

18515: Development and Evaluation of Broccoli Adapted to the Eastern US

Status: Submitted

Duration 10/01/2018 to 09/30/2023

Admin Advisors:

NIFA Reps:

Statement of Issues and Justification

Broccoli buyers in the eastern U.S. want local product, but depend on expensive and environmentally challenging transport from the West Coast of the U.S. and Mexico. Providing a local supply of high-quality broccoli requires three components: 1) a skilled grower base, 2) a developed distribution chain, and 3) improved varieties of broccoli. The USDA SCRI-funded Eastern Broccoli project is addressing all these elements, with the goal of developing broccoli production on the East Coast with a farm gate value of \$100 million a year by 2021. This Multistate project is intended to sustain the trial system established through the SCRI grant for as long as it proves useful. It will provide: economic development through production of high-value crop, particularly in economically depressed rural communities; social benefits that include greater access to locally grown vegetables and a more resilient broccoli supply for eastern consumers; and environmental benefits from reduced use of transportation fuel and irrigation water, and from improved rotations on vegetable farms.

The successful establishment of an eastern broccoli industry depends critically on the continued availability of hybrids adapted to eastern environments. New, adapted broccoli cultivars resulting from the project will be on the market by 2021, but continued breeding efforts will be necessary to meet new challenges and sustain the industry. To assess the performance of new broccoli hybrids developed for variable eastern conditions, the Eastern Broccoli Project (EBP) established a network of trial sites affiliated with public institutions along the East Coast from Maine to Florida. Turning the trial-site system into a USDA Multistate Research Project will ensure that the network continues to enable further improvement of cultivars targeted to eastern environments after SCRI funding ends. The strong grower base and distribution chain resulting from other portions of the EBP will ensure that demand for eastern-adapted cultivars remains high, so that developing, testing, and commercializing these new hybrids is a worthwhile endeavor for seed companies.

The fundamental reason why broccoli production in the East is far lower than the regional demand is that broccoli cultivars are not well adapted to the climate. In particular, warm nights during the growing season block the environmental cues that normally cause flower buds to develop in synchrony, resulting in poor quality, unmarketable heads. Climate change will increase the need for these adaptations. Developing new hybrids that perform well under these conditions has been a primary focus of the EBP. Higher demand for Eastern broccoli, expected increase in broccoli acreage in the East and the development of new breeding tools makes this a good time to create a self-sustaining, larger market for adapted seed.

The proposed Multistate Project will enhance communication and coordination among Eastern Broccoli trial collaborators and ensure that the network can continue to offer evaluation expertise in support of broccoli breeding efforts. Presently, all three public broccoli breeding programs and several seed companies make use of the project trial system to assess the performance of new hybrids that are targeted to eastern growing regions. There are many advantages for doing the work as a multistate effort: having closely coordinated protocols makes the results more powerful; multiple plantings and locations deliver a wealth of information across numerous eastern environments and production seasons each year, increasing breeding efficiency; coordination among trial sites can lead to further external (federal or industry) funding; and the trial network can be expanded to include other brassicas with potential in the region. This trial network provides seed companies with a better assessment of performance in the variable eastern environments than their own trials can provide. They also provide insight about the performance of competitors unreleased, anonymized, material. The network also fosters collaboration among researchers working on similar problems and gives them the expertise to advise Extension personnel in other eastern states. The consequences of not instituting this project include slower improvement of adapted broccoli and loss of expertise that is already in place. Testing broccoli hybrids would be more expensive and take years longer, which would discourage seed companies from developing cultivars for the east. The project has the potential to expand to other brassica crops, but that capacity would be lost if the project were discontinued.

This project will sustain and coordinate a network of eastern trials sites that offer expert, consistent evaluations of broccoli hybrids in support of the development of improved cultivars for eastern production and associated research. This process includes every public broccoli breeder in the US, but their numbers are severely limited. It is imperative to train new breeders who apply the latest molecular tools, use breeding strategies that are most appropriate for their crop, and master the art of breeding. We will train staff and graduate students who go on to breeding jobs.

Related, Current and Previous Work

The regional trial system was instrumental in identifying the best hybrids developed during the first five years of the eastern broccoli project. Quality and Yield trial protocols, data collection sheets, and rating reference cards were developed for EBP and these will continue to be useful for the USDA Multistate Research Project. At each location, personnel have been trained to evaluate broccoli with respect to quality and marketability. We have strong relationships with private breeding programs, with six who have entered material to date and two more that anticipate doing so in 2018. We expect this demand for our trials to continue as long as the market potential remains.

To date, 5 to 10 hybrids have shown superior adaptation in the extensive multi-regional yield trials. The commercialization process takes about five years from this point. Commercialization by seed companies involves producing enormous quantities of hybrid seeds, identifying which seasons and markets each hybrid is best suited for, negotiating intellectual property agreements, and developing marketing strategies.

Objectives

1. Screen new broccoli hybrids for better adaptation to the Eastern United States.
2. Test the commercial yield potential of Eastern-adapted broccoli hybrids.

Methods

Activities and procedures:

Public Broccoli Breeding Programs: Seed of parental lines and hybrids with good potential for commercial release will be produced. Broccoli germplasm with traits of interest (e.g., disease resistance; exerted head for easy harvest) from other U.S. public broccoli programs will be evaluated. Key personnel for this objective are public program leaders Mark Farnham of the US Vegetable Laboratory in Charleston, SC; Phillip Griffiths of Cornell University in Geneva, NY; and James Myers of Oregon State University in Corvallis.

Multi-regional Quality Trials. Broccoli hybrids from public and private seed-company breeding programs will be evaluated in locations representative of the variation seen across the East Coast. The evaluation protocol developed for the EBP will be employed by our experienced collaborators to identify broccoli hybrids that consistently produce high quality crowns over a range of eastern growing conditions (Figure 1). Yearly evaluations will be done with three test plantings (3 replications per test) at each of five eastern sites. A minimum of two commercial standard broccoli cultivars will be included in evaluations each year to provide a consistent reference for the growing conditions. The 15 environments per season will allow identification of material with stable performance under varied stress, as well as the potential planting season for which each is best suited. We anticipate 20 to 40 entries per year from all sources. Hybrids that have the greatest market potential in Quality trials will be entered in Yield trials, which are described below (Figure 1). Quality trials will be conducted by Mark Hutton in Monmouth, ME; Phil Griffiths at Cornell University in Geneva, NY; Jeanine Davis of North Carolina State University, in Waynesville, NC; Brian Ward at the Clemson Coastal Research and Education Center in Charleston, SC, and Lincoln Zotarelli at the University of Florida in Hastings, FL (Figure 2).

Dr. Carl Sams at the University of Tennessee will offer optional services to measure nutritional and flavor components, which can inform breeders' selection of hybrids for release and marketing. Samples will be analyzed to determine the concentration of sugar, glucosinolates, minerals, bitter (progoitrin and gluconapin) and sharp (indole metabolites) flavors.

Identify candidates for release. We will use results from Quality trials to identify broccoli hybrids that consistently produce high quality crowns in eastern environments. The best hybrids are considered candidates for Yield trials and possible commercial release. Ongoing efforts in breeding programs will result in additional candidates in the future.

Multi-regional Yield Trials. The production potential of candidate hybrids from public and private breeding programs will be determined in on-farm Yield trials that will be conducted in 11 commercial production settings typical of the region (Figure 2).

Measured traits will be yield, marketable yield, cause of non-marketability, and field holding. Hybrids will be tested at sites and in seasons that best match the potential market slot. A leading commercial broccoli cultivar will be included in Yield trials at all locations to serve as a check against which the performance of new hybrids can be measured. Yield trial sites can host field days to educate growers about production techniques and potential new cultivars.

Measurement of Progress and Results

Outputs

- New broccoli hybrids with superior performance Comments: Breeding progress will be measured in the performance of new hybrids relative to check cultivars in the Quality Trials. The value of the trials will be determined by the number of entries that breeders anticipate entering in both Quality and Yield trials. The success of Yield trial system will be measured by the number of those entries that are ultimately commercialized. Based on market size, we anticipate release and marketing of a new variety every year or two.

Outcomes or Projected Impacts

- New hybrids developed by the project will be on the market New hybrids developed by the project will be on the market. The continuation of the trial system beyond EBP SCRI funding will lead to the commercial release of additional and even better hybrids after 2021. Public breeding programs will release and license hybrids that are superior to those released earlier in this project, as well as new inbred lines with high value as parents of hybrids with commercial potential in the East. These inbreds will improve private breeders' ability to develop adapted hybrids. A breeding population enhanced with valuable traits that are not currently in commercial broccoli-breeding programs, and genomic information about it, will allow more efficient, targeted breeding. Commercial breeders will use the results of the Quality trials to inform their use of public material. Yield trial results will inform cultivar recommendations and support sales people advocating newly released cultivars. Eastern growers will harvest broccoli from the new cultivars in warmer seasons. New cultivars will be identified by presence in catalogs of primary seed distributors. Sales figures are not likely to be disclosed, but we plan to get market-share estimates from major growers and seed distributors.

Milestones

Outreach Plan

Trial leaders will meet yearly for the duration of the project to ensure consistency in evaluation standards across sites and to discuss trial results.

The outreach plan for this multistate project is broken-down into Objectives 1 and 2 as follows:

Objective 1. Bring new broccoli hybrids to market that are better adapted to the East United States.

Growers, wholesale and retail buyers, and other extension personnel working with vegetable growers are the primary outreach targets for this objective, so we will work closely with them throughout the year. The project supports their marketing efforts by providing objective performance data to seed distributors and prospective growers. Field days at trial sites are most effective for showing prospective growers new hybrids and production methods. Grower meetings and social media will call attention to available and suitable new cultivars and upcoming field days and related events. Wholesale and retail buyers will learn that local broccoli is becoming more available through articles in their trade publications. Seed companies will learn about the performance of public hybrids that may have commercial promise through site visits, trial reports, and discussions with public breeders.

Objective 2. Develop Eastern adapted broccoli hybrids with quality and productivity needed to keep the crop competitive into the future.

The primary target audience includes seed company breeders and marketing staff. Those personnel will have early information on which to base breeding and marketing decisions. Additional target audiences of this objective are other scientists working with broccoli and other extension personnel. Raw data will be submitted into appropriate public databases to inform other scientists working with broccoli of commercialization and breeding decisions. Other Extension personnel advising vegetable growers will be aware of new varieties.

Organization/Governance

Participants and Expected contributions. All project participants are expected to be involved for the entire period. Should individuals need to leave the project, we will seek replacements to cover their responsibilities. Historically, we have needed a replacement every 2-3 years. The list of participants and their roles follow.

- Thomas Björkman (Cornell University). He will work on genomics, conduct outreach and production support activities, assist with the NY Yield trial, and manage the Eastern Broccoli website and production guide.

- Mark Farnham (USDA Vegetable Lab), will enter public hybrids from his program in both Quality and Yield trials.
- Phillip Griffiths (Cornell University) will enter public hybrids from his program in both Quality and Yield trials.
- James Myers (Oregon State University) will enter hybrids in Quality trials.
- Mark Hutton (University of Maine) is leader for Quality and Yield trials in Maine.
- Jeanine Davis (North Carolina State University) will conduct Quality trials in western North Carolina.
- Brian Ward (Clemson University) is leader for Yield and Quality trials in South Carolina.
- Lincoln Zotarelli (University of Florida) is leader for Quality and Yield trials in northern Florida.
- Timothy Coolong (University of Georgia) is leader for Yield trials in Georgia.
- Christy Hoepting (Cornell University) is leader for Yield trials in western New York.
- Monica Ozores-Hampton (University of Florida) is leader for Yield trials in Immokalee, Florida.
- Ashley Edwards (Virginia Cooperative Extension) Yield trials in Virginia.
- Carl Sams (University of Tennessee) will, upon request, conduct chemical analyses of nutrient and flavor components of fresh broccoli.

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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

NE TEMP1834 “Genetic Bases for Resistance and Immunity to Avian Diseases” responses to reviewers

Reviewer 1.

“There seems to be a bit more emphasis on the MHC aspects of immunity and less on the innate aspects. Genetically, this seems to make sense, but practically there is an issue with the poultry industry and the use of MHC as a genetic basis for resistance and selection for growth and performance. There are no data that show that this two are compatible with each other.”

The committee continues to build upon its strong contributions in the area of the chicken MHC, because of the importance of this genomic region, the emerging information about new functions for genes in this region, and the availability of well-characterized chicken genetic resources for study of the MHC. The MHC is an excellent target for investigation of genetic resistance to infectious diseases in chickens, particularly because it is much smaller than in mammals, and some of its genes have much simpler expression patterns. This gives the MHC a more strongly detectable association with disease resistance than in many other species. For example, the MHC region codes for innate, adaptive and complement immune responses in chickens (1) and is used by the investigators in the project to understand immune responses in chickens against pathogens. This is basic research to understand which components of the innate and adaptive immune responses provide resistance to diseases in order to potentially use them in commercial chickens to prevent or control those diseases. As an illustration of the historic impact of chicken MHC research, this information has been applied in commercial companies to select for alleles that enhance host resistance to Marek’s disease.

The innate aspects of immunity are also well-represented within the project, including studies of natural antibodies, cytokines, and the functions of antigen-presenting cells.

We are unclear as to the meaning of the reviewer’s comment: “...there is an issue with the poultry industry and the use of MHC as a genetic basis for resistance and selection for growth and performance. There are no data that show that this two are compatible with each other.” If the reviewer is referring to potentially negative correlations between immune response and growth, the genome-wide association studies in this project will reveal genome regions that can be separately included in genomic selection programs to appropriately accommodate negative genetic correlations.

1. Kaufman, J. The Avian MHC. In: Avian Immunology, 2 ed. K. A. Schat, B. Kaspers and P. Kaiser, eds. Academic Press, Boston. pp 149-167. 2014.

“On the other hand, the fact that an acquired response cannot be induced without the induction of a proper and appropriate innate response seems to be forgotten within the proposal. This fact must be kept in mind.”

This comment was addressed in the prior response. We are very aware that innate response is linked with adaptive response and, importantly, we will study the non-specific (cross protective) nature of innate responses as a method of broadly preventing diseases (or enhancing vaccine response) in poultry.

As examples of innate response work in NE_TEMP1834 we mention:

- Natural antibody work
- High throughput analysis, mRNA seq, gene expression and pathway analysis
- Feather follicle challenge
- Innate immune factors driving MDV virulence, among others.

Reviewer 2.

Well written proposal, highly justifiable and essential. The scientists have been very productive as evidenced by publication record. This reviewer would have liked to see specific examples of the impact of previous work on poultry health.

We agree with the reviewer's comment and would like to have included additional examples of impacts on poultry health. The two main targets of research in this project are the highly competitive commercial poultry genetics companies and animal health companies. In both instances, the application of new processes, products and information is typically proprietary and maintained as trade secrets. However, there are many public indicators of the value that commercial companies attribute to the research of this project. There are several projects by members that are funded by companies, there are joint publications with industry scientists, and there is attendance at the annual Technical Committee meetings by representatives from poultry genetics and animal health companies. Additionally, the project has had a major impact on the scientific community by actively training the next generation of scientists working in poultry health and genetics, and in publishing results in peer-reviewed journals to provide a foundation for continued scientific progress.

Another question is there any cooperation between this group and the NC1170 (Advanced technologies for the genetic improvement of poultry)? Was there any consideration of exchanging a representative from each group at the annual meetings.

There is strong cooperation between these two multi-state projects (NE_TEMP1834 and NC1170). A paragraph describing this cooperation has been added to the proposal in the "Issues and Justification" section under: d) Advantages for doing the work as a multistate effort (The Essential Collaborative Nature of this Project)

Reviewer 3.

1. Please provide the objective titles in addition to the objective numbers under Related, Current and Previous Work. The objectives are not listed until after this section.

Done in the revision.

2. The objective numbers are duplicated when they are first listed.

Corrected in the revision.

3. The previous work and progress is considerable and the overlap with NC1170 is important as these two projects have significant overlap.

These two multi-state projects have different main objectives. Some of our members are part of both multistate projects and this is part of the strategy to ensure good coordination without duplicating work. There is strong cooperation between these two multi-state projects (NE_TEMP1834 and NC1170). A paragraph describing this cooperation has been added to the proposal in the "Issues and Justification" section under: d) Advantages for doing the work as a multistate effort (The Essential Collaborative Nature of this Project)

4. The writers need to go back through and be consistent in the station designations. CAD, CA-D or UC-D are used in multiple places where I know that is UC Davis because I know the groups. BRI and CA-COH are used interchangeably for Dr. Miller's group. The acronyms are listed near the end but those could go earlier and they should not use different acronyms, pick one and use it throughout. Similarly ADOL is sometimes USDA-ADOL but then at times referred to only as USDA which would be misleading as to which specific USDA facility is referenced. Would be better as just ADOL.

Suggestions were followed to have correct and consistent acronyms for each institution. Acronyms were listed earlier in the proposal.

5. Aims for Objective 1 seem to end abruptly and the last sentence is confusing at best. Objective 1 was re-arranged.

6. Maybe this reviewer is naïve but KLH is never defined. Keyhole limpet haemocyanin (KLH). Was defined in the aims for objective 1.

7. The aims for objective 2 seem to digress to past performance and use NE1334. In the methods section for objective 2 there is a list of activities that will be performed by each of the stations during subsequent years. It is now corrected to read as: NE-TEMP1834.

8. The introduction lists publications for only the past two years but in Objective 2 they list publications for last 4 years. I like the 4 year number better.

The proposal has a section "literature cited" listing all the publications for the last four years 2013 to 2017. The summary is 33 cooperative (multi-station or multi-member) and 204 individual station publications. Due to space constraints not all the 204 latter publications were listed in the proposal. The full list was added as an attachment.

9. Past leveraging is ever really listed but only summarized in the text? They list \$6M in grants in the text. I would have liked to see the number of postdocs and graduate students produced from past project.

Past leveraging is listed in the section: "Related Current and Previous Work". The suggestion to track post doc and graduate student training numbers is excellent, and a practice that we will implement annually so that we can list them for the next proposal.

10. For the milestones they list a 2023 milestone for 2018

The milestone for 2018 was added.

11. Page 41 they only list 8 participants (Appendix E) from 7 stations but they proposal is about many more participants and stations.

Project participants are in the process of re-submitting their appendix E, so that they are approved and active as of the start date of October 1, 2018.

NE_temp1834: Genetic Bases for Resistance and Immunity to Avian Diseases

Status: Under Review

Duration 10/01/2018 to 09/30/2023

Admin Advisors:

NIFA Reps:

Statement of Issues and Justification

a) The Ongoing Need for this Work, as indicated by stakeholders

The US per capita poultry (chickens, turkeys) consumption equals the combined per capita consumption of both beef and pork. The consumption of poultry worldwide has steadily increased since 1960. Given the high feed efficiency of poultry, this food source represents one of the most economically- and environmentally-sustainable means to provide protein to the growing human population. United Nations projects the 2050 world population to be 9.7 billion people up from the 7.3 billion people in 2015. Poultry breeders and producers seek to provide consumers with a wholesome product with higher production efficiency and lower production cost to meet this ever-increasing demand.

Disease remains a major issue for the poultry industry. Economic losses due to morbidity, poor performance, and mortality are significant with the added threat that some bacterial and viral zoonotic pathogens can cause human illness or death. While advances have been made in controlling many poultry pathogens, the impact of diseases is one major impediment for sustained productivity. The total disease impact on poultry production not only includes losses due to mortality, decreased meat and egg production, and condemnations at processing, but encompasses the increased costs in prevention (i.e. vaccinations, biosecurity, and eradication programs for exotic diseases). Moreover, with a major focus on antibiotic free (ABF) production, understanding and optimizing immune function has become of paramount importance to maintaining sustainable levels of production.

This project aligns with the producers' goal of the judicious antibiotic use in poultry meat and egg production, since healthier birds will reduce the events in which these drugs are necessary. The use of pharmacological agents (e.g., antibiotics) to treat disease poses its own challenges to animal production, the environment, as well as the wellbeing of poultry and the consumer.

Protection of poultry flocks against endemic and exotic diseases is a priority for meat and egg producers. The 2015 outbreak of highly-pathogenic avian influenza (HPAI) in the U.S. (Washington, Wisconsin, Minnesota, and Iowa) and subsequent smaller outbreaks in Montana, Tennessee, Alabama, Kentucky and Georgia, remind us of the vulnerability of these systems. In addition, consumer preferences are also driving commercial production systems to be more open (free range), elevating the risk for exotic disease introduction. The U.S. Poultry and Egg Association defines 28 critical needs for controlling disease and ensuring food safety in poultry. These needs include a focus on prevention of diseases, while decreasing the use of antibiotics, pesticides and anti-parasitic drugs.

This project addresses the genetic bases of disease resistance and immunity in poultry, as well as mechanistic understanding of innate and adaptive immune processes; issues having fundamental importance. Primary stakeholders (the most immediate users of these data, reagents, and tools generated in this project) are poultry breeding, vaccine, and allied animal health industries. Their frequent participation in the annual Technical Committee meetings, and their many collaborative research with the members, clearly indicate the high value that stakeholders ascribe to this project.

b) The Essential Nature of this Project

This work is essential to advance disease prevention and control strategies that ensure a sustainable poultry industry with

increased production for a growing world population. Genetic variability is inherent within populations of species and is a product of natural selection. This project addresses the important issues of genetic bases of resistance and immunity to diseases in poultry providing stakeholders with a better understanding of genetic variability within their stocks in order to produce future populations with sustainable, desired traits. Disease resistance as a genetic trait is very multifaceted. Sometimes one or a few genes, such as the major histocompatibility complex, affect disease responses. Other instances are more complex with many contributory genes, making heritability low, and difficult to improve by traditional genetic selection methods. Although there is a chicken genome sequence, much remains to be learned about loci that are naturally polymorphic and about the functional outcomes of interactions among polymorphic loci that are increasingly under directed selection. We will also be able to better understand immune responses to common poultry pathogens through this project. This information is crucial in order to strategize novel preventative strategies, which in some cases can be cross protective. Disease is one of the major limiting factors in large-scale and small-scale poultry production. Shifts toward managing birds in less-controlled environments, and reduction of antibiotics use in animal production are exacerbating disease issues. Currently pathologies that seemed to be controlled are recurring thereby disrupting commercial production. This project addresses the important issues of environmental and physiologic factors that regulate or affect immune system development, optimal immune function and disease resistance in poultry. If this work is not done, disease will increase, production efficiency will decrease, food safety will be greatly compromised, and as a result export markets will be closed. This project also addresses the need for development of methods, reagents and specialized genetic stocks to be able to assess, monitor and modulate immune system development, patterning and function. In contrast to biomedical model species, such as mice, few of the reagents needed for poultry research are commercially available. If immune development is not monitored, immune responses to vaccine and disease organisms could deteriorate, thereby increasing morbidity and mortality. Understanding immune system development as affected by genetic and environmental factors will find direct application in the breeding poultry stocks that have improved health and effective responses to vaccination. These improvements will contain production costs.

c) The Technical Feasibility of This Project

The NE-1334 Technical Committee collectively possesses a spectrum of scientific expertise to execute the collaborative research essential for the future of poultry production. Their a range of expertise encompasses many disciplines; including: immunology, infectious diseases, genetics, genomics, virology, kinomics, poultry medicine, physiology, nutrition, biochemistry, microbiology, and molecular biology. The work is technically feasible as it is rooted in methodologies that have been demonstrated as successful when used in other species, most notably human medicine. In the past decade, next generation sequencing, gene editing and many highly sophisticated methods have become now available to examine the expression and interactions of genes important in disease resistance. The techniques can be readily applied in investigations of disease especially in genetically defined experimental lines. The researchers work on the leading-edge of science, have demonstrated their expertise with the requisite infrastructure to successfully complete the described work, if sufficient financial support is made available.

d) Advantages for doing the work as a multistate effort (The Essential Collaborative Nature of this Project)

Conducting this work as a multistate project offers the advantage of pooling and sharing resources to address critical scientific questions. The members of the NE-1334 committee are well-established scientists conducting research in a range of disciplines to examine disease resistance at all levels. In addition, participants have unique skills or specialized resources such as genetic stocks and poultry-specific reagents that are needed to conduct the work. The multistate effort is required for the synergistic and collaborative conduct of research that is based upon the combination of biological materials (experimental lines of birds, antibodies, cell lines, pathogen stocks), facilities, equipment and expertise from multiple stations. No single station possesses all of these to address the major scientific issues for the project. Conducting this work as a multistate effort allows for the greatest efficiency of resource use from 27 independent laboratories from 17 U.S. states [AL (1), AR (1), CA (6), DE (1), GA (1), IA (1), IN (1), MD (1), MI (USDA-ADOL, 1), MO (1), NC (2), NY (1), OH (1), TX (1), VA (1), WI (1), WV (1)], and 2 other countries [Canada (2, ON, PEI) and the Netherlands (2, NL)] in the current NE-1334 group of scientists - each addressing complementary aspects of the problem. The truly essential, cooperative, multidisciplinary nature of the project is illustrated by the many joint-authored publications among participating stations. Between 2015 and 2016, the NE-1334 project members have produced 119 refereed publications and 145 abstracts many of which feature joint authorship among multiple stations. The extensive expertise of the NE-1334 Technical Committee members and collaborators is also very clearly illustrated by the members' contributions to books such as *Avian Immunology* (published in 2013) co-edited by a NE-1334 member plus authorship in 7 of the 22 chapters plus one of the two appendices. In addition, contributions of NE-1334 Technical Committee members and collaborators have been recognized by the Poultry Science Association (PSA) and the American Association of Avian Pathologists: PSA Early Achievement Award for Research, Hy-Line International Poultry Science Research Award, Embrex Fundamental Science Award, Evonik Degussa Award for Achievement in Poultry Science, Novus International, Inc. Teaching Award, two US Poultry Distinguished Poultry Industry career recognitions, Induction as Poultry Science Association Fellow and Bayer Snoeyenbos New Investigator Award.

In addition, this project synergizes and collaborates closely with other projects. There is very strong cooperation between NE1334 and the other most relevant multi-state project: NC1170, Advanced technologies for the genetic improvement of poultry. Multiple Technical Committee members of NE1334 are also members of NC1170 (including one who has been an active member of both projects for over 34 years), and their active participation in both projects allows for excellent coordination between the two multi-state project, ensuring good communication and collaboration while avoiding duplicative efforts.

e) Outcomes and Impacts of this Project

Impacts are expected to include but not be limited to: a better understanding of polymorphic loci and the consequence of selection on poultry health and production; new breeding strategies to produce more robust, disease resistant lines of poultry; improved efficacy of vaccines and other pharmaceutical agents; new vaccine programs for controlling existing as well as emerging diseases; a better comprehension of immune responses to specific antigens and a better fundamental understanding of how the avian immune system functions. These impacts aid future scientists by facilitating prevention or control strategies for current issues plus new problems that will arise. Improved disease resistance and enhanced prevention strategies will boost production efficiency, animal health and hence welfare. Reduced antibiotic use and improved poultry product safety will have favorable consumer reception. Much new knowledge in the basic and translational sciences, as well as, reagents and tools generated by this project, will constitute valuable resources to the stakeholders.

Related, Current and Previous Work

ACRONYMS

AR = Arkansas

UCD = Univ of California-Davis

DE = Univ of Delaware

GA = Univ of Georgia

IA = Iowa State

BRI = Beckman Research Institute, City of Hope

UMD = Univ of Maryland

NC = North Carolina State Univ

NL = Wageningen Univ

OH = Ohio State Univ

PEI = Prince Edward Island Univ

UA = University of Alberta

ADOL = United States Department of Agriculture, Avian Disease and Oncology Laboratory

VT = Virginia Tech

WU = University of Washington

WV = West Virginia University

WVC = Western University of Health Sciences

The NE-1834 multistate research project scientists design, create, maintain, and study unique poultry genetic lines. Some members carry out all of these functions and others a subset as an integral part of our research. These efforts have been our contribution and our responsibility to achieve the project objectives of understanding the genetic bases for immunity to avian diseases. Special genetic lines, established over the last 80 years, are at risk at many research stations. If lost, these unique avian genetic resources (e.g., congenic, recombinant, and inbred lines) are unlikely to be recreated. Since member scientists share these genetic resources in collaborative research, their elimination will impact the project, collaborators and the avian research community at large. The Technical Committee recognizes the imperative to conserve the resources currently available. Several Technical Committee members served on the Avian Genetic Resources Task Force and are now part of the National Animal Germplasm Program, Poultry Committee. The members are committed to the establishment of a national system of networked researchers and a site for orphaned stock conservation to support our objectives of understanding and improving resistance to diseases in poultry. Innovative technologies such as candidate gene identification, applications of recombinant DNA, monoclonal antibodies, DNA probes, QTL analysis, global and targeted transcriptome analysis, gene sequencing and other novel molecular approaches have been effectively used to identify and characterize many facets of disease resistance or immune function. These techniques expand upon the pioneering work conducted by NE-1834 members throughout the project history. Project results continue to be important and readily applicable in both research and industry. Commercial poultry breeders lead other animal breeders in terms of improvement of a variety of economic traits, including genetic resistance to disease. Further research on new methods to select for disease resistance in poultry must, and will, continue in the proposed renewal project for NE-1834. Recent scientific advances in understanding the immune system and enhanced knowledge about poultry pathogens promise imminent and significant improvements in poultry health, production efficiency, food safety and animal wellbeing through genetic selection. Extensive publication searches indexed in the comprehensive databases (Agricola, Biosis, CAB, CRIS, Health Index, and Medline) for the last five year period revealed substantial scientific contributions that NE-1834 members have made in genetics of disease resistance and immune response in poultry. A significant number of the current Technical Committee members are also part of the NC- 1170 multi-state project on advanced technologies for the genetic improvement of poultry. Through interactions with NC-1170, which focuses their research efforts more on genomics and system biology of poultry and elucidation of genetic mechanism that underlie economic traits, duplication can be avoided. The two groups overlap in the creation and sharing of poultry research populations and research tools, including gene transfer technologies, hence maximizing resources. Compared to other multi-state projects that focus on specific diseases [NC-1202; Enteric diseases of food animals; enhanced prevention, control and food safety] or animal welfare beyond animal health [NE-1042; Optimizing poultry welfare], the uniqueness of the NE-1834 group lies in examining genetic bases of resistance to diseases in avian species in the context of all levels of immune system development and function. Working relationships, either formal or informal, exist between NE-1834 stations and the international laboratories conducting similar research. This, too, assures coordination of efforts and avoidance of unnecessary research duplication. Most of the members also participate in meetings of the Avian Immunology Research Group which is an international conference that brings together researchers in avian immunology from all over the world to share advances in this field, build collaborations and avoid duplicate efforts. Participation of international contributors to NE-1834 from institutions in Canada (UA, PEI) and the Netherlands (NL) demonstrates the stature of the project. To highlight accomplishments achieved by the NE-1834 investigators during the past 4 years of this project, a summary of major contributions under each Objective and the need for continuation are presented below.

Objective 1 "To Determine how Allelic Variation Influences the Efficacy of Innate and Acquired Immune Functions"

Efforts by UCD, BRI, NL yielded a more extensive description of the organization and sequences of the B- and Y-MHC regions and MHC-B and MHC-Y haplotypes. Within this effort, the correct order of MHC genes on gga-16 was further defined revealing the presence of a here-to-fore unknown gene segment within the MHC. The nucleolus organizer region (NOR) was found to be tightly linked to the MHC-Y and separated from the telomeric MHC-B by a GC-rich region. Hence, the lack of linkage between the MHC-Y and -B is not the result of being separated by the NOR. This work debunked the untested hypothesis of the NOR being responsible for the lack of linkage of Y from B and opened up an entirely new avenue of research to explore the content of the GC-rich region, which likely includes repeat elements and other genes having important physiologic function relevant to the immune response and resistance or susceptibility to disease. Extensive sequence data for 14 MHC-B haplotypes, as well as, detailed definitions of the binding motifs for two MHC-B class I (BF) antigen-presentation molecules and candidate viral

peptides, opened new venue for the study of target cell recognition by Tc. This research also led to the discovery that only few of the MHC-YF class I proteins are expressed in chickens and that at least one of these may represent a new type of antigen presentation molecule with a hydrophobic binding groove able to present non-peptide molecules. Molecular definition of MHC haplotypes was extended beyond the use of the LEI0258 microsatellite marker based on finding micro-variation in the BF1 or BLb2 gene exons. For reliable molecular rather than serological MHC-typing, further characterization of gene exon variation is required in addition to the molecular MHC typing using the LEI0258 microsatellite marker. AR, UCD, BRI, WVC, DK, IA, ON, and VT investigators studied the sequences, expression and function of the products of other genes playing a role in immune function. Molecular characterization of chicken natural killer (NK) cells, heterophils and macrophages, as well as, cloning and characterization of avian cytokines and receptors (e.g. IL-19, IL-22, IL22BP, MIF, Nod1/2, TLRs, scavenger receptors) greatly increased our understanding of genes and their products involved in avian immune system development and function. The effort initiated and led by VT to sequence, annotate and analyze the turkey genome and immune related genes provides the much needed basic knowledge and tools for the study of disease resistance and immunity in turkeys. Further, part of the mannose-binding-lectin (MBL) promoter was cloned and sequenced to identify polymorphism in two inbred and various commercial and experimental lines. In total, 14 SNPs were identified which resulted in identification of six different promoter alleles. The allele A1 was found to be associated with low MBL in serum and it was found in inbred lines as well as in commercial lines. The phenotypic consequences of MHC-haplotypes in terms of immune system function and disease resistance were examined through live-bird challenges, quantitative trait locus mapping, single nucleotide polymorphisms (SNP) analyses, establishment of linkage maps based on microsatellite markers and SNPs, global microarray and qPCR assessment of gene expression (UCD, WVC, DK, IA, ADOL, NH). Using these approaches, many genes, genomic regions and signaling pathways associated with the host response to infection with pathogens such as E. coli, Salmonella, and Campylobacter in the chicken have been identified providing important direction for further study into these economically important bacterial infections. Similarly, microRNAs and signaling pathways related to avian influenza virus infection in chickens have been identified using high-throughput technology including microarray and next-generation sequencing. Other major contributions to our understanding of viral infections in poultry include NH's observation that Marek's disease incidence was affected by a single locus BG1 3'-untranslated region difference identified in congenic lines 003.R2 and 003.R4 with serologically identical MHC recombinant haplotypes. DK established that different MHC haplotypes were associated with different amounts of antibodies to infectious bursal disease virus (IBDV) and lower disease severity after experimental infection of chickens. A similar association of MHC-haplotype with antibody levels and pathology was also found in Newcastle disease virus (NDV) infected chickens and for parasite egg burden after a challenge with *Ascaridia galli*. Greater resistance to clinical illness and better viral clearance in infectious bronchitis virus (IBV) infected chicks with the B2/B2 compared to the B19/B19 MHC-haplotype were also reported by CA-WU and collaborators. These studies offer new opportunities for characterization of genetic regulation of resistance and immunity to pathogens in poultry.

Objective 2 "To Identify Factors and Agents Affecting Poultry Immune Development, Function, Dysfunction and Pathology"

The group has also made significant accomplishments regarding basic characterization of innate and adaptive immune functions and examination of the influence of genetic, environmental, nutritional and physiological factors on these processes. For these studies, availability of genetic lines with optimal and suboptimal immune responses to an experimental antigen has been very helpful. Lines selected for high or low antibody responses to SRBC were shown by NH to exhibit differential bursal gene-expression profiles revealing biomarkers unique for high and low SRBC-Ab responders as early as day 15 of incubation. Embryonic testosterone propionate exposure, which results in bursal ablation, influenced distinct pathways in birds from the high and low SRBC-Ab responder lines. USDA-ADOL compared MDV-susceptible (7-2) and resistant (6-3) lines based on expression analysis with a panel of immune-related genes revealing a much more vigorous, especially T cell-mediated, immune response in line 6-3 than line 7-2. Additionally, transient paralysis could be observed in both lines with high pathogenic strains of MDV. Using MHC-defined lines of chickens, known to respond differently to infection with pathogens or to have different innate immune activity to PAMPs or other immunostimulants, several members (BRI, WVC, UCD) were able to better define the nature of an effective or ineffective immune response. BRI and WVC in collaboration with others have helped to elucidate mechanisms underlying the activation of natural killer cells, T lymphocyte responses and macrophages. Evaluation of chicken monocytes as a factor in disease resistance showed that B2/B2 monocytes differentiated more readily into macrophages, were stimulated to significantly greater levels with either poly I:C or IFN γ , and exhibited differential upregulation of at least 9 pathways compared to B19 stimulated macrophages. Genetic lines prone to non-communicable diseases with immune system involvement (e.g. autoimmune disease, ascites, skin disorders, lameness, etc.) were used by AR, ADOL and NH for comparison of aberrant versus normal immune activities. AR identified IL-21, IL-10 and IFN γ as the signature cytokine profile associated with autoimmune vitiligo onset and progression in susceptible Smyth Line (SL) chickens. Global gene-expression analysis of the target tissue (feather) before and throughout SL vitiligo development established a role of innate and adaptive immunity, as well as, cellular stress. Independent of serotype, MDV infection administered at hatch was reliably associated with vitiligo expression in susceptible SL chicks. Based on HVT administration, the ability of MDV to trigger SL vitiligo is limited to infection during the first 6 weeks of life. Susceptibility to autoimmune SL vitiligo appears to be manifested in part in target cell (melanocyte) defects. Studies by NH using an atherosclerosis model also reports differentially expressed genes and soluble proteins found in aortic smooth muscle cells in atherosclerosis-susceptible

White Carneau and atherosclerosis-resistant Show Racer pigeons. Immune activity and pathogenic mechanisms initiated as a result of PAMP administration, viral, bacterial, or parasitic infections were also investigated in selected lines of chickens, commercial layers, broilers and turkeys by members and collaborators of NE-1034 (AR, UCD, DE, DK, NC, NL, NY, ON, PEI and VT). These studies yielded critical new knowledge regarding impact of physiological factors on aspects of disease susceptibility, disease progression, activities and interrelationships of innate and adaptive immune systems, virulence of pathogens, immunodominant epitopes, nature of effective or ineffective host responses and approaches for disease intervention and prevention. An eight year longitudinal survey of SPF flocks at NY infected with immunosuppressive chicken infectious anemia virus (CIAV) showed that antibody development to CIAV started in general on or after sexual maturity with significant differences in levels of seroconversion during this period. These findings suggest that the infection may remain latent and that reactivation is linked in part to sexual maturity. Studies on avian influenza (AI) by NY using the highly pathogenic H5N1 (VN1203/04) isolate showed that thrombocytes play an important role in the pathogenesis in chickens but not in ducks. ON conducted extensive study on gene-expression profiles during infection with different serotypes of Marek's disease viruses in a variety of tissues known to play a key role in infection, latency and transmission of these MDV. DE studied the effects of innate immune stimulants on the replication of MDV vaccine strains and overall vaccine efficacy. Inclusion of some select innate inducers (e.g. PAMPs) did not interfere with vaccine virus replication, despite potent induction of innate responses but did not have appreciable effects on cell-mediated immune function and MD protection. Gene expression profiling by USDA-ADOL between rMd5 and rMd5Dmeq infected chickens revealed that Meq functions as an immunosuppressive oncogene that results in down-regulation of many immunerelated genes and may be controlling the expression levels of p53 involved in regulating the cell cycle and tumor development. NC worked to characterize numerous circulating strains of the type- 2 turkey astrovirus isolated from commercial turkeys across the U.S. to identify potential virulence markers. In the infected host, astroviruses induced a reduction in the apical expression of sodium/hydrogen exchanger-3 which contributes to mal-absorption and diarrhea. The infected epithelial cells responded to infection by expressing inducible nitric oxide synthase (iNOS), which likely plays a key role in eliminating the virus in the immunologically immature host. VA assessed the differential genetic resistance to clostridial toxins in select chicken lines and conducted serum protein profiling and identification of potential blood markers in Eimeria-infected chickens from commercial genetic stocks. Influence of environmental factors on mucosal and systemic immunity has been a focus of research by AR, NC, NL, ON, and VA. AR and collaborators observed different vasoactive and cytokine responses to local pulmonary inflammatory activities induced by PAMPs or vascular occlusion in ascites-susceptible or -resistant broiler lines. ON developed a probiotic formulation that possesses immune stimulatory activities, which they plan to test in commercial settings in the near future. VA is evaluating the effects of antibiotic alternatives in commercial chickens (e.g. betaglucans, probiotics) using various delivery routes and disease models. Immunomodulatory effects of concurrent administration of model-antigens and PAMPs typically present in the air of poultry houses were observed by NL particularly in young broiler and layer chickens. Adaptive systemic immune responses were also shown to be affected by the absence of microflora in the gut following antibiotic treatment as well as by administration of probiotics. Through these studies, the period of 3-6 weeks of age was identified as a critical time in the development of mucosal immunity. Investigations into the role of nutrition in the immune responses of poultry by UCD established that nutrients that have primarily regulatory functions (vitamins A and D, and essential fatty acids) had greater effects on development of the immune system than nutrients that serve as anabolic precursors (amino acids, energy, minerals). Vitamin A deficiency during development diminished B lymphocyte maturation and the breadth of the antibody repertoire. The entire cost of the adaptive immune response (specific antibodies and lymphocytes) was easily fueled by the decay of the acute phase proteins produced during the innate response, suggesting that adaptive immunity has no net nutritional cost. NC demonstrated that changes in the intestinal microflora of poultry alter the amount of energy consumed by the immune response. PEI characterized local and systemic innate immunity in poultry during nutritional intervention using yeast derivate carbohydrates (YDC). In several comparative studies using broiler chickens fed conventional diets including an anticoccidial (Monensin), a growth promoter (BMD), and an anticoccidial plus YDC they found that the inclusion of YDC (23% mannans) affected several immunological parameters, including regulation of intestinal microflora, intestinal architecture, cytokine expression and enhanced neutrophil activity. DK conducted studies to characterize and examine the function of mannose-binding-lectin (MBL, innate immunity) in susceptibility of poultry to different pathogens. Using chickens selectively bred for a high or a low serum concentration of MBL, low circulating levels of MBL were associated with reduced ability of poultry to respond to pathogens such as IBV, E. coli, Pasteurella multocida, and Ascaridia galli. These results confirm that MBL, as shown in mammals, plays a major role in the outcome of various infections in chickens and may emerge as a biomarker for disease susceptibility. Similarly, investigations into natural antibodies (NAb) and health in poultry by NL showed that high levels of NAb, especially of the IgM isotype, correlate with lower mortality during a lay period. NAb levels were found to be very heritable (0.4), related to SNP in immune response-, behavioral-, and unknown-gene regions. Whether NAb originate from introduction of the intestinal microbiota or reflect homeostatic auto-antibodies is subject of future studies. Their studies also showed that NAb binding to protein extracts from chicken organs may provide a fingerprint for measuring the health status of individuals.

Objective 3 "To Develop and Employ Genetic Stocks, Methods, Reagents and other Tools to Assess Basic Immune Function, Characterize Immune Evolutionary Processes, Guide Genetic Selection, and Increase Resistance to or Protection Against Avian Diseases"

Efforts by the group resulted in development of a variety of tools and basic data for continued research on the genetic bases of resistance and immunity to avian diseases. A number of stations (AR, UCD, DK, IA, NC, NL, ADOL) developed, maintained, characterized, and made available unique genetic resources to the NE-1034 members and the entirety of the poultry research community. These included genetic lines that are highly inbred and contain MHC-congenic sets, are MHC-defined, and/or exhibit defined disease susceptibility/resistance characteristics. DK breeds of chicken were found to have five B21-like haplotypes including B131 (broiler origin) and BW1 (Red Jungle Fowl, *Gallus gallus gallus* origin). AL generated a better understanding of the mucosal immune system in chickens induced by IBV vaccines or Ad5 vaccine vectors expressing the avian influenza HA gene. AL found the head associated lymphoid system, i.e., conjunctiva-associated lymphoid tissues (CALT) and Harderian glands (HDGL), to generate immunity to avian pathogens after ocular or in ovo immunization. CALT generated more of a cell-mediated immune response and after priming seemed to contain cytotoxic effector memory T cells, while the HDGL generated more of a B cell response. The spleen played a minor role after mucosal IBV priming, but generated the highest IFN-gamma response after boosting, indicating induction of a central memory T cell response. IgA dominated the primary response, while IgG dominated the memory response to IBV. Advances made by the group to facilitate the study of immunity include development of chicken whole genome 44K gene expression array (UCD) that has been widely used in the poultry community; increased availability of genetic information to conduct targeted qPCR expression analyses of cytokine and other genes; and, B-cell spectratyping including IgA, IgG and IgM isotypes. Western blotting was optimized to measure post translational polymorphism of NAb and auto-antibody fingerprints. A minimally invasive procedure to monitor cellular immune responses in vivo using the growing feather as an in vivo test-tube was developed by AR. Based on analyses of antibody responses using a peptide array, AL reported that mutations in the S1 protein of IBV contributed to immune escape. VT identified innate immune markers correlating with disease resistance to coccidiosis. NC developed reagents for use in the study of turkey immunity, including polyclonal antibodies to the turkey iNOS protein. NY developed an antigen-antibody complex vaccine that does not cause damage in chickens lacking maternal antibodies to CIAV and protected against replication of a challenge virus. Over the last four years, DE's research on the evolving MDV resulted in identification and development of various MDV mutants for research. Included are mutations in the main oncogene (Meq) of MDV, Meq splice variants observed during MDV pathogenesis, and a glycoprotein L mutation common to high virulence MDVs. Studies with these MDV mutations focused on their effects on transactivation, target gene expression, cell shape and mobility, immune evasion and pathogenesis and the immune response. Use of these mutated MDV viruses has already provided insight into the impact of viral genes on tumor composition, mechanisms by which MDV regulates immune-associated genes, virulence, viral T cell epitopes presented, and effects on the early patterning of immune responses. While transgenic approaches to study the function of genes is not yet readily available for avian species, virus vectors approaches have been employed by members of the group. ON developed a prototype virus-vectored system for down-regulation the expression of IFN-gamma, a system that can be modified for down-regulation the expression of other cytokines in the future. WI has created a generation 3 self-inactivating lentiviral expression vectors for in ovo administration in preparation for expression of the Mx transcript from the highly pathogenic AI virus resistant Blue-winged Teal in chickens under control of an inducible promoter.

Objectives

1. To determine how allelic variation influences the efficacy of innate and acquired immune functions.
2. To identify factors and agents affecting poultry immune development, function, dysfunction, and pathology.
3. To develop and employ genetic stocks, methods, reagents, and other tools to assess basic immune function, characterize immune evolutionary processes, guide genetic selection, and increase resistance to or protection against avian diseases.

Methods

Over the past 30-40 years, the field of immunology and immunogenetics continuously has seen extensive advances, particularly with regard to immune system function and development in mammals. The increased understanding of immune system development and function stems primarily from studies on the mouse model, where manipulation of gene-expression seems to have unlimited potential in dissecting mechanisms of cell development and function. With the availability of the chicken genome sequence since 2004, holistic approaches such as genome-wide sequencing and whole transcriptome and proteome analyses have been carried out providing new insights into unique features of the avian immune system, disease resistance and susceptibility as well as the mechanistic aspects of immune system development and function in chickens. As additional avian resources are becoming available particularly through contributions of member stations, such as the recently published turkey genome and much improved chicken genome, poultry scientists are well positioned for further discoveries pertaining to avian immunity and their inherent responses to disease challenges. Since the complexity of the immune system's molecular and cellular components, genetic regulation underlying the functional responses of the immune system, and the interplay between genetic, immune system, environmental, physiological and nutritional factors have become apparent, such discoveries will significantly better our knowledge in this arena and further enhance the U.S. global competitiveness.

1. To determine how allelic variation influences the efficacy of innate and acquired immune functions.

Variation in DNA sequence can directly impact immune function, can modulate expression of genes that impact immune functions, and can serve as markers for other causative variants with which they are in linkage disequilibrium. This project will apply genetic, genomic and immunological approaches to assess the impact of allelic variation on response to specific pathogens or other immune stimuli. Several stations will focus on the major histocompatibility complex (MHC) region. **BRI** will investigate the role of MHC-Y haplotypic variation and, when possible, individual loci variation on immune responses in chickens. **WVC** will keep investigating the underlying genetic mechanisms of differences in innate immune functions, particularly macrophages, of B2 and B19 haplotypes. **WVC** will further analyze if epigenetic modifications/variations are responsible for gene dysregulation in B19 haplotypes after stimulation of macrophages with IFN γ . **UA** will expand its studies on analysis of the many alleles of the MHC class II region of mallard ducks by moving to high throughput approaches with additional animals. **UCD** will study the bases of resistance to respiratory diseases, specifically infectious bronchitis virus (IBV), using MHC congenic lines. In addition to in vivo challenges, **UCD** will perform in vitro challenges to investigate the specifics of the immune response without background noise. **UCD** will assess and compare cell activity in tracheal cultures and macrophages derived from relatively resistant and susceptible congenic chicken lines. In **UCD** and **IA**, genetic variants in diverse chicken populations that differ in immune response to viral infection will be identified using next generation sequencing and high density SNP genotyping, and associations between genetic variants and immune-related parameters such as viral load, antibody response, and others will be analyzed. **IA** will investigate the genetic basis of response to avian pathogenic *E. coli* (APEC), including the impact of allelic variation and allele-specific expression, and association of genetic pathways identified by RNA-seq with immune phenotypes. To identify candidate genes and gene regulatory networks for resistance to herpesvirus infection, **UMD** will analyze transcriptomic data in immune cells from infected and non-infected individuals of two reciprocal crosses. **WV** will use sequence and SNP analyses to identify and characterize alloantigen system genes whose products are detected by antibodies. **NL** will focus their efforts on looking into the associations, using keyhole limpet haemocyanin (KLH) of SNP's with natural antibodies of isotypes IgM and IgG. These associations will be assessed binding KLH and auto-antigens in laying hens.

2. To identify factors and agents affecting poultry immune development, function, dysfunction, and pathology.

There are numerous genetic, environmental, nutritional, physiological, management, and microbial factors that stimulate, regulate, and shape the immune response of poultry. The members of NE-TEMP 1834 have been at the forefront of research to understand basic mechanisms and unique features of the the avian immune response, to develop novel and effective means to promote poultry health and production. Over the past 4 years the members of NE-TEMP1834 have produced over 250 peer reviewed publications, and awarded approximately 15 competitive NIFA grants worth over \$6 million. The majority of these efforts are related to our understanding of factors and agents affecting poultry immune development, function, dysfunction, and pathology, and represent some of the most influential studies related to avian immunity over the past 5 years. As this highly successful project continues, the members of NE-TEMP1834 will continue to use a variety of poultry systems, immunomodulatory approaches, and pathogens to expand our understanding of the avian immune response. **AR** will determine immunopathology, immune system dysregulation, and the role of environmental factors in multifactorial, non-communicable diseases such as fibrosis/scleroderma, vitiligo, thyroiditis and other (auto-) inflammatory diseases. **AR** will determine basic innate-and adaptive immune system mechanisms in poultry and immunomodulatory effects of nutrients on immune system development and function. Methods will include a range from whole animal studies to histological, cellular and molecular examinations, including gene-expression at the transcriptome and protein level. **UCD** will investigate the effects of viral respiratory infection, specifically IBV, on the upper respiratory microbiome. **UCD** will examine the difference in immune function and development in genetically distinct chicken lines using RNA-seq and flow cytometry. Additionally, the molecular mechanism of disease resistance will be further investigated using CRISPR-cas9. **DE** will continue working with Industrial partners to test innate immune inducers that increase the resistance of poultry to various microbial agents. **DE** will use transcriptomic analysis of the effects of these inducers on innate signaling and ultimate patterning of acquired immune responses. **DE** will also employ a kinomic approach to study poultry health and disease from an immunometabolism perspective. This approach broadens our view of health, metabolism, disease pathogenesis and potential intervention strategies, and identify metabolic intermediates or immune modulatory compounds to be used therapeutically. **GA** will study the development of T-regulatory cells and the contribution of T-regulatory cells to Salmonella persistence, with ultimate goal of developing a nanoparticle based vaccine against Salmonella. **UMD** will generate deep sequencing libraries from avian immune cells in order to identify epigenetic markers, patterns of alternative splicing, and noncoding RNA such as microRNA and long intergenic noncoding RNA (lincRNA). Most importantly, **UMD** will ascertain the factors affecting avian immune development such as enhancer, repressor, insulator and transcription binding sites (TFs) and explore their influences on chromatin and the association with immune function, dysfunction and pathology. **NC** will investigate the interaction between the host's intestinal microbiome and development/function of the avian immune system. As a part of these investigations, **NC** will focus on how differences in host genetics affect this process, identifying key members of the microbiome, their metabolites, and ultimately their role in helping the bird resist colonization and infection by avian pathogens and foodborne pathogens that reside in poultry. **NL** will use homozygous SNP-typed TLR1A variant chickens, challenged with various types of infectious agents to understand its role in immunity and the production of natural (auto-) antibodies. Additionally, **NL** will investigate transgenerational epigenesis of specific immunity and innate immunity. **OH** use in ovo inoculation of d18 embryos with various

types of bacteria to investigate the impact of different pioneer bacteria on GIT immune system development. As part of this work the microbiome and proteome will be analyzed up to 10 days of age, leading to deeper insights into which types of bacteria promote appropriate immune development and improved sustainability. **PEI** will focus on the nutritional immunological factors regulating immune responses, animal health, and food safety, with the ultimate goal to advance our understanding of the nutritional, microbiological; and molecular components affect the chicken immune response. **VT** will focus on the impact of in ovo and in vivo delivered supplements on the gut microbiota and development of the immune system in poultry (chickens and turkeys). **VT** will assess how these treatments affect gut physiology (tight junction disassembly/restructure), the impact on cellular and body metabolism, feed intake and performance, and gut immune responses. As part of these studies **VT** will examine these effects under specific challenges such as; necrotic enteritis, coccidiosis, APEC, salmonellosis, in addition to blackhead and cellulitis in turkeys. **WU** will analyze epigenetic modifications during macrophage development and investigate what influence immune stimulants (liposomal vaccines, adjuvants and others) have on training of macrophages and other immune responses in different B haplotypes.

3. To develop and employ genetic stocks, methods, reagents, and other tools to assess basic immune function, characterize immune evolutionary processes, guide genetic selection, and increase resistance to or protection against avian diseases.

When paired with techniques such as next generation sequencing, SNP analysis, and qPCR, highly inbred chicken and turkey lines, sets of MHC-congenic lines, random breed lines and lines with distinct phenotypes allow the detection and selection of functional genetic elements that are related to immune function. To the extent that facilities and research budgets allow, **IA** will maintain, study and share with collaborators, several unique genetic stocks of chickens for research on the genetic basis of immune response and response to pathogens. The stocks include highly inbred lines, sets of MHC-congenic lines, and advanced intercrosses of lines with distinct phenotypes. **UCD** Regulatory elements such as enhancer, insulator, promoter in chicken genome will be functionally annotated and functional elements related to immune function will be identified. Genetic variants associated with disease resistance to virus infection will be used to genetically enhance broad immunity and resistance to specific pathogens in poultry. **BRI** MHC-Y was originally identified through polymorphic restriction fragments revealed in Southern hybridization. Until recently Southern hybridizations were the only means for revealing MHC-Y genotypes. **BRI** has developed simpler PCR-based methods for distinguishing MHC-Y haplotypes. These simpler methods make it easier to MHC-Y type large numbers of birds. **BRI** will continue to improve these methods and make them available for those wishing to define the MHC-Y haplotypes within genetic stocks and for use in defining the role of MHC-Y in genetic resistance to disease. **NC** To further the long-term goal of understanding how the genetic makeup of poultry determine its response to parasitic infections such as *Histomonas meleagridis*, experiments will be performed to identify SNPs associated with turkeys that can and cannot survive infection with a virulent strain of *H. meleagridis*. **NL** Chickens will be selected and bred such that homozygous genotypes will be obtained for a TLR1A variant on chromosome 4 using SNP-typing in combination with high natural antibody (Nab) levels to KLH (CC variant) and low NAb levels to KLH (GG variant). **ARK** To aid in research related to cellular and humoral immunity, genetic lines that spontaneously develop autoimmune diseases will be maintained and shared with project collaborators and techniques will be developed to monitor responses to antigen in blood and tissues. **WV** Congenic lines 003.R2 and 003.R4 will be maintained for use by project collaborators. Line 003.R4 differ from 003.R2 by a 225 bp insert in the BG-1 gene's 3' UTR resulting in variation in immune responses to various diseases. **ADOL** Experiments will be performed to develop assays to assess epigenetic modifications in a commercial or inbred chicken line, followed by analyzing the genetic variations associated with differential immune responses in B2 and B19 haplotypes. These assays will include ChIP seq, ATAC seq, PLAC seq and others. The genomic data will be made available to the poultry community at large as a resource for further research. **(ADOL, NC, WVC)** To study *Salmonella* colonization in broilers, two immunologically divergent lines of broilers based on selecting for a high and low phenotype of key innate immune markers in both sires and dams will be generated. Additionally, the role of the gut microbiome and intestinal mucosal response (secretory IgA [sIgA]) will be examined in the founder and the selected High and Low lines to determine the interplay between host genetics, the gut microbiome and local immune response, selection pressures, and *S. Enteritidis* colonization. **(UA)** To assess immune function in chicken cells, they will make an interferon reporter construct, mCherry-tagged IRF1 and IRF7 constructs, and many cloned genes and will also develop qRT-PCR primers for many chicken immune genes including IFN-beta, Mx, OASL. **(VT)** To continue improving the turkey transcriptome by further sequencing of additional tissue RNAs, The data will help in refining the global turkey transcriptome during early development, updating tissue-specific and overall annotations of both transcriptome and genome, and providing public tools for comparative analyses in poultry and other avian species. **(UMD)** By integrating different "OMICS" data, advanced methods on host-virus interaction with small number of chicken immune cells will be developed. In addition, methods on immune response modeling analysis will be established and share with project collaborators.

ACRONYMS

AR = Arkansas

UCD = Univ of California-Davis

DE = Univ of Delaware

GA = Univ of Georgia

IA = Iowa State

BRI = Beckman Research Institute, City of Hope

UMD = Univ of Maryland

NC = North Carolina State Univ

NL = Wageningen Univ

OH = Ohio State Univ

PEI = Prince Edward Island Univ

UA = University of Alberta

ADOL = United States Department of Agriculture, Avian Disease and Oncology Laboratory

VT = Virginia Tech

WU = University of Washington

WV = West Virginia University

WVC = Western University of Health Sciences

Measurement of Progress and Results

Outputs

- Assess the impact of allelic variation on response to specific pathogens or other immune stimuli Comments: This project will apply genetic, genomic and immunological approaches to assess the impact of allelic variation on response to specific pathogens or other immune stimuli.
- Understand basic mechanisms and unique features of the avian immune response, to develop novel and effective means to promote poultry health and production. Comments: The majority of these efforts are related to our understanding of factors and agents affecting poultry immune development, function, dysfunction, and pathology, and represent some of the most influential studies related to avian immunity over the past 5 years. As this highly successful project continues, the members of NE1834 will continue to use a variety of poultry systems, immunomodulatory approaches, and pathogens to expand our understanding of the avian immune response.
- Detection and selection of functional genetic elements that are related to immune function. Comments: When paired with techniques such as next generation sequencing, SNP analysis, and qPCR, highly inbred chicken and turkey lines, sets of MHC-congenic lines, random breed lines and lines with distinct phenotypes allow the detection and selection of functional genetic elements that are related to immune function
- Continue to maintain unique genetic resources including selected lines, inbred, congenic and recombinant congenic lines as well as experimental lines.
- Develop additional genetic material (e.g. line crosses) as needs arise.
- Generate atlases of transcriptional responses under normal and disease conditions including those to viruses (e.g. MDV, IBV, AI, AIV, and IBDV) and bacteria

(e.g. Salmonella, Campylobacter, and Clostridium) using RNA-Seq, microarrays, real-time PCR or other methods.

- Identify individual genes or quantitative trait loci (QTL) associated with disease resistance or immune response via next generation sequencing, microarray technology or emerging high throughput genotyping methods.
- Uncover and develop new single nucleotide polymorphisms (SNP) markers, microarrays, peptides, antisera, primer sets, and serum chemistry analyses to categorize immune responses in normal and disease states.
- Use genetic, environmental, dietary and immunostimulation methods to enhance protective immunity.
- Continue to identify new and characterize recently described immune response elements (e.g. cytokines, receptors, MHC molecules) and their involvement in resistance to disease.
- Use refereed publications, symposia, invited lectures and informal discussions at regional, national and international workshops and meetings to disseminate information to stakeholders and public

Outcomes or Projected Impacts

- Assess the impact of allelic variation on the response to specific pathogens By doing that we will be able to better understand immune responses elicited by chickens in response to a diverse range of pathogens and strategize on their prevention and control
- Promote poultry health By knowing and understanding processes and immunity elicited by avian species to diverse challenges (infectious and non infectious)
- Detection of elements that dictate the immune response in poultry Via molecular tool we will be able to detect these elements and maybe manipulate them to get the best of an immune response generating desired outcomes in terms of protection of the poultry population
- More, better and safer poultry products to feed a population in constant increase Our ultimate goal in this project is to producer more, better and safer poultry product in order to feed an increasing human population in the world
- Identify individual causative genes or quantitative trait loci will improve poultry health and animal agriculture in general through marker-assisted selection and breeding or technological applications.
- Development of new technologies to assess or improve the immune response.
- Appropriate immune responses and improved disease resistance through immune modulators will augment production efficiency.

Milestones

(2018): Better understand poultry immune responses to infectious and non infectious agents focusing on innate and adaptive responses using genetic stocks, molecular, serological and other techniques looking for resistance to diseases and conditions

(2019): Using complex molecular tools we will be able to better understand immunological pathways stimulated after infectious and non infectious challenges.

(2020): Better understand factors and agents affecting poultry immune development, function, dysfunction, and pathology

(2022): Detect distinct poultry phenotypes and /or functional elements related with immune function

(2023): Advance knowledge regarding basic aspects of innate and adaptive immune system development and function.

(2023): Improve transgenic technology to more effectively elucidate biological functions of known and unknown genes in the immune system.

(2023): By 2023 we should be able to better understand the effects of MHC against certain diseases and use this info to breed better chicken populations in commercial and non commercial settings

Outreach Plan

Industry stakeholders are invited to and frequently attend the annual project meetings. Their attendance provides an opportunity for information exchange. For example, representatives of breeder organizations can learn of the latest genetic advances in disease resistance from the project scientists. Technical Committee members gain knowledge of emerging field problems that the project can address through experiments. The combined efforts of the NE-1834 stations will generate new scientific data. Refereed publications, online data bases of genetic lines and genome/transcriptome information, symposia, invited lectures and informal discussions are some methods used to disseminate information. Project investigators have made significant scientific contributions to the improvement of poultry immune responses as well as the genetics of disease resistance. Cooperation among project members and with other researchers will remain a hallmark of NE-1834. This cooperative effort will include sharing scientific expertise and genetic resources held at numerous project stations. The addition of several international members has expanded the research scope and global dissemination of research findings.

Variation in DNA sequence can directly impact immune function, can modulate expression of genes that impact immune functions, and can serve as markers for other causative variants with which they are in linkage disequilibrium. This project will apply genetic, genomic and immunological approaches to assess the impact of allelic variation on response to specific pathogens or other immune stimuli. Several stations will focus on the major histocompatibility complex (MHC) region. Continuous communication and collaboration will be performed. Historically this communication is by direct collaboration of participants and / or by presentation and diffusion of the results at scientific meetings, including our annual meeting. This constant contact allows to strategize and plan in order to avoid research topics duplication.

There are numerous genetic, environmental, nutritional, physiological, management, and microbial factors that stimulate, regulate, and shape the immune response of poultry. The members of NE-1334 have been at the forefront of research to understand basic mechanisms and unique features of the avian immune response, to develop novel and effective means to promote poultry health and production. As an example of this fruitful collaboration, over the past 4 years the members of NE-

1334 have produced over 250 peer reviewed publications, and awarded approximately 15 competitive NIFA grants worth over \$6 million. The majority of these efforts are related to our understanding of factors and agents affecting poultry immune development, function, dysfunction, and pathology, and represent some of the most influential studies related to avian immunity over the past 5 years. As this highly successful project continues, the members of NE1834 will continue to use a variety of poultry systems, immunomodulatory approaches, and pathogens to expand our understanding of the avian immune response.

When paired with techniques such as next generation sequencing, SNP analysis, and qPCR, highly inbred chicken and turkey lines, sets of MHC-congenic lines, random breed lines and lines with distinct phenotypes allow the detection and selection of functional genetic elements that are related to immune function. What has made our project so successful is the level of collaboration and interaction of our group. Even though the members of the group come from diverse poultry fields all their expertises are imprinted in the work we do. This high level of planning and coordination is due to the communication we have via meetings and collaborations.

Organization/Governance

The planning and supervision of the Multistate Research Project shall be the responsibility of the Multistate Technical Committee. The membership of this committee shall consist of an Administrative Advisor, which this time will be an active member of this project, a technical representative of each participating agency or experiment station, and a representative of the USDA National Institute of Food and Agriculture (NIFA). The voting membership shall consist of the Technical Committee Representatives. The Technical Committee shall be responsible for review and acceptance of contributing projects, preparation of reviews, modification of the multistate project proposal, and preparation of an annual report for transmittal by the Administrative Advisor upon approval to NIFA. Annual written reports will be prepared by each technical committee member and distributed at the annual meeting. A limited number of the compiled annual reports will be available upon request from the Administrative Advisor. The Technical Committee will meet yearly and elect a secretary, who will serve the year after election and as the chairperson the following year. An Executive Committee will be formed to conduct all business of the Technical Committee between annual meetings. The Executive Committee shall consist of the current Technical Committee Chairperson, the Secretary, and the two immediate Past Chairpersons. The chairperson may name other subcommittees as needed to perform specific assignments. They may include subcommittees to develop procedures, manuals, and phases of the multistate project, to review work assignments; to develop research methods, to prepare publications, and to write proposals. Other agencies and institutions may participate and vote at the invitation of the Administrative Advisor. Minimum expectations for Technical Committee members are submission of a written annual report every year, and attendance at an annual meeting including presentation of research results at least one year out of two. Collaborators may include emeritus members with an interest in attending annual meetings, scientists who wish to contribute by virtue of having special expertise or interest, and those who engage in research interactions with an individual Technical Committee member. Collaborators should submit a written report every year, and present their progress when attending the annual meeting. Guests who attend an annual meeting through special connection to the Technical Committee (i.e. host institution) are invited to make a brief presentation of their interests and ongoing research.

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237 total publications from NE-1334 Project participants 2013-2017

***= 33 cooperative publications among 2 or more project participants**

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Land Grant Participating States/Institutions
GA,CA,AR,NC,WV,IA

Non Land Grant Participating States/Institutions
City of Hope Beckman Research Institute, Western University of Health Sciences

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Ashwell, Christopher		North Carolina - North Carolina State University		303 303	3220 3210	1040 1040	0.20	0.00	0.00	0	0
Drechsler, Yvonne		Western University of Health Sciences	1,2,3	304 311	3299 0	1090 1080	0.00	0.00	0.00	0	0
Erf, Gisela F.	Yes	Arkansas - University of Arkansas		311 311 311	3210 3220 3230	1090 1090 1090	0.30	0.00	0.20	0	0
Koci, Matthew D		North Carolina - North Carolina State University		311 311 311 311 311	3299 3299 3299 3299 3299	1040 1080 1090 1101 1100	0.20	0.00	0.20	0	0
Lamont, Susan J.	Yes	Iowa - Iowa State University	1,3	311 311	3299 3299	1080 1090	0.10	0.00	0.00	0	0
Miller, Marcia		City of Hope Beckman Research Institute	1	311	3299	1080	1.00	1.00	0.00	0	0
Selvaraj, Ramesh K.	Yes	Georgia - University of Georgia		311	3299	1090	0.10	0.10	0.05	0	0
Taylor, Robert	Yes	West Virginia - West Virginia University		311 311 303 303	3210 3220 3210 3220	1090 1090 1090 1090	0.10	0.00	0.00	0	0
Zhou, Huaijun		California -Davis : University of California, Davis		303 304 306 311 304 311	3210 3210 3210 3210 3220 3220	1090 1101 1080 1040 1080 1080	0.00	0.00	0.00	0	0

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
311-3299-1090	0.1	0.1	0.05
311-3299-1090	0	0	0
311-3299-1101	0	0	0
311-3299-1080	1	1	0
311-3210-1090	0.1	0	0.2
311-3220-1090	0.1	0	0.2
311-3230-1090	0.1	0	0.2
303-3210-1090	0	0	0
304-3210-1101	0	0	0
304-3220-1080	0	0	0
306-3210-1080	0	0	0
311-3210-1040	0	0	0
311-3220-1080	0	0	0
Grand Total:	2.00	1.10	0.45

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
311-3299-1040	0.04	0	0.2
311-3299-1080	0.04	0	0.2
311-3299-1090	0.04	0	0.2
311-3299-1100	0.04	0	0.2
311-3299-1101	0.04	0	0.2
303-3210-1090	0.03	0	0
303-3220-1090	0.03	0	0
311-3210-1090	0.03	0	0
311-3220-1090	0.03	0	0
303-3210-1040	0.1	0	0
303-3220-1040	0.1	0	0
311-3299-1080	0.05	0	0
311-3299-1090	0.05	0	0
304-3299-1090	0	0	0
311-0-1080	0	0	0
Grand Total:	2.00	1.10	0.45

Program/KA	Total FTE
0	0
311	0.03
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
Grand FTE Total: 0.1	

NE_TEMP1835: Resource Optimization in Controlled Environment Agriculture

Status: Under Review

Duration 10/01/2018 to 09/30/2023

Admin Advisors: [\[Adel Shirmohammadi\]](#)

NIFA Reps:

Statement of Issues and Justification

Statement of Issues

Agriculture is threatened by its sensitivity to climate change and irregular weather patterns. Accurate projection of yields and harvest time are essential to pair production with market demands and forecast financial margins. In controlled environment agriculture (CEA), farmers can closely control most aspects of the growing environment and adjust production to weather and market fluctuations. The ability to control environmental and agricultural inputs results in increased resource efficiency per production area and reduced shrinkage compared with outdoor field production. Hence optimized efficiency and use of low-cost alternative resources is fundamental for the successful future of CEA operations. Adaptation to climate change to overcome potential risks demands strategies that match current and projected conditions.

CEA is an economically important sector in agriculture in the U.S. Despite the economic crisis in 2008, the USDA Census reported that the number of operations and sales in specialty crops increased by 7.5% and 17%, respectively, from 2009 to 2014. IBISWorld reported that the hydroponic industry is on the growth stage in the market, which is characterized by many new companies entering the market, rapid technology change, growing acceptance by consumers, and rapid introduction of products and brands. CEA is particularly important in northern climates where year-round production is only possible in protected agriculture, and in urban areas where space is limited.

While we have advanced the understanding of energy, light, and water utilization in CEA we still must further understand the potential of emerging technologies on energy and water optimization. The results of our previous project expanded knowledge on (1) the effect of irrigation practices and nutrient management on plant health, (2) the energy footprint and efficiency of different type of lights and production systems, and (3) sensors to monitor energy use. For the next project, we propose evaluating new greenhouse lamps, wavelengths of light, and layout designs and their effect on plant growth. We also aim to evaluate and design low energy alternatives to ventilate high tunnels, cool and heat greenhouses, and sensors to monitoring environmental conditions and plant growth. We will evaluate organic fertilizers for production of edible crops and test alternative growing media for container production. Finally, we will evaluate alternative irrigation methods and water sources to reduce the amount of fresh water for crop production. We will evaluate non-chemical alternatives to control diseases. Ultimately, we **aim** to measure energy and water consumption and develop saving strategies. We will deliver recommendations to commercial growers.

Our team consists of 24 members who represent 18 different experiment stations. Four members (FL, NH, IN, IL), are representatives of three new stations, who joined our group in the 2016–2017 period. Five new members (2 OH, IA, NC, MI) joined our group in this upcoming period. Our team has produced a total of 158 scholarly outputs which include 11 dissertations/theses, 1 book, 10 book chapters, 45 refereed journal articles, 20 symposium proceedings, and 71 presentation papers. Our team has also dedicated to translate research outcomes to stakeholders via 170 outputs, including 72 popular articles, 28 sponsored-workshops, 28 participations in workshops, and 42 other creative works. We have a long standing history of collaboration and productivity and the continuous enrollment of new and young faculty reflects the steady growth of our field of research.

The environmental conditions in each of our stations vary by location, nonetheless by conducting research as a multistate group we are able to develop robust models and then tailor research projects and recommendations to our local stakeholders. Moreover, our team brings together a complementary knowledge base that is essential for optimizing resource management in CEA. Members include greenhouse engineers, plant scientists, and an economist, all with practical experience in solving problems in multidisciplinary environments and direct contact with stakeholders.

Justification

A major benefit of growing plants in CEA operations is the ability to use sensors to monitor the production environment to make informed decisions about fertilization, irrigation, heating, cooling, and lighting. Over the last decade, new technologies have emerged in the industry with the potential to provide growers with alternatives to improve production efficiency and profit

margins. However, science-based guidelines are needed for growers to make informed-decisions about the feasibility of implementing these technologies in their operations. Growers can achieve consistent production (i.e. increase crop cycles per year, reduce time from seed to harvest, and improve flowering regulation) by reducing temporal and spatial variation in the greenhouse environment. Our team believes that sensors and control strategies are essential for efficient production in CEA operations. CEA production systems range in technological complexity from high tunnels to highly controlled environments (e.g. plant factories). Strategies to control fertilization, irrigation, heating, cooling, and lighting will vary by CEA production systems. We aim to work with the whole range of CEA operations.

Heating, cooling and lighting options determine energy consumption and are strongly correlated with plant growth rate. Therefore, the efficiency of heating, cooling, and lighting have a direct impact in the bottom-line of businesses. Fan performance and ventilation alternatives to achieve homogeneous temperatures and humidity, temperature prediction models using thermal environment in high tunnels and greenhouses, and alternative lighting wavelengths, intensity and duration can be used to regulate plant growth and maximize outputs (production) per input (energy).

Water management is closely tied to nutrient management, particularly in greenhouse production where plants receive nutrients via fertigation. Automated-irrigation scheduling using sensor-based set-point irrigation has the potential to reduce water volume significantly. Despite the low-cost of water, we anticipate that economic benefits may result in terms of reduced labor, fertilizer injection, and disease incidence. Fertilizer reduction can also lower the environmental impact related to fertilizer runoff into natural habitats, and fertilizer mining and processing. CEA facilities can be designed or easily retrofitted to maximize water use efficiency, while at the same time minimize or eliminate leachate from contaminating the outdoor environment (e.g., recirculating ebb and flood irrigation). We propose to determine set-point irrigation control of different irrigation species in propagation and evaluate how alternatives substrates and water sources impact water use in container production.

In 2017, the National Organic Standard Board voted to allow USDA Organic certification of hydroponic production systems. This news provides CEA operations a gateway to a growing market. However, high efficiency in organic greenhouse production is not possible yet. Matching nutrient availability with crop demand is a major challenge in organic greenhouse production, which can have negative effects in plant quality and productivity. Nutrient release from organic compounds is linked to microbial activity, hence associated with environmental conditions and cultural practices that affect microbiological processes. We propose to develop nutrient release curves of organic-sources of fertilizer under multiple environmental conditions and provide recommendations on how to improve predictability of nutrient release.

Even though the U.S. is a pioneer in CEA, there is still a lot of room for resource optimization. For example, the water footprint global average (gal per pound) for tomato production is 25.6, the average in the U.S. is 15.2 which is 15 times more than the average in the Netherlands where the majority of crops are grown in greenhouses. Our team believes that optimization of resources used in CEA can result in significant reduction of agricultural inputs (water, fertilizer, and energy), increased production efficiency (days to harvest, crops per year, yields per square foot greenhouse), and lowered production costs.

Related, Current and Previous Work

Our approach has been to conduct applied research that is relevant to industry needs. We have provided valuable greenhouse engineering technology to the industry throughout our history through technology transfer. Some examples include the use of air-inflated double-layer polyethylene films as greenhouses cover material, advances in hydroponic production systems and supplemental lighting, floor heating for greenhouses, and the use of energy curtains. Many of the technologies originally developed by members of this project are now industry standards for improving sustainability and conserving energy. Our team has trained several professionals who are now leaders in the CEA industry.

Alternative Energy Sources and Energy Conservation

Energy is the second largest input cost in greenhouse production. Unheated high tunnels were found to be a suitable energy-efficient alternative to heated greenhouses for finishing cold-tolerant bedding plants in the spring in northern U.S. locations (IA, IN, NJ, NY) and, in some cases, crops finished in high tunnels led to high plant quality (Currey et al., 2014; Gerovac et al., 2015). The energy efficacy (light output per unit electricity consumed) of supplemental lights has increased greatly in the past 20 years. Some, but not all, light emitting diodes (LED) have higher energy efficacy than traditional high-pressure sodium (HPS) fixtures (Wallace and Both, 2016). The decision on when to add supplemental light to the greenhouse is often based on a time clock approach or instantaneous light threshold. However, a coordinated daily predictive approach to achieve a target daily light integral (DLI) can reduce lighting energy costs by more than 30 percent compared with the light threshold. As LEDs improve in energy efficiency and initial cost, commercial adoption will become more widespread. However, more research is needed to: test energy efficacy of fixtures, optimize light spectrum modification to maximize crop growth, and control lighting.

Research on supplemental lighting (Mitchell et al., 2015; Kubota et al., 2016; Wallace and Both, 2016) has resulted in a proposed product label (Both et al., 2017) for lamps designed to deliver photosynthetically active radiation to promote crop production (FL, NJ, OH). The label provides information about operating characteristics that will be useful to growers considering using a supplemental lighting system and we hope manufacturers will adopt it. Work on measurement standards (Both et al., 2015) has resulted in a set of measurement guidelines researchers can use for research and reporting of those measurements in the scientific literature. A collaboration with researchers in Japan (Ishii et al., 2015) has further improved our understanding of natural ventilation in open-roof greenhouses. As a result of the rapid developments in the lighting industry (especially regarding LED lamps), work on supplemental lighting is continuing and includes the evaluation of lamp operating characteristics as part of a collaborative project with researchers from Cornell and Rensselaer Polytechnic Institute (GLASE project) (NY, NJ). Additional work on ventilation will continue as part of a multi-institutional SCRI grant that investigates berry production in high tunnels (IL, NJ, OH).

We evaluated plant growth response to the environmental parameters in lettuce and spinach grown in heated greenhouses in hydroponics with control of the nutrient solution (CT, NH). Lettuce had a greater contribution of irradiance, and lesser contributions from temperature and solution nitrate to relative growth rate compared with spinach (Gent, 2017). In other words, lettuce was affected more by irradiance and spinach was affected by temperature and solution nitrate concentration. On a separate experiment, we (NH) evaluated hydroponic lettuce growing in naturally sunlit greenhouses was subjected to two temperature treatments (NH). Minimum temperatures were 20° or 10°C, and ventilation temperatures were 26 and 16°C, for warm and cool treatments, respectively. Warm compared to cool temperature increased relative growth rate by 85% for small plants of 2 to 5 g fresh weight (fw) compared to 30% for medium plants of 20 to 60 g fw. Warm temperature increased specific leaf area for plants grown under a low light integral of 4.5 mol·m⁻²·d⁻¹, compared to a high light integral of 15 to 17 mol·m⁻²·d⁻¹. Dry matter content was 20% greater for plants grown under cool compared to warm conditions. Total reduced nitrogen was 7% less under cool temperature. The concentration of nitrate was 40% lower, while the concentration of sugars was about 50% higher, under cool compared to warm temperature. Cool temperature increased the tissue concentrations of malic acid and potassium, but had no effect on phosphorus.

We (CT) evaluated the effect of daily light integral on the composition of hydroponically grown lettuce (Gent, 2014). We identified that except for nitrate, metabolite concentrations on a fresh weight basis increased with irradiance, and the changes resulting from irradiance were greater when harvested in the afternoon than in the morning. Our results suggest that lettuce for human consumption is best harvested in the afternoon after growth under high light, when it has the least nitrate and more of other nutrients. On spinach, sugars, sucrose, glucose, and fructose, had a binary response with high values in the day and low values in the night (Gent, 2016). Spinach grows slower in the fall compared with summer because of the effect of low temperature on metabolism of sugars and nitrate.

Virginia Cooperative Extension provided access to and funding for energy audits and renewable energy feasibility studies for 66 agricultural operations in Southside and Southwest Virginia. Between 2014 and 2016, 71 farms were accepted into the energy program, 5 farms withdrew, 64 of the 66 remaining farms completed an energy audit with 2 farms delaying their audits, 31 farms have used approximately \$214,000 in grant funding along with over \$610,000 in individual funds to implement energy retrofits, and 35 farms have funds remaining in their energy accounts for additional improvements. The 64 completed farm energy audits identified potential annual energy savings of 873,968 kWh in electricity and 429,847 gallons of propane with efficiency improvements resulting in a projected 3,151 MTCO₂e greenhouse gas emissions reductions and an annual energy-cost savings of \$850,734. Approximately 46% of the energy conservation measures had a payback period of less than 5 years. The Agriculture Energy Efficiency Initiative has delivered 20 educational programs on energy efficiency practices and technologies. Partnerships are in place with USDA Rural Development, Virginia Department of Mines, Minerals and Energy, Old Dominion Electric Cooperative, and other organizations to support this project.

Water and Nutrient Management

While fertilizers and fertigation practices have improved agricultural productivity, nutrients that are not taken up by the crops and run off into the environment have caused environmental concerns that put at risk the long term sustainability of water bodies and soils. Our approach has been to evaluate alternatives to (1) reduce initial water use while maintaining plant health and quality (CT, ME), (2) use non-traditional water sources such as recycled or recirculated water (CT), (3) improve the efficiency of fertilizer applications (CT, ME, NH, NY, OH). We investigated several parallel approaches frequently sharing the results and incorporating feedback from other members into new research.

ME built a capacitance sensor automated fog system for propagating stem cuttings (ME). This novel system allows growers to more precisely control the environment during propagation. We tested this system and found that the amount of water applied during propagation impacts root quantity and length of the native plant, Twinflower (*Linnaea borealis*). We also determined that

the environment in which stock plants are grown affects root quantity and length (Foster et al., 2017). We acknowledge that we need to evaluate more crops and we also need to understand how we should adjust light in propagation settings to make it a more comprehensive propagation method.

ME developed water guidelines for herbaceous perennial plants using a capacitance sensor automated irrigation system. These guidelines allow growers to determine the right amount of water to apply to important ornamental crops. For example, the herbs rosemary (*Rosmarinus officinalis*) and English lavender 'Munstead' and 'Hidcote' (*Lavandula angustifolia*) were larger when grown at high soil moisture contents (Zhen et al., 2014; Zhen and Burnett, 2015). Although both of these plants are considered to be drought tolerant, survival was poor at low soil moisture contents (volumetric water content = 10%). English lavender produced more flowers when grown at a volumetric water content of at least 30%. Growth of the perennials cheddar pink (*Dianthus gratianopolitanus* 'Bath's Pink') and columbine (*Aquilegia canadensis*) was also greater when plants were grown with more water (Zhen et al., 2014). However, these plants survived in drier soils and appeared to be more drought tolerant than English lavender and rosemary.

NE conducted a 3-year study to determine if final biomass of basil could be predicted based on electronic sensor inputs and other factors. Five basil (*Ocimum* spp.) cultivars were grown using a capillary mat (CapMat) fertigation bench system that consisted of a sandwich of white reflective, black plastic polyethylene top, capillary mat, black 6 mm polyethylene, in a double polyethylene-glazed Quonset style greenhouse (ENT-3). We installed Decagon GS3® electronic sensors at the root balls of the containers to sense and monitor volumetric water content, electrical conductivity, and media temperature. We used height as the response parameter, which we collected weekly. There were three plants per pot in this study. Plant height responses did not follow a classical sigmoidal growth model. We concluded that approximate reasoning modeling (Fuzzy Logic discussed under objective 2) followed the growth process closely.

CT experiment stations (Storrs and New Haven) collaborated to study the effects of salinity and irrigation management on zinnia, pansy and poinsettia. We demonstrated that partial saturation is an effective water management regime even when raw water quality is poor as represented by elevated salinity (Gent et al., 2016; Macherla, 2014; Macherla and McAvoy, 2017). Whether using a partial-saturation or full-saturation irrigation management regime, dissolved sodium (Na) has dramatic deleterious effects on zinnia health that become visually evident as the electrical conductivity approaches 10 mS/cm and tissue Na concentrations approach 8,000 ppm.

VA team surveyed nursery and greenhouse operators to evaluate BMPs use and barriers to implement new BMPs. We are currently using these results to develop publications and resources to assist producers in improving production efficiency and reduce environmental impact (Mack et al., 2017).

High diversity and incidence of plant pathogens have been reported in low-quality water resources (i.e. recycled and recirculated water) (Hong, 2014). Growers inject chemical sanitizers in the irrigation system to prevent spread of propagules. However, plant sensitivity to sanitizers is in general unknown. CT-Storrs estimated the phytotoxicity threshold of *Impatiens walleriana* to residual concentrations of free chlorine in the irrigation water (Raudales, 2017). We applied chlorine between 2 and 32 mg/L of free chlorine in every irrigation. Plants irrigated with 2 mg/L were no different from plants irrigated with no chlorine residues. Plants irrigated with 4 mg/L of free chlorine were smaller by weight and did not present any visual lesions on the foliage or flowers. Plants irrigated with 8-32 mg/L presented clear phytotoxicity symptoms. Results indicate that irrigation water with chlorine levels greater than 4 mg/L affect quality and yield of *Impatiens walleriana* is 2 mg/L.

Fertilizer Optimization

In previous research, alternatives to constant liquid fertilizer (CLF) for bedding plants and vegetable transplants were investigated to reduce nutrient leaching as well as develop fertilization strategies for organic crops (NY). Controlled release fertilizers incorporated into the potting mix could reduce leaching of garden mums by 5-fold (Mattson et al., 2014). Use of controlled- and slow- release fertilizers was most effective to replace CLF for bedding plants and vegetable transplants with low to moderate fertility (Mattson, 2014). For heavier feeders (ex: petunias, garden mums) additional liquid fertilizer appears necessary for optimal plant growth (Mattson et al., 2014). Commercial substrates vary greatly in their base fertility (starter nutrient charge), pH, and physical properties and growers should obtain media analysis before using new products (Li and Mattson, 2016). Substrate amendments can further enhance plant performance but must be examined on a crop-by-crop basis. For example, seaweed extract applied as a weekly drench (but not foliar spray) enhanced post-production quality of petunias and tomatoes (Li and Mattson, 2015). We summarized research and commercial practices for organic fertilization practices (Burnett et al. 2016, ME, NY, VA). For vegetable transplants, organic amendments added to the substrate may provide decent fertility for 4 to 6 weeks, but must be further amended or supplied with liquid organic fertilizers after this time. With increased interest in hydroponic vegetable production, more research is needed to further optimize fertilization of conventional and

organic hydroponic production systems.

Microgreens (young leafy greens harvested at the emergence of a true leaf) are a relatively new profitable niche crop for greenhouse operations. However, very little information is available in the scientific literature regarding cultural practices. The influence of liquid fertilizer (applied at 0, 50, 100, 150, and 200 ppm N from a complete liquid fertilizer) was evaluated on three brassica microgreens (arugula, mustard and mizuna) (Allred, 2017). All species exhibited increased fresh weight as fertilization increased from 0 to 200 ppm N, with fresh weight about double at 200 ppm N than the unfertilized control. Seeds were sown in a peat/perlite based soilless media. Substrate depth within a flat was also studied and significantly enhanced yields were found when a flat was filled nearly to the top (as opposed to shallow filling to save on substrate).

Container-grown ornamental and edible crops are at the highest quality at the end of production in the greenhouse or nursery. Plant quality often declines in retail and for consumers, typically resulting from stress, insufficient watering or fertilization. In retail, marketable plant quality lasts on average three to six days, resulting in crop losses near 15% (Healy, 2009). Even though problems occur during post-production, poor plant experiences result in dissatisfied customers and lower repeat business. In addition, contract growers only receive payment for plants sold in retail, and have greater incentive to promote plant quality post-production. Unless growers take action to extend plant quality into post-production, they will likely encounter more negative customer experiences, increased shrink (plants not sold), and reduced profitability. Past research has shown that providing fertilizer with residual activity and agrichemicals that prevent wilt or increase substrate water-holding capacity improve the post-production performance of container crops (Argo and Biernbaum, 1993; Armitage, 1992; Blanchard and Runkle, 2007; Million et al., 2001; Nell, 1994; van Iersel, 2009). We (NH) have focused on understanding how plant genotypes affect the pH of the growing media (Dickson et al., 2017). We further conducted a pilot study (NH, unpublished) that has shown potential to manipulate nutrient concentrations and form, application method, and type for improved post-production performance and added value. There is a need for further research on optimizing new fertilizer and agrichemical strategies for post-production, focusing on practicality and cost for growers.

Costs of soilless substrates used in greenhouse crop production have increased significantly over the past decade. In addition, key components such as sphagnum peat moss are considered non-sustainable. New tree wood materials offer potential to reduce substrate costs and decrease dependence on peat (Caron, 2017; Jackson et al., 2009; Schmilewski, 2017). New manufacturing processes have improved the consistency and quality of wood chip and wood fiber products as well as decrease material cost. Pine trees are also a renewable resource and in some cases produced locally (Barrett et al., 2016). Wood materials have potential to immobilize nitrogen, influence substrate-pH buffering capacity and water-holding capacity. Using new wood fiber and wood chip substrates will likely influence fertilizer and irrigation practices for the grower, in retail, and for consumers. Project results indicated that peat-based substrates amended with wood fiber and coconut coir may require growers to adjust their fertilizer and irrigation program. Recommendations on fertilizer nitrogen form, substrate lime type and rate, and irrigation frequency for different blends of peat, coir, and wood fiber blends were developed.

Education

Over the years, members of this project have collaborated formally and informally in many education programs.

The results of light research that we have conducted over the years has been summarized in a new book where six of our members (IA, MI, NC, NJ, NY, OH) were first authors and co-authors of multiple chapters (Lopez and Runkle, 2017).

In 2015, team members from AZ, OH, and NJ received funding from the USDA-NIFA Higher Education Challenge Grant Program. We used these funds to develop a series of "Controlled Environment Plant Production Engineering/Technology Education Modules". We uploaded all the modules in the "Horticultural Engineering Technology" YouTube channel, that we created (https://www.youtube.com/channel/UCsD2oKzVv1B_GtummyKKE8LA/videos). Up to Jan 2018, the channel had a total of 15,456 views.

Many of our members have partial extension appointments and organize annual greenhouse workshops and short courses in AZ, CT, IA, NH, NJ, NY, OH. In all of these instances, we invite members from other stations to participate as speakers.

CT, MA & NH are conducting an eight-part webinar series focused on root-zone management to cover local needs of greenhouse growers in the northeastern US.

Virginia Tech and CT-Storrs conducted a workshop on hydroponic production of greenhouse vegetables that included lectures and hands-on demonstrations for about 40 people in each location. Comments after the workshop included appreciation for the

training and decision support tools. As a result of the workshop, one attendee has joined our team as an online graduate student starting this fall. All presentations have been posted on the Fresh Produce Food Safety YouTube channel.

Maine presented information on water requirements of various herbaceous perennials at New England Grows, which is a large regional trade show. This information was a summary of research in Maine over the past ten years regarding how much water a variety of perennial plants need for growth and development. These recommendations were developed by growing perennials in a moisture sensor automated irrigation system at various soil moisture levels (varying from 5% to 40% soil moisture).

A series of three extension articles regarding visual symptoms of nutrient deficiencies in hydroponic lettuce, basil, and arugula was developed (NY). The project was completed by a female undergraduate student who grew plants in hydroponic nutrient solutions lacking particular elements of interest. Photos and written symptoms of nutrient deficiencies were documented. The article series has been published both at e-Gro.org and Inside Grower magazine.

Objectives

1. Objective 1. To evaluate and develop strategies to improve energy efficiency in controlled environment agriculture.
2. Objective 2. To reduce fresh water use and evaluate alternative fertilizers and growing substrates for the production of greenhouse crops.
3. Objective 3. To train growers and students to utilize emerging controlled environment agriculture technologies.

Methods

Objective 1. Evaluate and develop strategies to improve energy efficiency in controlled environment agriculture.

We will study several approaches to improve energy efficiency in controlled environment agriculture, with a focus on lighting and air quality (temperature and humidity).

AZ, FL, MI, NC, NY, NJ and OH will collaborate to determine energy efficacy of commercial lighting systems. Several commercially available horticultural LEDs are on the market but their energy efficiency varies widely. The best fixtures are twice as efficient as the lowest efficiency fixtures. LED fixtures also vary in their light spectrum with two typical approaches: 1) primarily red/blue light in different ratios and 2) broad spectrum (white) light (obtained from phosphor-coated blue LEDs). The ratio of red/blue can impact plant height and yield. NY will study effect of red/blue ratios on model vegetable and flower crops as well as compare plant performance of several higher efficacy LEDs (both red/blue and broad spectrum) compared to baseline HPS lamps. Data on crop yield, installation, maintenance, ongoing energy costs, and life expectancy will be used to determine economics of LED adoption.

We will use a 2-meter integrating sphere and a dark room to evaluate the efficacy and light distribution of a variety of lamps designed for horticultural applications. Testing reports will be generated and made available to growers and the research community. Outreach activities will be conducted toward the lamp manufacturers in order to encourage them to use the proposed product label. We will continue to contribute to lighting standard development efforts focused on the green industry that are currently undertaken by several organizations such as ASABE, UL, DLC, and IES.

We will also develop a computational fluid dynamics (CFD) model to evaluate various ventilation strategies in high tunnels. Several novel ventilation designs have been installed at a research facility operated by colleagues at Penn State, and these designs will be used to develop and further test the CFD model. The final model should be able to help determine the optimum ventilation strategies for crops grown in high tunnels. Improved ventilation strategies will improve the overall efficiency of this popular production system.

Several strategies to make supplemental lighting decisions given a background of sunlight will be compared, including: time clock (lights on for a fixed number of hours daily), light threshold (lights turn on/off when instantaneous light reaches a set threshold), daily light integral (DLI, coordinated control of light and shade curtains to reach a target DLI). A greenhouse energy model simulation (previously developed by NY) will be used to determine potential energy costs when using each of the 3 control methods using example floriculture, lettuce, and tomato crops. We will then compare crop uniformity/quality using the three methods to grow a model floriculture and lettuce crop in our greenhouses. Data on crop yield/quality, control system costs, and energy costs will be compiled to determine economics of various control strategies.

We will develop dynamic light recipes to improve lettuce growth rate and quality (morphology and phytochemical content) in indoor production systems. Most research has focused on characterizing plant responses when exposed to a single light recipe

(*static spectrum*). We will contribute by researching and the matching spectrum to the different plant growth stages (lag, log, and plateau) to maximize output (nutrient, flavor, growth) and improve system energy efficiency. We called this the *dynamic spectrum*. This research project is focused on finding dynamic light recipes to improve lettuce growth and quality.

We will investigate the interactions between daily light integral (DLI) and CO₂ concentration to improve propagation efficiency of grafted vegetable seedlings. Indoor nursery using electrical lighting is a standard commercial technology for pre- and/or post-grafting processes in Asian and European countries that have advanced nursery technologies. The advantages include 1) uniform plant growth, 2) higher density production, 3) highly contained environment, 3) complete independence from outside climate, and 5) conditioned flowering for earliness; yet the disadvantages are 1) high capital costs, higher energy consumption and 3) limited availability of systems. The objective of this project is to maintain high plant quality while reducing electricity demands from electrical lighting by increasing concentration of supplemental CO₂ in order to increase plant grow efficacy (g per kWh).

We will work on the optimization of strawberry nursery production in full controlled environment systems. The current open-field strawberry nursery production system is a multi-year and multi-location operation and limited to select regions in the U.S. The current system has inherit risks. For example, disease contamination of strawberry nursery material; and lack of plant uniformity and supply due to variable environmental conditions. In this research project, we aim to designing precision indoor propagation (PIP) tools to optimize strawberry nursery production and to develop disease-free propagation material. The project main objective is to optimize the environment to increase runner/tip production while reducing production cost. Current efforts are focused on photoperiod, light intensity, and temperature optimization.

We will work on optimizing site-specific solar radiation modelling for its application in the horticultural, agricultural and photonics industries. The objective of this project is to measure irradiance conditions under a range of structural materials, different locations, sites, and times of day/year. Measurements will be performed with partners, in Finland, the US, UK and mainland Europe. The objective of the project is to predict light quality inside the greenhouse based on outside irradiance using modelling. Validation of models is done by measuring under various scenarios and comparing measurements to models of what the irradiance is predicted to be.

We aim to improve environmental optimization to increase phytochemicals in non-commercial plant species. The objective of this research program is to increase the affordability of Vertical farm products by providing more nutritionally dense produce by significantly increasing the phytochemical contents and biomass accumulation while reducing the resource inputs. We will characterize the responses of exotic germplasms grown under optimized indoor conditions in hopes to have a greater content of beneficial phytochemicals than commercial cultivars.

Objective 2: Reduce fresh water use and evaluate alternative fertilizers and growing substrates for the production of greenhouse crops.

We (CT, IN, ME, MN, NH, NY, OH) aim to develop alternatives to preserve fresh water use for production of greenhouses by studying irrigation methods to reduce fresh water use, treatment options to use low-quality water, mechanisms to improve fertilizer efficiency, and enhance conditions to promote beneficial microbiomes.

One primary objective is to reduce the amount of water used during vegetative propagation of ornamental plants in the horticulture industry. We are exploring two approaches that will provide growers with alternatives to over-head mist, the traditional approach. Both of these would reduce water use during this phase of production. One of these systems is a sensor-automated mist system, which we plan to further explore. We would like to use our sensor automated mist system to help growers refine over-head mist systems and more accurately determine how much water is truly needed during propagation. The second system is called submist; in this system, water is applied to the base of cuttings that are in enclosed tubs rather than overhead. Preliminary results indicate that roots form more quickly when plants are propagated in this system compared to traditional overhead mist.

We will evaluate the feasibility of using phytoremediation in line in hydroponic production systems. We will assess if halophytes can be used in hydroponic systems to remove sodium and chloride from reclaimed water. We will evaluate crops that not only have the potential to remove salts inline, but also have a commercial value. Our first experiment will consist in growing lettuce and purslane in hydroponic systems where nutrient solutions contain a range of chloride and sodium levels.

Several commercially available conventional and organic hydroponic nutrient solutions will be tested for production of greenhouse leafy greens and fruit-bearing crops. Daily pH and EC measurements and periodic nutrient solution elemental

analysis will be taken to determine potential for salt buildup and nutrient imbalances of the given crops. Based on initial findings, one conventional and one organic nutrient solution will be further tested for hydroponic lettuce grown using nutrient film technique (NFT). Different strategies for nutrient management (frequencies of replacing nutrient solution) will be undertaken to test several salt thresholds for nutrient solution replenishment and impact on crop yield.

Our team will also study the microbial activity in hydroponic solutions. We will evaluate if microbes in the solution can be primed to accelerate nitrification, used to improve nutritional quality of the crop for human consumption and resistance to plant diseases.

A range of fertilizer and application strategies designed to provide nutrients during post-production will be evaluated at the University of New Hampshire. Fertilizer types include various controlled release (single and dual polymer coat), slow release organic, and water-soluble fertilizer formulations. Effects on substrate-pH, substrate nutrient level, plant performance (leaf greenness, growth, flowering, size and compactness), and longevity will be evaluated in both simulated and actual retail environments. Production simulations and partial economic analyses will be used to evaluate fertilizer strategy practicality and potential cost-benefit.

New wood chip and fiber substrates will be evaluated for potential nitrogen drawdown and effects on plant performance for container-grown crops. Projects will test substrates varying proportions and types of wood materials blended with sphagnum peat in combination with different fertilizer strategies. Fertilizer strategies will consist of a range of applied nitrogen concentrations and form (ammonium and nitrate). Nitrogen immobilization will be measured in substrates using VDLUFA standard methodology. Plant performance (plant growth, leaf greenness, flower number, size and compactness), substrate-pH buffering, and nitrogen immobilization will be measured during production and simulated retail environments to determine the effects of wood materials for growers and consumers.

We will develop best nutrient management practices for hydroponic and bioptic production systems and provide growers with crop production options that will improve yield, quality, and profitability, while significantly reducing fertilizer use and minimizing discharge (IN). Our activities include reducing the use of nitrogen and phosphorus fertilizers, determining the efficiency of open and closed systems, enhancing food safety, and conducting cost-benefit analysis in different production scenarios.

We will develop best nutrient management strategies for economically and environmentally viable hydroponic and bioptic food-production systems. The proposed research gives producers significant tools to make decisions from start-up through production and sales. Best production management practices, economic and food safety analysis will increase the likelihood of successful operation producing local food for Indiana and beyond. It will also significantly increase the diversification of food crops and production systems, while protecting its fragile environment.

Objective 3: Train growers and students to utilize emerging controlled environment agriculture technologies.

All team members have committed to involve undergraduate in independent-research related to this project. We strongly believe that the future of the industry depends on highly-qualified individuals.

We will continue to train graduate students and involve them in this project. This year we will begin the NE-1385 travel grant scholarship to incentivize graduate student attendance to our annual meeting.

We will continue to host short courses on energy efficiency, water management, and general controlled environment production practices. Our members with partial extension appointments will continue to organize annual greenhouse workshops and short courses in AZ, CT, IA, NH, NJ, NY, OH. In all of these instances, we will invite members from other stations to participate as speakers. Upcoming events include Northeast Greenhouse Conference and Cultivate with attendance over 800 individuals.

Many of our members have outfitted greenhouse sections with several alternative production systems (horizontal and vertical hydroponic growing systems, as well as traditional pot-and-media based systems) that the students use to learn about different growing techniques. We all have agreed to do more hands-on training sessions in CEA-related courses to increase practical training.

We are currently collaborating on a seven-part article series on Urban Agriculture. The articles will be published in Produce Grower Magazine, a well-known trade journal with a readership of 9,749. We agreed to use the proceeds of these publications to provide travel grants to graduate students to attend to our annual meetings.

Measurement of Progress and Results

Outputs

- We will develop recommendations for optimal lamp choices and layouts for greenhouses and indoor production facilities
- We will improve ventilation alternatives for high-tunnels that result in better cooling in the summer and reduced heat loss in the winter.
- We will develop practical production guidelines to increase the efficiency of organic fertilizers in production of container-grown ornamentals and hydroponically-grown vegetables.
- We will develop practical management guidelines yield and quality of vegetables grown in recirculating hydroponics and aquaponics systems.to improve production efficiency and increase
- We will develop recommendations for application of flexible wavelength lighting and selective cover materials or shading elements for greenhouses.
- We will develop strategies to reduce water use in propagation of ornamentals and vegetables.
- We will accelerate propagation timing by reducing water use.
- We will generate new knowledge about environmental management practices that enhance beneficial microbes in hydroponic solutions.
- We will develop management guidelines to use low-quality water for irrigating greenhouse crops.
- We will production guidelines to adjust nutrient programs to non-peat-based substrates.
- We will organize education programs that target CEA growers around the US, our target populations will include Hispanics, Native Americans, and new farmers.
- We will publish a hydroponic production book and an eight-part article series on urban agriculture.
- We will enhance undergraduate research training in the area of controlled environment plant production to prepare the students for independent studies.
- We will submit at least 3 grants to enhance our collaboration within the team.
- We will produce at least 10 scholar outputs every year.

Outcomes or Projected Impacts

- Improved ventilation, lighting, heating, cooling and dehumidification strategies will improve energy efficiency in greenhouses. This will allow growers to reduce their energy consumption by 10-30%, resulting in significant savings.
- Production time (day to harvest) will reduce by at least 15% and therefore annual yields will also increase by at least 10%. This gain in production time is expected in response to tighter environmental controls and more production cycles per year (more yields per area per year).
- The water footprint of CEA-grown products will reduce by at least 30%. We expect these results in response to increase water recirculation within the farm, increase recycling water from other sources (i.e. reclaimed water), and reduce use of fresh and potable water.
- Fertilizer recommendations for hydroponic production will reduce leaching and fertilizer usage by 10-20% and allow growers to remain competitive in this rapidly growing field.
- The development of the proposed guidelines for controlled agriculture and hydroponics in will become part of a joint publication article series on Urban Agriculture planned for Produce Grower, and a short book on hydroponics. These publications will help to keep growers competitive and aware of the latest research in controlled environment agriculture.
- Information gathered and shared through this project will empower growers to improve production practices through the use of (remote) monitoring and control of the greenhouse environment and plant status. The realtime and web based greenhouse and climate control platforms developed will help growers to reduce 10% of their labor use dedicated to greenhouse climate monitoring, recording, and management.

Milestones

(2018):Teach undergraduate course on controlled environment crop production practices.

(2018):Transfer basic knowledge and newly generated results to local, regional, and national stakeholders.

(2018):Test several lamps that are specifically designed for horticultural applications.

(2018):Develop a list of herbaceous and woody plants that can be propagated in sub-mist irrigation.

(2018):Evaluate non-chemical control options to manage waterborne pathogens and algae in hydroponic systems.

(2018):Finish first draft of hydroponic book.

(2019):Contribute to lighting standard developments. Promote the use of the lighting label.

(2019):Develop a preliminary ventilation model for high tunnels.

(2019):Evaluate phytoremediation as an alternative to remove sodium and chloride from reclaimed water in hydroponic systems.

(2019):Compare root formation timing and plant physiological traits under sub-mist compared with overhead mist.

(2019):Refine a ventilation model for high tunnels. Develop ventilation recommendations for high tunnel growers.

(2019):Develop guidelines on managing conventional and organic fertilizer source, and substrates for hydroponics and container-grown crops during production and post-production.

(2019):Develop guidelines on managing on nutrition when using alternative growing-media substrates.

(2019):Evaluate silicon supplementation for control of root-borne pathogens in soilless substrates and hydroponic systems.

(2019):Compare yield and system efficiency between hydroponics and bioponics.

(2020):Conduct a series of outreach efforts to promote the results of the studies on ventilation, water treatments, irrigation, and nutrient management.

(2020):Develop Si application guidelines for growers.

- (2020):Develop practical management guidelines and economic analysis of nutrient programs for promoting retail and consumer success with container crops.
- (2020):Evaluate technical and financial characteristics of wood chip and fiber substrates in container production.
- (2020):Compare LED fixtures with HPS fixtures in terms of energy efficiency, crop response, and financial benefits as compared with the 2016 HPS baseline.
- (2020):Develop production guidelines and best management practices for hydroponics/biaponics of major greenhouse vegetables
- (2021):Guidelines for managing salt build-up and nutrient imbalances for NFT production of hydroponic lettuce to reduce water usage while maintain crop yield/quality.
- (2021):Develop propagation protocols for appropriate moisture content for annuals plants using sensor automated propagation system.
- (2021):Develop guidelines on using plant growth regulators, surfactants, and adsorbents to prevent wilt and promote container-crop longevity during post-production and retail.
- (2021):Develop production guidelines and best management practices for hydroponics/biaponics of many greenhouse vegetables
- (2022):Conduct research determining whether microbial inoculants are beneficial in hydroponic production and if the presence of these microbes affects the nutritional quality for consumers. Transfer results to producers.
- (2022):Develop models predicting the impact of irradiance, temperature and CO₂ concentration on leafy green and ornamental crop photosynthesis. Integrate these models into the USDA-ARS 'Virtual Grower' software program available to all growers.
- (2022):Develop and test lighting control strategies that reduce energy usage as compared to timeclock/threshold while maintaining crop quality/yield.
- (2022):Conduct a series of outreach efforts to promote the use of LED lamps for horticultural applications.
- (2022):Develop guidelines on non-chemical alternatives to prevent clogging caused by biofilms in irrigation systems.
- (2022):Develop models predicting the impact of irradiance, temperature and CO₂ concentration on leafy green and ornamental crop photosynthesis. Integrate these models into the USDA-ARS 'Virtual Grower' software program available to all growers.
- (2022):Evaluate potential phytotoxicity of various agrichemicals used to promote post-production performance of container crops.
- (2022):Evaluate the effect of residual fertilizer and agrichemical strategies when using wood fiber substrates on plant performance during production and post-production at commercial growers and retail operations.
- (2022):Cost-benefit analysis as a decision tool for hydroponic/biaponics crop production.
-

Outreach Plan

We work closely with the controlled environment agricultural industry throughout the United States. Several team members have partial Extension appointments, providing additional connections with grower associations, industry suppliers, and the Land-grant Extension network. We use these connections with the industry to develop questions that are based on actual industry needs and to communicate the new knowledge that we generate.

We plan to continue sharing the results of our research through a variety of methods to reach both our peers at research institutions, as well as greenhouse growers, industry suppliers, and Extension personnel. Results will be published in refereed articles and Extension publications.

A large number of our members organize annual education programs for greenhouse growers. In this venues, we collaborate with our peers and cross state borders to share our knowledge.

Our team is currently collaborating on a seven-part article series on Urban Agriculture. The articles will be published in Produce Grower Magazine, a well-known trade journal with a readership of 9,749. In addition, we plan to publish a book on Hydroponic Production for greenhouse growers that includes the results of our previous work on water and energy savings, ventilation and cooling, and alternative energy use.

We will work closely with Cultivate has an attendance of over 10K, and the Northeast Greenhouse Growers Association and the Michigan Growers Association annual meetings which have an attendance of over 800. We will also write articles for trade magazines with readerships of >18K (Greenhouse Grower, GrowerTalks and GPN Magazine) and repost in HortiDaily which has a readership of > 20K world-wide.

Over the years, members of this project have provided valuable greenhouse engineering technology to the industry throughout our history through technology transfer. Some examples include the use of air-inflated double-layer polyethylene films as greenhouses cover material, advances in hydroponic production systems and supplemental lighting, floor heating for greenhouses, and the use of energy curtains. Many of the technologies originally developed by members of this project are

now industry standards for improving sustainability and/or conserving energy. Several key industry members received part of their education at the institutions (and in some cases were instructed by members of our team: involved in this project.

We are distinctively qualified to develop strategies that address resource management in controlled environment agriculture. Our group consists of plant scientists, agricultural engineers, and an agricultural economist. Our group includes early, mid, and late career researchers. The extent of diversity in terms of area of expertise, career stage, demographics, and location provides a valuable contribution to the industry in which practical and research-based solutions are quickly developed and spread in time and space.

Organization/Governance

The technical committee has organized itself by annually appointing an incoming secretary, who then serves as the secretary for the following year (including the next annual meeting). The secretary will complete a one-year term and then serve as the committee chair the following year. We do not have the position of vice chair (chair elect). Therefore, officers served for two-year terms. This limited the time commitment requested from incoming officers and since the committee was small and informal, sufficient institutional memory could be tapped in case procedural questions came up. Annual meetings were organized on a rotating basis after the membership was polled for availability and interest. This organizational model has worked well for many years, and we plan to continue with it if our proposal is approved for continued funding. Many of the participating members meet each other at other scientific meetings throughout the year, ensuring sufficient opportunity for interaction in addition to the annual project meetings.

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Land Grant Participating States/Institutions
AZ,MI

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Kacira, Murat	Yes	Arizona - University of Arizona	1,3	205	1499	2020	0.10	0.00	0.00	0	0
				401	1499	2020					
				402	1499	2020					
				404	1499	2020					
Lopez, Roberto G.	Yes	Michigan - Michigan State University	1,3	203	2199	1020	0.10	0.00	0.00	0.1	203
				205	2199	1020					205

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
0-0-0	0	0	0
205-1499-2020	0.03	0	0
401-1499-2020	0.03	0	0
402-1499-2020	0.03	0	0
404-1499-2020	0.03	0	0
203-2199-1020	0.05	0	0
205-2199-1020	0.05	0	0
Grand Total:	0.20	0.00	0.00

Program/KA	Total FTE
0	0
0	0
203	0.03
205	0.03
Grand FTE Total:	0.1

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP1835: Resource Optimization in Controlled Environment Agriculture

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 strong multi-disciplinary group with both research and extension team members. The proposal is well written.

Your Recommendation:

Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP1835: Resource Optimization in Controlled Environment Agriculture

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:

Good

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

Excellent

5. Overall technical merit:

Good

Comments

To be honest, it is difficult to review the scientific merit of the project. I could not even determine all of the project participants from the information to which I had access. However, I could discern that many of the project participants are leaders and colleagues of mine in the field of controlled environment agriculture and will undoubtedly make significant and scientifically-sound strides towards the goals outlined in the project.

Your Recommendation:

Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP1835: Resource Optimization in Controlled Environment Agriculture

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 research grant is focused on controlled environment agriculture across 18 different experiment stations, covering most of the US. The research is broken down into 3 main objectives energy (heat and lighting), water/fertilizer and education. The background of the researchers are very strong and is highlighted with the number and range of publications. This research is greatly needed as most of the growers are still using 1970's based technology in their greenhouses but a lack of scientific documentation available to the public limits the growers ability to make informed decisions. Currently, industrial literature is the dominate means of education but a unified effort of these experts would greatly bring the CEA field to a modern edge. i strongly support this effort and wish them all the best on their project.

Your Recommendation:

Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP1835: Resource Optimization in Controlled Environment Agriculture

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:

Good

Comments

I have read the Multi-state Research Project proposal "Resource Optimization in Controlled Environment Agriculture." My first impression is that this proposal is both very aggressive and very ambitious in its goals and projected outcomes.

Outreach: I was glad to see that the outreach and educator stakeholder section was carefully thought out. I would recommend placing additional emphasis on "alternative outreach vehicles" such as eGro, and take a look at using the various groups that are relevant in Facebook, such as the Floriculture Faculty, Southern Horticulture, Garden Professors, etc. A great many of the topics presented in this proposal have applications at the consumer and grower level.

Outputs: This is a very ambitious list of goals. I was encouraged that the publishing of the Hydroponic book was included as a measurement of results. This is an important and much needed resource at the national level. The research regarding using beneficial microbes in hydroponic solutions is also very relevant and is in its infancy. This research could have huge impact in this area of production.

Objectives 1). Efficiency Strategies. Water conservation, sensor controlled management and revisiting sources for use as production inputs are all necessary, and high profile needs in the industry. Controlled environment models are still in their infancy, and with new technology (such as LED's and photosynthesis management, the need to clarify their use, and place in the industry is imperative. Phytoremediation in-line with hydroponic solutions is a new concept that may or may not pan out, but is worth exploring as long as scrutiny is applied in the area of plant pathology. The use of supplemented microbial populations may work, but I suspect it will take many years to learn how to control and balance microbe populations in an open hydroponic system.

Objective 2). Water/Fertilizer Use. Clearly important to our environments well being and to the sustainability and financial well being of our industries production businesses. This has been a huge issue since the beginning of hydroponic research in the 1960's. Water conservation will continue to be a high priority, and members of this team are well positioned to do the next level of research needed to bring the technology to widespread useage in the industry. My only issue with the scope of this section involves the use of wood chips. This research has been evaluated by two universities for almost 15 years, and the descriptor of the proposed work certainly doesn't imply or delineate how the proposed research will differ from the last 15 years of wood chip/wood fiber research for nursery crop production use. In my opinion, this is not anywhere near as important as some of the other aspects of the proposal. I would recommend looking at alternative substrates that have not already had extensive, multi-university program testing. There are many waste stream byproducts and harvest byproducts that are possibly useful.

Objective 3). Grower and Student Training. This section might have benefited from a bit more detail, but the core concept that should be realized by the group is the development and addition of formal classroom courses in hydroponics, water use and efficiency and controlled environment management to university programs. Extension programs are expected here and the team is well equipped to deliver these, but where the real change occurs in the industry is in the students that graduate in the field and make decisions downstream as managers. A strong coordination of these formal classes, including multi-university collaboration, would have the greatest impact on the industry based on what we have seen in the past.

Overall, this is a very productive team, and they show every indication of having the capacity and ability to deliver on the project goals and outcomes. Their past, published work has been high quality and impactful.

Your Recommendation:

Approve/continue project

NE_TEMP1838: Development of a Weed Emergence Model for the Northeastern United States

Status: Under Review

Duration 10/01/2018 to 09/30/2023

Admin Advisors: [\[Margaret E. Smith\]](#)

NIFA Reps:

Statement of Issues and Justification

Need for Project

Unlike crops, which have been selected for uniform emergence, weed species have adopted variability in timing of their emergence; even the seed matured on the same plant may germinate at different times. This “bet-hedging” strategy, with which a weed avoids putting all its seed in one basket of emergence timing, will enable weeds to escape control measures that are applied at the “wrong” time. Post-emergence management carried out too early, i.e. before most problem weeds have emerged, will yield low returns for the effort, investment, and ecological cost of the management (herbicide off-target effects, soil compaction, etc), as weed seeds that have yet to germinate are often unaffected. Providing seedling emergence information so that farmers can effectively time their weed management operations can increase efficacy of control, reduce labor costs, and minimize any negative environmental impacts (e.g. reduce the likelihood that repeat applications of an herbicide or cultivation may be required for late germinating/emerging weeds). There is, therefore, an urgent need for the development of time-specific weed management tools to help address the frequently asked, yet to be answered, question of *when is the “right” time to control weeds?*

Weed seedling emergence is a complex process regulated by a multitude of internal (e.g. species-specific parameters such as base temperature, base water potential) and environmental (e.g. soil temperature and moisture) factors. A range of modeling approaches, varying from simple empirical to advanced mechanistic models, have therefore been adopted to quantify the extent and time of emergence for a significant number of weeds. Weed seedling emergence models enable farmers to determine the percent emergence of a specific weed species by a given date, taking into account the weather, management actions, and field conditions to that point. No weed seedling emergence model exists for the Northeastern region of the United States, despite recent advances in our understanding of regional weed emergence patterns and developments in fine-scale weather prediction and mapping. Data exist to create a weed forecasting product similar to those available for insect and disease threats to Northeastern agriculture, which would enable farmers to approach weed management with more precision and planning. Populations of weeds respond differently in different regions to climate and habitat, requiring that emergence models be modified for a particular region. In the past decade, decision support tools have been built to help farmers manage weeds effectively in the Midwestern United States and Europe; these would serve as a road map for the Northeastern model. Recent advancements in climate and weather models and computational power have generated detailed weather data that are available to the general public free of charge. In the northeast, daily weather data are now available on a 4 × 4 km grid across the region using the Applied Climate Information System (ACIS) Web Services (DeGaetano et al 2014). These databases provide an unprecedented opportunity to estimate parameters directly relevant to seedling emergence such as growing degree day and hydrothermal time, from soil temperature and moisture data at very fine spatial resolution

The overarching goal of this project is to work collaboratively across the northeast region to optimize farmers’ ability to manage weeds in agricultural systems, in the face of the challenges of changing climate and herbicide resistant weeds. *In this proposal, our goal is to develop a user-friendly, online interface for the real time prediction of weed emergence in the northeastern US. The seedling emergence model takes GPS location, soil type, tillage, crop data, and accesses weather history to provide percent emergence of the farmer’s problem weeds at that location.*

Importance of the Work

Weed management is a priority issue for Northeastern farmers, particularly with the increasing prevalence of organic production, the rise of herbicide resistant weeds, and the recent increase in small farms and urban farming. With interest in local food increasing particularly in urban areas, developing methodologies for weed management in the Northeast with specific, regionally-focused data and tools could provide great benefits to local growers and consumers, while reducing negative impacts on the environment. Weed management remains the major challenge for organic farmers that impacts yields (Baker & Mohler 2014; Jenkins & Ory 2016). Yield losses to weed competition are an increasing problem for conventional farmers as well, as the incidence of herbicide resistant weeds continues to increase (Heap 2017). The failure to account for the temporal variability of emergence can result in mistimed application of control measures with poor control levels necessitating repeated applications that are not only costly to the farmers, but also hazardous to our environment. Better timed and more

effective use of herbicides and/or cultivation are likely to delay the development of herbicide resistance and increase yield.

Modeling seedling emergence has long been recognized as a practical approach for tackling the problem of timing in weed management programs (Forcella et al., 2000; Hardegree et al. 2003; Maisin et al. 2005). However, after two decades of effort in development of weed emergence models, these models have not been adopted by growers or extension specialists and the decision about the application timing is still made upon imprecise guesswork. We believe limitations with the availability/accessibility of input data and ease-of-use have been the two main impediments to adopting emergence models. That is, 1) these models have not been presented in a format that can be accessed and used easily by the growers, and 2) the required input data, mainly weather data, have not been available or easily accessible at the proper temporal and spatial resolutions. However, thanks to recent technological and analytical advancements, these barriers no longer exist and we are in a unique position to deliver weed emergence models to farmers in a simple and usable format. Further, as increasing variability in weather presents farmers with more extreme and unusual weather patterns, a functional and more site-specific weed emergence model will help farmers better time weed control measures and protect crop yields. Information on the temporal pattern of seedling emergence can also help improve the efficiency of site-specific weed management by guiding the timing of the ground and aerial mapping of weed infestation.

Technical Feasibility

Weed seedling emergence models have been published for several other regions, with varying degrees of success and modernity; the details are found in the "Related, Current and Previous Work" section of this proposal. The key components for a hydrothermal emergence model are well-established links between weed emergence, growing degree days, and soil moisture; weather modeling at sufficiently fine scale to be useful to individual farmers; and a model for soil moisture across the region of interest. The multistate project team possesses all of the technical skills necessary to develop such a model for the Northeast.

Cornell University has been at the forefront of seed burial and movement research (Mohler citations), and has secured funds to initiate the first phases of this project through NE-IPM ("Establishing a Northeastern IPM Weeds Working Group", a 2-year grant for \$20,000) to bring together a network of weed scientists from around the region to plan the research needed to develop and validate the weed emergence model. This group met in December of 2017 and January of 2018, and developed the project as set forth in this proposal. Dr. DiTommaso and Dr. DeGaetano also secured funds from 2016-2018 to collect pilot data for this project and create a proof-of-concept model, which can be found at: <https://alexsinfarosa.github.io/weed-modelV2/>. Dr. DiTommaso has handled the weed ecology end of the project, while Dr. DeGaetano of Cornell's Atmospheric Sciences department has led the modeling component of the draft model, and has access to 4km grid-scale weather prediction models and soil moisture models for the Northeast.

This proof-of-concept model was based on data from a project in which another multistate partner, Dr. VanGessel, participated (Myers et al 2004, Myers et al 2005). Dr. VanGessel also tested the Midwestern weed emergence model, WeedCast, against data from Delaware and found that it was not very reliable for the Northeast, which was the initial impetus for this project. Dr. VanGessel has also conducted research on weed emergence of winter annuals (VanGessel et al. 2015) and horseweed (VanGessel et al. 2007).

Thierry Besancon has ongoing research focused on environmental parameters that govern germination/emergence of volunteer cranberry (*Vaccinium macrocarpon*) seeds and Carolina redroot (*Lachnanthes caroliniana*), two types of weeds that are challenging for New Jersey cranberry growers, either because of genetic pollution of the cranberry beds or because of direct competition with the cranberry vines. Dr. Besancon provides New Jersey growers with weekly recommendations on efficient weed management strategies for various specialty crops, which would greatly benefit from data generated by an emergence model for some of the most troublesome weed species. With data from the model, he could provide growers with timely information on mechanical or chemical weed control strategies, improve the efficiency of these strategies by controlling weeds at the most sensitive stage, and reduce reliance on herbicides.

Jacob Barney has worked on seed germination and biology in a variety of ecosystems of both agricultural and invasive weeds. In particular he has extensive experience evaluating the intraspecific variation of hydro-thermal dynamics of weeds. He also has experience evaluating the role of propagule pressure, seed size, and density on weed emergence (Barney 2016).

Mohsen Mesgaran is a modeler with extensive experience in developing both physiological and statistical models for seed germination and seedling emergence of weeds. Evaluating eight hydrotime time models on four weed species, he found that the most common model in the literature, which assumes a normal distribution of base water potential among seed sub-

populations, provides the poorest prediction of germination dynamics (Mesgaran et al., 2013). He therefore developed a new hydrothermal model that not only gives better fits to germination data but also provides useful insights about the adaptive strategies evolved by weed to respond to unpredictable environments (Mesgaran et al., 2017). In collaboration with Andrea Onofri (Perugia, Italy), he also contributed to development of new statistical methods (e.g. “cure” model; Onofri et al., 2011) to overcome statistical issues arising from the use of traditional methods (e.g. ANOVA) for the analysis of germination data as well as provided guidelines on the proper experimental design for germination/emergence studies (Onofri et al., 2014). He also has experience in developing software for data analytics including the ExDet tool (<https://www.climond.org/ExDet.aspx>) that enables the comparison of multidimensional climate envelopes across space and time (Mesgaran et al., 2014).

Multistate Advantages

Weather patterns and soil types are highly variable across the Northeast, making the collection of data from across the region critical for developing and refining the hydrothermal weed emergence model. Additionally, Cordeau et al. (2017) found that populations of weed species had different emergence patterns in different Northeastern states; whether that difference is due to genetic variability within the species or plasticity in emergence patterns depending on climatic conditions is not yet known. As a result, it is critical to reproduce the same basic weed emergence experiment at all sites across the region. To create weed management solutions for optimization of farmers’ weed management by linking weed emergence to weather data, several years of data from around the region will be needed to capture both spatial and temporal variability. Participating researchers will include Eric Gallandt (Maine), Richard Smith (New Hampshire), Mark VanGessel (Delaware), Antonio DiTommaso and Matt Ryan (New York), Thierry Besancon (New Jersey), Bill Phillips (Maryland), Jacob Barney (Virginia), and Mohsen Mesgaran (California). Having thorough coverage of the region will ensure that the model responds appropriately for farmers throughout the Northeast.

Likely Impact

Optimizing farmers’ ability to manage weeds in agricultural systems, despite the challenges of changing climate and herbicide resistant weeds, is likely to improve farm profitability and reduce environmental impacts of inefficient weed management programs. The weed emergence model produced by this multistate effort will provide farmers with real-time information on weed emergence in their specific farm field, enabling them to target weed management actions more timely and hence increase the efficiency of control measures. This version of the model will focus on summer annual species. The proposed decision support system will benefit both conventional and organic farmers. In conventional farming, where herbicides are the main means of weed control, our predictive tool has great potential to reduce herbicide use by avoiding applications that are too early or too late to provide any effective weed control. The tool can therefore reduce both costs and environmental impacts of multiple treatments and delay development of herbicide resistance. Organic farmers can use the tool to reduce the number of cultivations thus reducing impacts to soil health from disturbance and compaction. For all users, the ability to quantify emergence patterns for common weeds will bring a level of predictability to weed management similar to other modeled crop pests such as powdery mildew, late blight, and apple scab. Although the use of unmanned aerial vehicles (UAV) for mapping weeds has received soaring popularity in recent years, the picture they provide on the distribution of weeds largely depends on the timing of snapshots taken from the field, which if captured at the wrong time, the picture will not be a realist representation of the weed infestation. Our tool can reduce the costs (e.g. avoid flights that are too early) and contribute to the success of this growing technology by guiding the flight operation timing based on the predicted level of seedling emergence. The proposed tool can also serve as a “time management” system for field operations where growers (particularly new farmers) can plan their weed management practices ahead of time through the information provide on the potential weed problem given the near time weather forecasts.

Related, Current and Previous Work

Many studies have been published on weed seedling emergence rates and patterns. The most practical models for farmer use are hydrothermal models; these models map seedling emergence against soil moisture and growing degree days (Forcella 1998; Maisin 2010). These models use information on temperature, precipitation, basic soil type and time of plowing to predict what percentage of specified weed seedlings will have emerged in a given location at a given point in time during the growing season. In general, these regionally specific models have had good predictive ability in their target regions (Grundy 2003; LeBlanc et al. 2004; Martinson et al. 2007; Maisin et al. 2010, 2014; Royo-Esnal et al. 2010; Clay et al. 2014; Werle et al. 2014). The Midwestern US model of weed seedling emergence, WeedCast, uses information on the previous year’s crop, tillage, crop residue, basic soil type, water capacity, and temperature to provide seedling emergence potential, emergence timing, and seedling growth (Forcella 1998). While WeedCast has correlated well with data from the north-central United States, its outputs have not matched as well with data collected in New York State or Delaware (DiTommaso & VanGessel, unpublished). Due to its early publication date, it also lacks a user-friendly interface; farmers are required to upload individual Excel files for each metric needed, and supply their own soil data. The Italian model, AlertInf, is based on hydrothermal time

as described above, and is fine-tuned for the Veneto region of northeastern Italy (Maisin et al. 2014). Since these tools were released, new weed seedling emergence data specific to the Northeast (e.g. New York, New Hampshire, Maine, Delaware) have been collected (Cordeau et al. 2017; DiTommaso et al. 2018), and the aforementioned weather mapping advances allow the general public access to fine-scale weather data (e.g. Northeast Regional Climate Center <http://www.nrcc.cornell.edu/>). We believe these factors make development of a northeast-specific weed seedling emergence tool technically feasible. Weed ecologists from across the region are committed to developing this product; the proposal includes researchers from Maine, New Hampshire, New York, New Jersey, Delaware, Maryland, California and Virginia. Art DeGaetano and his team at the Northeast Regional Climate Center have developed a 4km scale soil moisture model to use with the National Weather Service's 4km grid weather data and forecasting models. Art's team has also helped develop other weather-dependent modeling tools for farmers. This interdisciplinary group of weed ecologists and climate modelers have the expertise, experience, and tools necessary to develop a regional weed seedling emergence model for the Northeast.

Objectives

1. Link weed emergence timing data with modern weather prediction models to create an online tool for farmers that will help them plan their weed management for optimal weed control. This tool will cover three weeds that are problematic across the region as well as a few weeds of concern for the northern or southern portions of the region.
2. Collect data across the region to validate and refine the weed emergence model, from researchers and farmers.

Methods

The overarching goal of the project is to work collaboratively across the Northeast to optimize farmers' ability to manage weeds in agricultural systems, despite the challenges of changing climate and herbicide resistant weeds. Recent weed ecology advances have opened the door to more targeted weed management decision support tools, and developing such tools will bring weed management in the Northeast into the 21st century. For this project, our two objectives support the larger goal by 1) linking weed emergence timing with weather prediction tools to create a decision support tool for farmers' weed management, and 2) collecting the data necessary to test and refine the model so it is useful to Northeastern farmers.

Objective 1. Data from Dr. VanGessel and colleagues at Penn State were used to create a preliminary seedling emergence model by fitting existing weed emergence data to precipitation, temperature and soil data for a given farm location (ART CITATIONS). This model serves as a proof of concept for the proposed tool. To develop the beta model, data collected by the DiTommaso lab from two extreme weather years in New York State (2016 & 2017) will be used to extend the preliminary model's functional range. The beta model will be transitioned from draft format to a web-based application, allowing user input of necessary parameters and linking to real-time weather observations and 3-7 day forecasts. At the end of Year 1, data from the Multistate project will be used to update the model, and all participating states will test the model and provide qualitative feedback on its fit with their knowledge of weed behavior in their states. This feedback will be incorporated into the model, and the resulting beta model will be launched on the NEWA website (<http://www.nrcc.cornell.edu/>) in Year 2, where farmers from participating states will be asked to provide feedback on the model's accuracy and ease-of-use. In Year 3, data from research and farmer input will be used to further validate and refine the model, and a fully functional version will be installed on the NEWA site.

Objective 2. All regional collaborators will establish research plots to validate/refine the emergence model. This proposal focuses on warm season annual weeds, which are difficult to control and are projected to become more problematic with climate change. Data will be collected from earliest available crop planting date until the regional date of soybean canopy closure. Dates will vary by site, as the study area stretches from Virginia to Maine and each year's weather influences planting date. Each site will have two treatments, one with biweekly disturbance to capture the weed emergence after tillage events through the planting season, and one with no tillage to inform weed emergence in no-till situations. Eight 1 square meter plots of each treatment will be planted, for a total of sixteen plots per site. Either ¼ or ½ square meter subplots will be sampled per plot, depending on the density of weed emergence. Data will be collected weekly; all emerged weeds of the selected species of interest will be identified and counted, and all emerged plants removed without disrupting the soil (clipped or pinched). Each participating institution will plant a mixture of three species of common interest across the region, selected by the cooperators: common lambsquarters (*Chenopodium album*), pigweed (*Amaranthus retroflexus*) and large crabgrass (*Digitaria sanguinalis*). In addition, southern states will also incorporate morningglories (*Ipomoea* spp.) into the weed mix, while northern states will plant common ragweed (*Ambrosia artemisiifolia*). All species except large crabgrass are among the Weed Science Society of America's top ten most troublesome or common weeds. Finally, each location will add any additional species critical for their state that is not part of the larger study; these species will be added to the model for that state.

Planned Participation of Multistate Partners

Cornell University

School of Integrated Plant Science, Soil and Crop Sciences section

PI: **Antonio DiTommaso** – Professor, Soil and Crop Sciences section

PM: **Caroline Marschner** – Invasive Species Specialist, Soil and Crop Science section

Matthew Ryan – Assistant Professor, Soil and Crop Sciences section

The Cornell University Soil and Crop Sciences researchers will take the lead on this proposal. The team will work at two sites that cover two categories of soil texture, and will plant all the weeds that are used in any state unless planting them will cause their introduction to the region (this excludes Palmer amaranth, which is not yet in New York State). The experiment will be managed by Dr. DiTommaso's lab, with technical and field support from Dr. Ryan's staff.

School of Integrated Plant Science, Horticulture Section

John Wallace, Assistant Professor

Dr. Wallace is stationed at New York State's Experiment Station in Geneva. He will be planting the basic project design in muck soils to capture the unique qualities of those soils for the model. He will include common ragweed and horseweed (*Conyza canadensis*) into the trials.

Department of Earth and Atmospheric Sciences

Art Degaetano – Professor

Alex Sinfarosa – Climate Application Programmer

Rick Moore – Research Support Specialist

Keith Eggleston – Research Support Specialist

The team from the Department of Earth and Atmospheric Sciences will be responsible for the modeling and application development portion of the project. Working with NY-IPM research funds, they have already developed a pilot model for weed emergence that incorporates soil temperature, one round of weed emergence data, and a recently developed weather model that uses a four-kilometer grid. They will expand their weather modeling applications to include forecast data, integrate any necessary soil models to accurately predict seedling emergence, and use the weed data collected by participants to test and refine the existing draft model.

New York State IPM program

Bryan Brown – Integrated Weed Management Specialist

Dan Olmstead – Coordinator of the Network for Environment and Weather Applications (NEWA)

NYS-IPM has funded the pilot research that led to the current proof-of-concept model now being tested. Bryan Brown will provide advice and data to the project. Dan Olmstead's NEWA program will be hosting the draft and final model applications on their website, which currently hosts a suite of similar models for pests and pathogens such as apple maggot, downy mildew, and late blight.

University of Maine

Eric Gallandt—School of Food and Agriculture, Professor of Weed Ecology

Dr. Gallandt will be establishing the basic project design, incorporating common ragweed as the northern regional weed of interest.

Virginia Tech

Jacob Barney— Dept. of Plant Pathology, Physiology, and Weed Science, Associate Professor

Dr. Barney will be planting the basic project design, incorporating the morningglories and adding Johnsongrass (*Sorghum halepense*). Johnsongrass is present through most of the southeast and mid-Atlantic into New York, and is a serious agronomic weed in Virginia.

University of New Hampshire

Richard Smith— College of Life Sciences and Agriculture, Associate Professor

Dr. Smith will be establishing the basic project design, incorporating common ragweed as the northern regional weed of interest.

Rutgers University Experiment Station

Thierry Besancon— Assistant Extension Specialist in Weed Science

Dr. Besancon will be establishing the basic project design, incorporating ragweed and adding hairy galinsoga (*Galinsoga quadriradiata*), a very common and tough-to-control weed species in NJ vegetable cropping systems.

University of Delaware

Mark VanGessel— College of Agriculture & Natural Resources, Professor and Extension Specialist

Dr. VanGessel will be establishing the basic project design, incorporating both morning glories and common ragweed as both are an issue in his state. He will also add Palmer amaranth (*Amaranthus palmeri*), a serious problem weed especially in southern states. Most of our collaborators will be unable to include this weed, but Dr. VanGessel's study site already has Palmer amaranth.

University of Maryland

Bill Phillips – Plant Science & Landscape Architecture, Assistant Clinical Professor

Dr. Phillips will be establishing the basic project design, incorporating both morning glories and common ragweed as both are an issue in his state.

University of California, Davis

Mohsen B Mesgaran – Assistant Professor, Department of Plant Sciences

Dr. Mesgaran will bring weed emergence modeling expertise from the weed ecology perspective, and help with experimental design.

Measurement of Progress and Results

Outputs

- Three years of emergence data from seven states on five high-impact summer annual species and a few additional species of importance to individual participating states.
- Both a draft and final model of weed emergence based on temperature and precipitation data, incorporating the interactions of temperature and precipitation with soil.
- A review paper of the current state of weed emergence science.

Outcomes or Projected Impacts

- Increased effective weed control for farmers, ideally coupled with a reduction in weed management activities, particularly herbicide use. We anticipate that farmers and extension agents will use the tool to time their weed management activities for maximum weed control within their available window of treatment. We will conduct a small survey of farmer users of the tool to measure actual impact, with success defined as a reduction in herbicide applications or cultivations, and/or reported assistance with farm operation planning or reduced problems due to weed populations. We also hope that this will serve as a tool to help extension agents communicate scientifically sound weed advice to their farmers; we will conduct a small survey of extension users of the model to measure its use for extension activities, with success defined as a reported assistance in delivering specifically targeted weed advice to extension recipients.

Milestones

(2019):Finalize field research plans

(2019):Season 1 data collection

(2020):Season 1 data incorporated into draft model; model uploaded to website

(2020):Finalize year 2 field research plans

(2020):Recruit farmers and extension personnel to test draft model

(2020):Complete season 2 data collection

(2021):Incorporate season 2 data and stakeholder input; upload modified model

(2021):Finalize year 3 field research plans

(2021):Outreach to increase model use

(2021):Complete season 3 data collection

(2021):Review paper of the current state of weed emergence science submitted.

Outreach Plan

The project will produce a tool for use by farmers, extension personnel, crop consultants, and the general public; the web-based weed emergence tool will be housed on the Network for Environment and Weather Applications ([NEWA](#)) website. An integral part of the project is wide participation for draft model testing in year 2, and outreach to make stakeholders aware of the final product in year 3. Multistate partners will actively extend their knowledge of the tool and encourage participation and use among stakeholders at a wide range of venues, from grower meetings to extension conferences to scientific conferences. Some of these venues include:

Weed Science Society of America annual meeting
Northeastern Plant, Pest and Soils annual conference
Crop Science Society of America annual meeting
New York Cooperative Extension Agricultural In-Service
Delaware Cooperative Extension In-Service
Cornell University Aurora Field Day
Delaware University's Weed Science Field Day
Empire Vegetable Expo
New York's Certified Crop Advisor meeting
What's Cropping Up
Extension Insider (a weekly internal New York extension publication)
Field day / twilight tours / field walks
County extension meetings

Organization/Governance

The multistate research group will have an elected Chair, Chair-elect, and Secretary; we will elect them for the duration of our funded cycle. Administrative guidance will be provided by an assigned Administrative Advisor and a NIFA Representative.

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Land Grant Participating States/Institutions
MI

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Burns, Erin	Yes	Michigan - Michigan State University	1,2	213	2300	1140	0.20	0.00	0.00	0	0

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
213-2300-1140	0.2	0	0
Grand Total:	0.20	0.00	0.00

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

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP1838: Development of a Weed Emergence Model for the Northeastern United States

Rate the technical merit of the project:

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

As the authors of this proposal noted, WeedCast, an emergence model developed for the US Midwest, had never been widely adopted or used by farmers. Before embarking on such an ambitious project to develop a new weed emergence model for the NE region, I think user need should be carefully assessed. The authors of the current proposal claim that an unfriendly user interface was the main reason WeedCast wasn't adopted widely. However, I am not convinced this is the only reason for lack of interest in this sort of tool, as no supporting documentation was provided for this claim. I think, as a first step, some fairly extensive grower surveys should be conducted to assess whether or not a weed emergence model would indeed be desired and used by NE growers.

Although the authors didn't mention it, the Weedometer, an online tool for estimating weed emergence, is currently available (<http://weedecology.wisc.edu/weedometer/>). The user interface for this tool is exceptionally simple – one need only input the weed species and one's location. As a test, I chose common waterhemp with my location and the model prediction was extremely accurate. In fact, the Weedometer estimated the exact date of reported waterhemp emergence in my area. And yet, to my knowledge, this tool hasn't been widely adopted, even though it has existed for at least 15 years. The reason can't be that the interface is too difficult – nothing could be easier.

Another first step would be to identify the main reasons WHY WeedCast doesn't perform well in the NE region. A plethora of factors potentially contribute to weed emergence timing including genetics, temperature, soil moisture, soil chemical and physical properties, seed burial depth, tillage (including timing, depth, degree of inversion, and frequency), seed dormancy, and complex interactions among all these variables. The modeling process would be simplified if one focused on the factors that contribute MOST to emergence timing variability. I suspect that one of the biggest contributors to emergence timing variability might be genetic variability. Otherwise, WeedCast should have produced good predictions for the NE.

Therefore, another preliminary step might be to conduct reciprocal common gardens experiments at a wide geographical range of sites using many weed species biotypes. I suggest using both Midwestern and NE biotypes planted in a series of locations in both regions. Such an array of experiments would allow one to gauge WHY the WeedCast model performs poorly in the NE. This would be a starting place for developing a new model. Or instead of reinventing the wheel, one could work to parameterize and validate the existing model for the NE. I think that the Midwest regional group might be able to collaborate with the NE group to accomplish these objectives.

If genetic variability underlies much of the variability in weed emergence timing, how would weed biotype information be incorporated into an emergence model? One would need to know which weed biotypes occur at a given location. Could these biotypes be identified via DNA analyses then mapped? This alone would be an enormous undertaking.

Another issue with the NE proposal is that no methods are presented to account for genetic variability. No mention was made of growing various biotypes in reciprocal plantings. In fact, a few species would be grown by only one researcher at one site. This proposed approach will have limited success if genetic variability is a big component of emergence timing variability. Furthermore, the proposal authors claimed that herbicide resistance management would be enhanced by an accurate weed emergence model, and yet few of the species they chose to focus on are major resistance problems. However, I understand that researchers are reluctant to introduce new species to their respective locations.

The proposal lacks sufficient detail describing exactly how the proposed model would be developed, parameterized, and validated. All three of these steps are required to develop and test a model. This lack of detail suggests that the participating researchers may be inexperienced in regard to developing emergence models. To this point, I think that more progress could be made by collaborating with the researchers who developed the Midwest models. Instead of reinventing the wheel, progress could be made by building on and improving existing models.

Overall, I think that this proposal addresses a potentially interesting and relevant issue. However, in its current state the proposal demonstrates a lack of logical thinking about how best to tackle the problem. I suggest a major revision, and reconsideration of the main objectives.

Your Recommendation:

Approve/continue project with revision

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP1838: Development of a Weed Emergence Model for the Northeastern United States

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

Weed emergence models have long been advocated as important tools for making weed management programs more effective and efficient; but, weed emergence models are rarely used in management decisions because, in part, farmers and consultants believe that the emergence forecasts do not pertain to their locations. For farmers and consultants in the Northeast US, concerns regarding applicability are warranted. This is because weed emergence models have yet to be developed and disseminated for weed species and biotypes in the Northeast US.

This project will deliver weed emergence models to farmers and consultants in the Northeast. The new emergence model will make use of recent advances in weather forecasting, thereby improving the spatial resolution and accuracy of the emergence predictions. The new emergence model will also employ current approaches for developing relationships between soil moisture/temperature and weed emergence. By combining current approaches in emergence modeling with new weather forecasting tools, this project represents a critical advancement in integrated pest management.

Additional strengths:

The experiments make use of existing knowledge and are devised to incorporate farmer feedback for model improvement. Further, the eventual emergence models fit in the pest-forecasting framework utilized by regional IPM programs for insects and diseases.

The project team, which includes weed scientists, extension specialists and climate scientists, has the expertise to carry out and disseminate the proposed work.

Data analyses supporting model development will mostly be performed by weed science and climate researchers at one university (Cornell University).

Comment for improvement:

In addition to weather forecasts at high spatial resolution, the weed emergence model will need to incorporate spatial information on soil type, as well as information on relationships among weather, soil type and soil conditions. The proposal indicates that soil types have been considered in the development of preliminary data, but it is not clear how the eventual emergence model will provide end-users to customize soil type settings. Although specifics are not provided, the proposal states that researchers in the Department of Earth and Atmospheric Sciences at Cornell University will integrate soil models. I am confident that these researchers will be able to integrate soil maps and models, but because procedures are not provided, I scored "achievable goals/objectives" as "good".

Your Recommendation:

Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP1838: Development of a Weed Emergence Model for the Northeastern United States

Rate the technical merit of the project:

1. Sound Scientific approach:

Approve/continue project with revision

2. Achievable goals/objectives:

Fair

3. Appropriate scope of activity to accomplish objectives:

Good

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

Good

5. Overall technical merit:

Unacceptable

Comments

This is a very important topic and with great potential to generate valuable information for decision making of weed managers. It would be valuable for PIs to pay attention to differences in emergence due to environmental factors and genetic differences among populations. Without understanding the influence of these two factors on seedling emergence, it is difficult to generate seedling emergence predictive models that have local and regional accuracy.

The selected species, although considered weeds, are commonly not the ones driving weed management decisions in the proposed area with the exception of common ragweed. PIs should explain better how information about emergence timing of these species will drive the overall decision making for weed control.

There are existing emergence and dormancy/germination models for common ragweed, redroot pigweed, and johnsongrass. The PIs made no reference to those models, or how their model will incorporate that information or how their model is better than existing ones.

There is no mention of how the models will be validated. This is a common mistake in "modeling" projects. Just describing the observed emergence based on thermal or hydrothermal time does not generate a predictive model. Assessing the robustness of the model against independent data, preferably generated from different locations and with different climatic conditions is important.

It is a concern that most of the PIs have no expertise on weed emergence modeling. Dr. Mesgaran is part of the project providing some help with experimental design, but the rest of the PIs seem to be limited to collecting emergence data. It is not clear who will be doing the complicated task of developing the model.

It is greatly appreciate that this is a regional project covering a large extension. This could generate very valuable data to understand differences in emergence across locations. Although, it is also positive that the PIs propose to work with growers to test the draft model and get feedback about its value and use, my experience working on seedling emergence modeling is that it is very important to have a fully validated model before encouraging their use by growers. Having an inaccurate model will discourage growers from using these tools in the future. PIs should develop a strategy for avoiding this issue.

There are multiple citations with the reference missing.

Your Recommendation:

Approve/continue project with revision