensure that implications of existing research permeate the policy arena, as a basis for evidence-centered health promotion.

Objectives (5%): Over the past 5-year project period, NE1962 group objectives have focused on utilizing basic and applied research and synthesis to generate evidence illustrating the critical role of park and outdoor recreation in society with respect to three key areas:

1. Physical activity and associated preventative health benefits
2. Environmental literacy (particularly among youth), also documenting the long-term influences of early lifespan connections with nature
3. Community vibrancy and resilience

Accomplishments predominantly based on the past five-year project period.

Outputs (10%): The project has produced numerous deliverables between 2018 and 2021 that include **nearly 90 publications, over 60 conference presentations and trainings (see Table 1).**

Table 1. Number of Outputs based on NE1962 Project Objectives, demonstrating output growth from 2018 to 2021	**2018-2019**		**2020-2021**	
	Publications	Presentations & Trainings	Publications	Presentations & Trainings
1. Preventive Health Benefits	19	7	26	17
2. Environmental Literacy	1	5	13	13
3. Community Vibrancy and Resilience	11	9	16	12
TOTALS	31	21	55	42

The most significant NE 1962 product is a soon-to-be-published edited volume titled "***The Transformative Power of Parks***," built on contributions of NE1962 members (the co-editors are seven group members from seven different states) and many more. Published by Sagamore-Venture, this ebook will synthesize the current state of knowledge regarding the broad benefits of parks and recreation and identify opportunities for generating equitable outcomes across diverse communities. Focusing on the overarching themes of *Health & Well-being, Environmental Literacy & Stewardship, Equity & Inclusion, and Community Resilience & Vitality*, the book features more than 50 chapters written by over 100 authors from diverse sectors (e.g., academic institutions, government agencies, not-for-profit organizations, industry) around the world. Each chapter uses scientific evidence, case studies, and other sources to illustrate how parks and recreation can transform the way people live and interact with social and ecological systems.

The book will serve as a resource to help practitioners, policy-makers, researchers, students, and other key stakeholders across multiple disciplines understand, communicate, promote, and realize the diverse benefits of parks and recreation. NE1962 members determined that this unique and unprecedented book project was the best way for the group to achieve its goals.

Short-term outcomes (5%): The NE1962 project has bolstered awareness of connections between outdoor recreation, parks, greenspace, and a variety of health-related outcomes in many ways. Group research projects have addressed and advanced the following themes and topics:

- Visitor use monitoring and impact assessment
- Examination of efforts to increase equity and inclusion in public spaces
- Development of park audit tools to facilitate health promotion
- Assessments and evaluation of planning for nature-based health promotion
- Impacts of outdoor programming on youth development
- Nature therapy treatments for veterans with post-traumatic stress disorder
- Development and expansion of Nature Rx (nature prescription) programs
- Effects of park use and nature-based recreation on coping strategies and health outcomes during the COVID-19 pandemic

Medium-term outcomes (5%): The project has changed behavior by providing a forum where researchers across the country working on similar topics can regularly meet and exchange ideas. In earlier years, the project did this via annual meetings often linked to a major interdisciplinary meeting in the field (e.g., National Environment and Recreation Research Symposium). However, in the past two years, the focus has shifted to our book project and collaborative publications and presentations have essentially doubled over the last 2-year period (Table 1).

Long-term outcomes (5%): The project has changed conditions by uniting researchers and projects previously separated by geography, disciplines, and time to synergistically achieve larger goals. A wealth of research on nature-based health promotion had been conducted before NE1962 formed and continues to proliferate today. The greatest and perhaps most unique asset of NE1962 is its effort to pull these disparate entities together, amplifying the impact of the research and the collective voice of researchers in these related fields. The book project embodies this diversity and inclusive spirit, with contributors from multiple sectors and countries around the world. Rarely have so many diverse perspectives been assembled to address a common theme.

Impacts (10%): The project is having a significant impact on the world by emphasizing the critical role that parks and greenspace play in promoting human health and community

well-being. This impact is best exemplified through the Transformative Power of Parks book, which synthesizes anecdotes, evidence, and best practices across multiple sectors to tell a cohesive story about parks and outdoor recreation benefits in a global society. For example, the NE1962 book editors are working with the National Recreation and Park Association to ensure that all proceeds support the organization's "Park Build Community" program, which focuses on park development in historically marginalized neighborhoods. Additionally, the book will include a foreword and endorsement from Gil Penalosa, former Chair of World Urban Parks and influential founder of the innovative 8-80 Cities community health concept.

Added-value and synergistic activities across mission areas

Multi-disciplinary activities (10%): All work conducted by the NE1962 group is inherently multi-disciplinary, as group members are eager to study nature-based health promotion from a wide variety of academic disciplines and perspectives. This interdisciplinary exchange has been an asset of the group since its inception in 2012, but the benefits have been realized at unprecedented levels during the development of the edited book.

Multi-functional integrated activities (10%): In addition to research outputs and the edited book project, NE1962 has facilitated collaborations among members in other ways. For instance, information sharing across institutions has impacted educational curricula, influencing undergraduate course design and even inspiring new courses focused on the intersection of nature, health, and wellness.

Additional partnerships, associations, or collaborations (10%): The NE1962 has effectively done what many previous efforts have struggled to do… bring together people from different backgrounds to answer shared research questions and achieve mutual goals. Countless research collaborations have emerged from these conversations (note the number of related publications noted above). As one tangible example, NE1962 has ignited conversations about nature prescription programs in a variety of settings, helping to fuel a new Campus Nature Rx network that lays the foundation for additional collaborative opportunities in the future.

Committee synergy that would not have been accomplished with individual work (10%): Significant and important individual research focused on the nexus of nature and health has been conducted for decades - often by members of the NE1962 group. However, the multistate group has provided a unique forum for these researchers to pool ideas and, in some cases, data, to achieve broader impacts. Undoubtedly, the best example of this is the comprehensive edited volume, which will effectively showcase the transformative power of parks in society.

Evidence of multi-institutional and leveraged funding with examples of sources (10%): The researchers that participate in NE1962 are largely social science researchers (i.e., non-STEM by Carnegie Classification) and therefore large research expenditure is not a good measure; however, pursuit of a comprehensive edited volume that can impact the field globally is a tremendous leverage of the multistate approach. The group continues to search for ways to leverage funding. For example, several NE1962 members co-hosted a meeting in March 2022 with the National Park Service's (NPS) *Healthy Parks Healthy People* leaders. The meeting was to facilitate discourse among researchers across multiple universities in central NC (NC State University, Duke, the University of North Carolina, North Carolina Central) and diverse disciplines (recreation and leisure, park management, natural resource conservation, epidemiology, public health) to develop a research consortium that could partner with NPS and pursue larger projects. Similar conversations should continue in the years to come, ideally involving other federal agencies, including the US Forest Service and the EPA.

Summary of Participating Institutions and Units (5%)

Table 2: 48 Institutions & Organizations

Agnes Scott College	Southern Utah University
Antioch University	St. Lawrence University
American Society of Landscape Architects	The Ohio State University
Arizona State University	Trust for Public Land
Augsburg University	USFS Pacific NW Research Station
Azim Premji University, India	University of Alberta, Canada
Bureau of Land Management	University of Alaska Fairbanks
City of Quebec	University of Arizona
Clemson University	University of Colorado Boulder
Colorado Mesa University	University of Colorado, Colorado Springs
Cornell University	University of Connecticut
Eastern Washington University	University of Connecticut Extension
Greenways Incorporated	University of Copenhagen, Denmark
Health & Technology Partner	University of Florida
Kansas State University	University of Illinois
Kristianstad University, Sweden	University of New Mexico
National Geographic Society	University of Northern Iowa
National Park Service: Rivers, Trails and Conservation Assistance Program	University of Otago, New Zealand
North Carolina State University, USA	University of Ottawa, Canada
Park Rx America	University of Montana
Parklands Foundation of Charleston Co.	University of Wisconsin-Madison/Extension
Penn State University	University of Wisconsin-Stevens Point
South Dakota State University	Utah State University
South Dakota Dept of Game, Fish, & Parks	West Virginia University