Report and Recommendations
NORTHEAST MULTISTATE ACTIVITIES COMMITTEE MEETING
May 31, 2023
11:00 AM -12:00 PM ET Zoom Teleconference

Members: Matt Wilson (WVU-Chair), Puneet Srivastava (Maryland), Jason White (CT-New Haven), Blair Siegfried (Penn State), Cindy Fitch (WVU/NEED), Ali Mitchell (NEED) [Non-voting, ex officio: Rick Rhodes (NERA), David Leibovitz (NERA)]

Request to approve FFY2024 Off-the-Top Budget (MAC recommendation to NERA)

- NE59: *FFY2024 Support for the Northeastern Regional Center for Rural Development* (Director: Stephan Goetz. See memo of April 13, 2023, Blair Siegfried to MAC, $40,788)
    - NERCRD is housed at Penn State. The requested funding amount supports some of the Director's salary and some staff salaries; fringe is supported by Penn State. The request of $40,788 has not changed since at least 2016.
    - In NIMSS, NE59 appears as a Coordinating Committee. Participation does not require reporting to NIFA. NERCRD has historically been supported by regional off-the-top funding.
    - For FFY25, NERA could consider creating a 5-year multistate project to support NERCRD rather than using off-the-top funding and the coordinating committee mechanism. The North Central region uses this mechanism to fund its regional rural development center.
    - **The MAC unanimously recommends approval of the NE59 off-the-top budget request for FFY2024. The MAC will also recommend the exploration of using a multistate project to support NERCRD in future years.**

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

- NE_TEMP2333: *Biological Improvement of Chestnut through Technologies that Address Management of the Species and its Pathogens and Pests*, 10/2023 – 09/2028 [Renewal of NE1833, AA: Brad Hillman – Rutgers]
    - USDA, in cooperation with state and private agencies, established a reparation effort in 1910 to re-establish the American Chestnut tree. This project has directly impacted the reparation effort for multiple 5-year cycles.
    - This project was the last Northeast group to win the National Excellence in Multistate Research Award (over 10 years ago).
    - Peer reviews were all very supportive and did not call for substantive edits by the technical team.
    - **The MAC unanimously recommends approval by NERA of NE_TEMP2333.**

- NE_TEMP2334: *Genetic Bases for Resistance and Immunity to Avian Diseases*, 10/2023 – 09/2028 [Renewal of NE1834, AA: Bob Taylor – West Virginia]

- Peer reviews (4) were supportive; one reviewer called for light revisions by the technical team.
- The group is highly functional and productive, with over 160 publications over its past 5-year cycle.
- **The MAC unanimously recommends approval by NERA of NE_TEMP2334.**

- NE_TEMP2335: *Resource Optimization in Controlled Environment Agriculture*, 10/2023 – 09/2028 [Renewal of NE1835, AA: Puneet Srivastava - Maryland]
  - This proposal is arranged into a set of three concrete objectives with embedded sub-objectives. The group has been historically active.
  - Peer reviews were generally supportive. One reviewer was concerned about overlap with a North Central extension/research activity in controlled environment ag. The two groups are complementary and there are participants who are engaged with both.
  - **The MAC unanimously recommends approval by NERA of NE_TEMP2335.**

- NE_TEMP2336: *Improving Quality and Reducing Losses in Specialty Fruit and Vegetable Crops through Storage Technologies*, 10/2023 – 09/2028 [Renewal of NE1836, AA: Chris Watkins – Cornell]
  - This project addresses the critical issue of safe, high-quality fruit and vegetable storage. Objectives are clearly defined. Reviews were generally supportive and expressed great enthusiasm for the work of the group, one reviewer calling the proposal a successful example of fundamental applied science.
  - Cooperative Extension has been urged by USDA to position itself for engagement in the food loss / food storage working space, as funding opportunities will become available.
  - **The MAC unanimously recommends approval by NERA of NE_TEMP2336.**

- NE_TEMP9: *Conservation and Utilization of Plant Genetic Resources*, 10/2023 – 09/2028 [Renewal of NE9, AA: Olga Padilla-Zakour]
  - Each 1862 AES region supports a germplasm center using regional off-the-top funding. NE_TEMP9 is the proposal to renew funding support for the Northeast's germplasm center (located in Geneva, NY; Cornell AgriTech) for a 5-year cycle. The Northeast center works closely with the national network of regional plant germplasm centers.
  - The requested budget is increased slightly (~7.0%) from its previous cycle. That increase is a bit less (on a % basis) than increases to the Multistate Research Fund, 2018 to 2022 (~7.8%).
  - Reviews were generally supportive and called for light revisions. The technical team responded appropriately and captured the responses in a memo attached to the proposal.
  - **The MAC unanimously recommends the approval by NERA of NE_TEMP9.**

Northeast Administrative Adviser assignment recommendations to NERA

- NRSP_TEMP_11: *Building Collaborative Research Networks to Advance the Science of Soil Fertility: Fertilizer Recommendation Support Tool (FRST)* – **new NRSP proposal seeking a Northeast AA**
- NE1832: *Biological Control of Arthropod Pests and Weeds* – **expiring 9/2023 with an intention to renew**
    - Jan Nyrop (Cornell AgriTech, retired)
    - Jason White agreed to serve as the NE1832 Administrative Adviser
    - **The MAC unanimously recommends to NERA that Jason White is appointed as the Administrative Adviser for NE1832.**

Informational Items

- NE nomination for the 2023 Experiment Station Section Award for Excellence in Multistate Research

  NE2140: *Sustainable Management of Nematodes in Plant and Soil Health Systems* (AA Anton Bekkerman)

- Draft proposals undergoing MAC ready check and peer review:
    - NE_TEMP2338: *Weed Emergence in a Changing Climate*, 10/2023 – 09/2028 [Renewal of NE1838, AA: Margaret Smith – Cornell]
    - NECC_TEMP2312: *Northeast Coordinating Committee on Soil Testing*, 10/2023 – 09/2028 [Renewal of NECC1812, AA: Rick Rhodes – NERA]

- Renewing projects ready for MAC ready check:
    - NE1832: *Biological Control of Arthropod Pests and Weeds* (former AA Jan Nyrop) – **ending 9/2023**

- Expiring multistate activities with no confirmed intent to renew:
    - NECC29: *Northeastern Corn Improvement Conference* (AA Margaret Smith) – **ending 9/2023**
    - NEERA1603: *Northeast Pasture Consortium* (AA Margaret Smith) – **ended 9/2021**

- Expiring multistate activities not seeking renewal:
    - NE1944: *Management of the Brown Marmorated Stink Bug*, 10/2018-9/2023 – **project ending 9/2023**

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DATE: April 13, 2023

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

TO: Members of the Northeast Multi-State Activities Committee (NE-MAC)
Matt Wilson
Puneet Srivastava
Jason White
Blair Siegfried
Cindy Fitch
Ali Mitchell

RE: Action Item for NERA Meeting

By means of this memo, I am requesting off-the-top funding in the amount of $40,788 for the Northeast Regional Center for Rural Development, for the period October 1, 2023 through September 30, 2024, for NE-59, Regional Research Coordination, Northeast Region. The regional funds are used to support the salaries of the Center directors and staff. Penn State pays all the fringe benefits associated with these personnel services. This means that the bulk of the USDA-NIFA special research funds for rural development are used to support the program.

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

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

cc: Directors of Agricultural Experiment Station, NE Region
S. Goetz

NE_TEMP2333: Biological Improvement of Chestnut through Technologies that Address Management of the Species and its Pathogens and Pests

Status: Submitted As Final

Duration	10/01/2023 to 09/30/2028
Admin Advisors:	[Bradley Hillman]
NIFA Reps:	

Statement of Issues and Justification

Chestnut blight, incited by *Cryphonectria parasitica* (Murr.) Barr, devastated the American chestnut tree (*Castanea dentata* (Borkh.) Marsh) in the first half of the 20thcentury, killing approximately 4 billion dominant and codominant trees in the hardwood forests of the eastern United States. Prior to blight, the tree had many uses, producing sawtimber, poles, posts, fence rails, cord wood for fuel, paper and tannin extraction, and nuts for humans, livestock and wildlife. It also can be characterized as a member of our charismatic megaflora; many people mourn its loss and participate in citizen-science projects to restore it. Restoration of the American chestnut would be a demonstration of an application of science for the public good in the face of continuing environmental degradation due to the advent of industrial and now postindustrial economies and the accompanying influx of exotic pests.

The United States Department of Agriculture (USDA), in cooperation with state and private agencies, began work in the 1910s to restore the chestnut tree after recognizing that it was on an inexorable path to destruction caused by the blight. As part of their work, exotic species of *Castanea* were introduced, which has resulted in a nascent orchard industry in numerous states from coast to coast in the US. Although the aggregate production of edible chestnuts is still too small to be tallied separately by the USDA, in 2015, the United States had 919 farms producing chestnuts on more than 3,700 acres. The states with the most chestnut acreage were Michigan, Florida, California, Oregon, Virginia, and Iowa. Most of those trees are not afflicted by blight, but are affected by other pests and diseases, which need management. Additionally, specialized cultivation techniques for the trees are required, and infrastructure to process and market chestnuts needs further development. NE-1833 members have performed research and obtained funding to address these needs and have formulated extension recommendations.

The NE-1833 project and its predecessors have been the central organization coordinating chestnut research since its inception as NE140 in 1982. For 40 years the project has exemplified what the original USDA model for Regional Research, now Multistate Research projects, was intended to accomplish; as detailed below, it has been and continues to be successful at every level. Members span numerous disciplines in forest pathology, plant sciences, microbiology, molecular genetics and biochemistry, and the annual meeting provides an opportunity for members to be exposed to this diversity. NE-1833 has provided a forum for new and established researchers to develop collaborative relationships and to share resources and expertise. NE-1833 meetings are well attended, and about 30 presentations are typically made by participants each year. Despite pandemic-related disruption in 2020 and 2021, the group still met virtually to share ideas and continue the work. International visitors and collaborators have often been included in these presentations, and two international symposia were organized and hosted by NE-1333, the immediate predecessor to NE-1833. As a result, numerous multi-state and international research efforts have been undertaken by NE-1833 members. The project was initiated to explore the diversity of hypoviruses and their efficacy for controlling blight on American chestnut at different locations in its natural range. That original goal persists, but range-wide studies additionally include breeding and evaluation of disease-resistant progeny as well as studies of orchard chestnuts for nut production. Additional activities requiring a multistate effort have been able to leverage rapid developments in genome sequencing technology and have led to the development of specific and useful genomic tools for *Castanea*.

The NE-1833 project comprises three objectives: 1) develop and evaluate disease-resistant chestnuts for food and fiber through traditional and molecular approaches that incorporate knowledge of the chestnut genome; 2) evaluate biological approaches for controlling chestnut blight from the ecological to the molecular level by utilizing knowledge of the fungal and hypovirus genomes to investigate the mechanisms that regulate virulence and hypovirulence in *C. parasitica*; and 3) investigate chestnut conservation and reestablishment in orchard and forest settings with special consideration of the current and historical knowledge of the species and its interaction with other pests and pathogens.

Objective 1: Develop and evaluate disease-resistant chestnuts for food and fiber through traditional and molecular approaches that incorporate knowledge of the chestnut genome.

Restoration of American chestnut depends on producing a founder population that has adequate blight resistance, forest competitiveness, and genetic diversity to adapt to a large natural range and a changing climate. It is also important that the restoration population has resistance to phytophthora root rot (PRR) caused by the oomycete pathogen *Phytophthora cinnamomi* (Westbrook et al. 2019; Gustafson et al. 2022). This disease is most prevalent in the Southeastern U.S. but is expected to spread northward as the climate warms (Burgess et al. 2017).

For forty years, the American Chestnut Foundation (TACF) has pursued backcross breeding to introgress resistance to *Cryphonectria parasitica* and *Phytophthora cinnamomi*, from Chinese chestnut (*C. mollissima)*, into a genetically diverse population of American chestnut. In parallel, scientists at the State University of New York college of Environmental Science and Forestry (SUNY-ESF) have worked since 1990 on transgenic approaches to enhance blight and PRR resistance in American chestnut (Steiner et al. 2017).

To date, the most promising transgenic approach to enhance blight resistance has been the insertion of the oxalate oxidase (OxO) gene from wheat, which detoxifies oxalic acid produced by the chestnut blight fungus and significantly reduces the severity of chestnut blight stem cankers (Newhouse et al. 2014; Powell et al. 2019; Newhouse & Powell, 2020). In 2020 and 2021, the research team at SUNY-ESF submitted petitions to three federal regulatory agencies (USDA, EPA, and FDA) to obtain non-regulated status of the 'Darling 58' (D58) transgenic variety of the American chestnut containing the OxO gene (Newhouse et al. 2020). The experimental evidence presented in the petition indicates that D58 progeny pose no significant plant pest or food safety risks. Darling 58 progeny may be deregulated and distributed to the public as early as 2023. If and when D58 is deregulated, TACF and ESF plan to breed D58 progeny with a genetically diverse population of American chestnuts over three to five generations with the aim of representing 99% of the climate adaptive genetic diversity in the wild population of *C. dentata* (Westbrook et al. 2020; Sandercock et al. 2022). Furthermore, we plan to breed D58 progeny with backcross trees selected for resistance to *P. cinnamomi* to combine resistance to the two major diseases of American chestnut (Westbrook et al. 2019). The combination of biotechnology with breeding is a promising strategy to produce restoration-worthy trees. The initiatives outlined in the current and future work section describe our current best practices and future projects aimed at generating disease resistant and genetically diverse populations of American chestnut for restoration.

NE-1833 participants maintain longstanding commitments to education, outreach, transparency, collaborations, and research toward safe and effective American chestnut restoration that date back to the conception of the original NE-140 project. Researchers at ESF have been working closely with federal regulators from all three agencies in the Coordinated Framework for Biotechnology: USDA-APHIS, EPA, and FDA. The D58 chestnut has provided a unique opportunity for both university researchers and federal regulators to understand how this process, historically applied to annual agricultural crops, can be extended to a wild tree intended for wild release and persistence in the environment. This series of rigorous reviews by all three agencies will help ensure safety and demonstrate to the public that the development and distribution of Darling 58 chestnuts is being done responsibly and transparently.

The project has always welcomed and incorporated public feedback while acting on scientifically sound advice. Indeed, the decision to pursue regulatory approval and public distribution has been driven and sustained by public feedback. As part of the deregulation petition for D58 there have been public comment periods (PCP) that have received supportive input from thousands of individuals and over 100 agencies (including The Sierra Club and The Nature Conservancy). In addition, dozens of commenters state that, while they are generally opposed to genetically-modified organisms as a general rule, they see the transgenic American chestnut as an amazing application of the technology and offer their support. In the first PCP (August 2020), the support from unique, individual commenters was strongly supportive (62%), a trend that continued in the second PCP (85% of unique comments are supportive as of 1/9/23, with over 5,000 unique comments contributed in total to date). This information is provided in more detail, with hyperlinks to comments, in the Outreach section, below.

Objective 2: Evaluate biological approaches for controlling chestnut blight from the ecological to the molecular level by utilizing knowledge of the fungal and hypovirus genomes to investigate the mechanisms that regulate virulence and hypovirulence in *C. parasitica*.

Chestnut blight appears to have been controlled by naturally occurring hypoviruses on*C. sativa* in Europe but not on *C. dentata* in North America, except in specialized settings. Research by NE-1833 members and their European colleagues contributed to the view that control in North America was hampered by the much larger number of strains of *C. parasitica* in different vegetative compatibility groups than occurred in Europe. Other factors hampering control in North America versus Europe may have included greater competition from other hardwood species, greater susceptibility to blight in *C. dentata* and differing forest management practices.

The RNA sequence of a hypovirus was first determined by members of NE-140 (the original precursor of NE-1833), and a number of species of virus were found based on sequence analysis. Viruses in families other than the Hypoviridae, including mitochondrial viruses, were found infecting *C. parasitica,* some associated with reduced virulence and biocontrol. Transformation of *C. parasitica* with cDNA of Cryphonectria hypovirus 1 resulted in transmission of the DNA in ascospores and regeneration of RNA viruses in progeny. This completed Koch's Postulates for the hypovirus. Unfortunately, while transformed fungus strains could produce progeny that infected adjacent chestnut trees with hypovirus-containing *C. parasitica,* disease remission did not occur in the general populations.

Regarding the molecular basis of fungal pathogenicity, NE-1833 researchers working in this and previous project cycles have found numerous fungal genes involved. Their protein products were components of complex signaling or response mechanisms critical for basic cellular functions, for example, G-protein signaling pathway components, some of which were also affected in expression by the presence of the hypovirus. Additionally, hypovirus-infected mycelium was found to fail to transition metabolism with colony age in the same manner as the uninfected mycelium. Thus, there are now known to be many genetic factors in the fungus that can influence pathogenicity, and several of these are now known to be affected by hypovirus infection.

To address the issue noted above concerning the variation in vegetative incompatibility groups in North American populations of *C. parasitica* and the potential negative impact on biological control potential, the strain Ep155 was crossed with a European strain and six vegetative compatibility loci, known as Vic genes, were genetically mapped in the progeny. The DNA of strain Ep155 of *C. parasitica* was sequenced and the European strain resequenced. The six Vic genes were identified, and a "super donor" strain prepared with five inactivated Vic genes (four Vic genes were knocked out). Demonstrating the efficacy of the superdonor strain should be able to transmit hypoviruses to strains with any combination of Vic genes. The strain is being tested in the forest for disease control and development of this potential tool is described in the current work section.

Strain Ep155 is regarded as highly pathogenic and has been used most effectively as the prototypic strain in the studies of fungal pathogenicity determinants. It is also used to screen chestnut trees for blight resistance in combination with a strain of low pathogenicity known as SG2-3. To investigate the genetic differences between these two strains that lead to the different phenotypes, the two strains were crossed and 96 progeny evaluated for pathogenicity. As part of NE-1833 all of these strains were sequenced to a high depth and preliminary work to identify quantitative trait loci (QTL) with the intent to test for pathogenicity effects by gene knockout. This should help lead to further understanding of mechanisms of pathogenicity in the fungus. The mechanisms of virulence reduction by hypoviruses in the fungus also remain an active area of investigation, as do other aspects of virus activity in the fungus. Reannotation of the fungal genome by NE-1833 researchers is facilitating transcriptomics and other detailed analyses in the above studies.

Blight cankers on chestnut are perennial and have been observed to persist more than 40 years. A rather complex community develops in cankers, especially as they age. NE-1833 members have documented numerous species of invertebrates and microorganisms in cankers. The blight fungus itself becomes a host for various viruses and similar entities, and multiple strains can be isolated from cankers. The development of these communities and association of their composition with canker longevity is a promising area for metagenomic investigation. Sadly, work at Shenandoah University in this area was significantly compromised by the COVID pandemic and resulting sample losses caused a significant setback to this part of the work.

Objective 3: Investigate chestnut conservation and reestablishment in orchard and forest settings with special consideration of the current and historical knowledge of the species and its interaction with other pests and pathogens.

In addition to the activities discussed under Objectives 1 and 2 above, research is ongoing on gall wasp, silvics, juvenile*versus* adult chestnut blight resistance, genetic variation in American chestnut, and integrating resistance with hypovirulence to control blight, *inter alia*.

It has been found that introduced and native parasites of the Asian chestnut gall wasp (*Dryocosmus kuriphilus*) control the pest after the first few years of infestation. Despite a plant quarantine, Michigan is now in the third year of gall-wasp infestation. While nut harvests are markedly decreased during those first few years of infestation, insecticidal treatments also would destroy the parasites, so the recommendation is to NOT spray insecticides to control gall wasp. Dispersal of parasites as a biological control is recommended, but none are being produced currently. There have been efforts to bring parasite-infested boughs to new areas of gall-wasp infestation, to introduce parasites earlier than occurs naturally. There is variation between cultivars in their susceptibility to gall wasp.

In general, silvicultural evaluations of American chestnut in several states have found that it is a very rapid grower, frequently much faster than planted oak and walnut, though naturally regenerated woody vegetation often grows faster than planted chestnuts in forest settings. Chestnut growth varies with site, like most hardwoods, and competitive dynamics will differ with site and light availability. Earlier research found that exotic chestnut species do not grow well in native forests, unlike American chestnut. This finding was part of the motivation leading to the proposal to backcross resistance from exotic into American chestnut.

The results of inoculating young seedlings of the three Chinese*Castanea* species do not match up with blight severity on mature specimens of the three species in China; this result needs more detailed experimental evaluation. Low levels of blight resistance occur in a few American chestnut trees. Intercrossing of these to enhance that resistance has been pursued for a long time. In combination with hypoviruses, impressive levels of blight control have been observed on some pure American chestnut trees with low levels of resistance. The hypothesis that hypoviruses coupled with resistance in backcross progenies will diminish blight severity is being evaluated.

Castanea species native to the U.S. (*dentata*, *pumila* var. *pumila,* and *pumila* var. *ozarkensis*), *Castanea* species imported from elsewhere (*crenata, mollissima, henryi, seguinii*, and *sativa*), and *Castanea* hybrids are maintained and studied by NE-1833 scientists and their citizen-scientist collaborators. These trees are in: Alabama, California, Connecticut, Delaware, Florida, Georgia, Indiana, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Mississippi, Missouri, New Jersey, New Hampshire, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, Vermont, Virginia, West Virginia, and Wisconsin. Strains of *C. parasitica* are shared by members of NE-1833 (according to APHIS PPQ permitting and restrictions), and important strains are deposited with the American Type Culture Collection. Strains with genetic markers are available, and information on the genetic determinants of vegetative incompatibility (*vic* genes) is available for use in population studies. *Hypovirus* types from France, Italy, MI, WV, KY, and China are studied and shared by NE-1833 members.

Members are renowned for their work on chestnut, *Cryphonectria,* and fungal viruses. In the duration of the NE-1833 project to present (2018 – end of 2022) NE-1833 members collectively published 49 peer-reviewed technical articles in scientific journals and participated in numerous articles in the popular press that have been distributed in both print and digital media through venues as diverse as the Washington Post and New York Times to the Sierra Club to modernfarmer.com and granitegeek.com. Student training also continued with 4 Ph.D. dissertations and 11 M.S. theses completed during the current project, and a large number of undergraduates contributing to the work at the participating institutions.

Venues for scientific presentations, although somewhat limited or sometimes virtual due to the COVID pandemic during the project period, nonetheless included the Plant and Animal Genome Conference, the American Phytopathological Society, the Mycological Society of America, the Fungal Genetics Conference (sponsored by the Genetics Society of America), the Ecological Society of America, the Society of American Foresters, various venues of the The American Chestnut Foundation (TACF), the Entomological Society of America, the American Society for Microbiology, and the American Society for Virology. The results of research have been extended to growers, especially in Pennsylvania, Michigan and Missouri, and to volunteer citizen scientists in 21 states guided by TACF and the American Chestnut Cooperators' Foundation (ACCF).

In recognition of these successes, researchers currently part of NE-1833 received the ESS Excellence in Multistate Research award in 2010 (NE-1833 was then known as NE-1033). The cohort of scientists from 12 years ago has undergone some natural turnover but remains strong with exceptional young scientists joining the project. Despite the significant impact of the COVID pandemic on research operations and the ability to meet in person, NE-1833 has largely met the milestones detailed in the project description and will continue to work on similar collaborative projects in the next five years. Data generated under the auspices of the NE-1833 project have been used by members to gain intramural and extramural funding for all aspects of chestnut biology and restoration.

In summary, the NE-1833 project remains a productive group of collaborators that has provided new and meaningful information to all clients interested in chestnut biology and restoration, from the bench scientist to the professional orchardist and to the individual volunteer grower of chestnut for restoration. In the next five years, we will continue to pursue collaborative projects under our three stated objectives leading to increased production of chestnuts in orchards and furthering the restoration efforts of the iconic American chestnut.

Related, Current and Previous Work

Objective 1

A: Diversify transgenic blight tolerant populations and incorporate phytophthora root rot resistance via breeding transgenic with backcross trees

We have begun introgressing the OxO gene into a diverse population of American chestnut by “speed breeding” D58 progeny with diverse wild type and backcrossed American chestnuts from across the species range. Simulations suggest that outcrossing a single transgenic founder over five generations to 700 diverse wild type parents will increase the effective population size to >1000 and minimize inbreeding coefficients to less than 1% (Westbrook et al. 2020a). Genome-environment analyses based on whole genome sequencing on a sample of 356 American chestnut individuals suggests that the *C. dentata* native range is composed of 3 to 4 locally adapted subpopulations (Sandercock et al. 2022; Sandercock et al., unpublished). Preliminary analyses suggest that breeding with 209 total wild type individuals or 92 individuals from the northeastern zone, 87 from the central zone, and 30 from the southwestern zone is sufficient to match climate adaptive allele frequencies in each zone at $R^2 = 0.99$ (Sandercock et al., unpublished). In 2022,**Virginia Tech** researchers performed whole genome sequencing on 350 backcross progeny representing most of the ~250 wild type parents used in TACF’s breeding program to determine how much of the adaptive diversity is represented. Under permits from the USDA, the multistate cooperators on this project have taken Darling 58 progeny to the third generation of outcrossing to over 100 wild type and backcross American chestnut parents.

Researchers at the **University of New England**, **SUNF-ESF, TACF's Meadowview Research Farms**, and **Penn State** have established systems to grow seedlings under long day and high fertilizing conditions to induce transgenic Darling 58 seedlings to produce catkins and pollen within the first growing season. Between these locations, we expect to produce and cryostore over 2000 vials of pollen before the start of the 2023 growing season. This pollen supply is enough to pollinate more than 200,000 female flowers.

Scientists at **TACF** and **Virginia Tech** have collaborated on an extensive genotyping and phenotyping effort encompassing over 5,500 American chestnut backcross hybrids planted in 88 orchard locations in 20 states. A total of 421 backcross trees that descended from a range wide sample of unique wild type American chestnuts have been prioritized for breeding with D58. These trees inherited between 85% and 100% of their genome from *C. dentata*. TACF has also collaborated with **RAPiD Genomics** to develop a genotyping method that will enable us to select against residual inheritance of genes from Chinese chestnut and select progeny that inherited all of their genome from *C. dentata*. Genotyping also enables us to select progeny that inherited less than 10% of their genome from the D58 founder tree. Selecting against the D58 genome minimizes the potential for deleterious inbreeding in the restoration population and ensures that the trees that we deploy for restoration have locally adapted genetics. Upon deregulation of D58, we plan to plant 6 + seed orchards collectively composed of 5000 + D58 progeny derived from crosses with a range wide sample of 200 + American chestnuts. These seed orchards collectively will have the capacity to produce >1 M open pollinated seed by age 10 years.

We have begun breeding D58 progeny with the PRR resistant selections to generate 'dual resistance' progeny with resistance to both chestnut blight and PRR. Through a combination of progeny testing and genomic prediction, we've also identified 146 backcross trees with elevated resistance to *P. cinnamomi* ranging from 25 to 77 on the 0 American chestnut to 100 Chinese chestnut scale. In 2021 and 2022, **U.S. Forest Service**, **Clemson University**, and **TACF** collaborators inoculated 220 OxO positive progeny from ten crosses between Darling 58 progeny and PRR resistant backcross trees with *P. cinnamomi* at the U.S. Forest Service Resistance Screening in Asheville, NC. To date, a total of 65 seedlings have survived the greenhouse inoculations with intact root systems and were planted at a permitted TACF orchard site in Georgia. Our plan is to eventually intercross the partially PRR resistant survivors and select for higher levels of PRR resistance and OxO homozygosity among the progeny.

Finally, Breeding D58 with Ozark, Allegheny, and Alabama chinquapins holds promise as a method to enhance blight resistance in these blight susceptible and sexually compatible species. To introgress OxO into chinquapin populations, we plan to outcross D58 with chinquapin varieties over 3 + generations and use genetic markers to select for maximal chinquapin ancestry in each generation.

B: Compare traditional breeding as a standalone strategy to the combined biotech/breeding approaches to enhance blight and phytophthora resistance

Even if D58 is approved, we anticipate that some landowners will prefer non-transgenic trees for species restoration. The traditional breeding program to introgress blight resistance into American chestnut from Chinese chestnut has been challenging due to the latter's complex genetic architecture of blight resistance. Estimates of trees' genetic resistance are negatively correlated with the proportion of genome inheritance from *C. dentata* (Westbrook et al. 2020b). Simulations suggest that controlled crosses between backcross trees selected for blight resistance (hereafter referred to as best x best crosses) will produce progeny with average blight resistance index values of 50 and approximately 85% American chestnut ancestry. Genomic selection of the most resistant 10% of the progeny from best x best crosses is expected to further improve average blight resistance indices to ~ 65 while also potentially reducing *C. dentata* inheritance to 75%.

Another traditional breeding option for improving blight resistance in American chestnut is to perform controlled pollinations between partially blight resistant wild type American chestnuts and select for enhanced resistance in the progeny through repeated cycles of breeding and selection. In the late 1990's and early 2000's **TACF** performed controlled pollinations between large surviving American chestnuts and planted the progeny at Meadowview Research Farms. Current TACF staff recently assessed the long-term blight resistance of 48 progeny of intercrosses among 12 large surviving wild trees. All the progeny were inoculated with virulent strains of the blight over a decade ago and the trees ranged in age from 16 to 25 years old. We visually assessed these trees for survival of the main inoculated stem, percent of the tree canopy that was healthy, presence/absence of large cankers, exposed wood, stump sprouts, and blight fungal sporulation from cankers. The blight resistance data from the individual trees and their relatives to estimate the genetic resistance of the progeny of large surviving trees relative to typical blight-susceptible American chestnuts, resistant Chinese chestnuts, and partially resistant American chestnut backcross hybrids. A blight resistance index was created from the sum of the individual traits and scaled this index from 0 = average of typical blight susceptible wild type American chestnuts to 100 = average of blight resistant Chinese chestnuts. The blight resistance indices of the 10 most resistant progeny of the large surviving trees varied from 43 to 25. Nine out of 10 of these progeny and all trees had 100% American chestnut ancestry (Westbrook et al. unpublished). For context, the blight resistance indices of the top 5% most resistant backcross selections (137 selected trees) varied from 40 to 80 (average 50) while American chestnut ancestry varied from 60% to 100% (average 88%). These results confirm that blight resistance in American chestnut is heritable.

TACF is planning to compare the resistance of progeny D58, best x best backcross trees, and large surviving American chestnuts using small stem assays for blight resistance replicated at two greenhouse locations in 2023. If we find that the blight resistance index values of best x best progeny exceeds our minimum standard 40, then it would be worthwhile to pursue both transgenic and non-transgenic approaches in parallel to be able to offer different types of trees depending on landowner preference.

C: Develop a basic understanding of the biology and genes underlying blight and phytophthora root rot resistance in Castanea

Understanding of the biology of resistance and susceptibility to chestnut blight and phytophthora root rot in *Castanea* is a prerequisite to additional biotechnology aimed at enhancing resistance to these diseases in American chestnut. To discover candidate genes underlying resistance to chestnut blight and PRR, researchers from **Virginia Tech**, **University of Kentucky**, **TACF,** and **Oak Ridge National Laboratory** are currently pursuing a combination of quantitative trait locus mapping in American chestnut backcross populations, RNAseq and metabolomic timecourses, genome scans for signatures of natural selection, and comparative genomics. This work has been aided by the recent completion of a chromosome scale annotated reference genome for *Castanea dentata* and two haplotype resolved genome assemblies for *C. mollissima* by colleagues at **Hudson Alpha Institute of Biotechnology**. These genomes are available on Phytozome (https://phytozome-next.jgi.doe.gov/).

The quantitative trait locus mapping completed to date indicates that blight resistance is controlled by tens to hundreds of small effect loci on all twelve chromosomes, while phytophthora resistance is controlled by fewer larger effect loci concentrated on chromosome five. We found regions of overlap between QTLs for blight and PRR resistance and signatures of natural selection in resistant *C. mollissima* and *C. crenata*. In the RNAseq timecourse, we identified over 3,000 differentially expressed genes (DEGs) in response to blight inoculation in either *C. mollissima* or *C. dentata*. A subset of 700 DEGs were specifically differentially expressed in *C. mollissima* and an additional 800 DEGs were specific to *C. dentata.* The species specific DEGs may contain genes important for blight resistance and susceptibility in *Castanea*. We identified a smaller subset of 58 hub genes whose expression was strongly correlated with the expression of genes specific to the *C. mollissima* response. As this work continues, our aim is to narrow the list to dozens of well supported candidate genes to target for gene knockouts with CRISPR or transgenic insertion to test for enhanced resistance or susceptibility in *C. mollissima* and *C. dentata*.

D: Develop additional founder lines containing the OxO gene and a higher throughput system to do genetic transformations in American chestnut.

The Darling 58 variety was developed by transforming a single founder wild type American chestnut from New York state. Researchers at **University of Georgia** and **SUNY-ESF** are collaborating to transform up to three additional American chestnut genotypes from Georgia, Virginia, and Pennsylvania with the OxO gene to reduce the potential for deleterious inbreeding and founder effects. Some of the new OxO events in these founder lines will be expressed with a wound and pathogen inducible promoter (win3.12) from *Populus* (Carlson et al. 2022). Inducible OxO expression has the potential to reduce growth penalties and other deleterious effects that might result from constitutive OxO expression. Next steps for the development of these new transgenic founder lines include verifying transgene expression and copy number, developing whole plants from transformed somatic embryos, long read sequencing to determine the transgene location, high light pollen production, initial breeding with wild type trees, and conducting blight resistance assays on the progeny. Once these steps are complete, we plan to petition for non-regulated status to use these new founder lines in our larger breeding effort to introgress OxO into wild populations of *C. dentata*.

Any further biotechnology aimed at enhancing disease resistance in American chestnut will be aided by developing a more efficient transformation system. Recently, it was demonstrated that gene editing could be performed on live plants by simultaneously inducing and transforming meristems by injecting stems with developmental regulators and gene editing constructs (Mahar et al. 2020). This method bypasses the time-consuming development of somatic embryos and potentially could be adapted to multiple genotypes. In 2023 and 2024, scientists at **Virginia Tech** and **University of California Davis** are planning pilot studies to test if meristem induction and transformation could work in American chestnut.

Objective 2

Hypovirus infection of *C. parasitica* often reduces canker expansion rates (Anagnostakis & Waggoner 1981). Heiniger and Rigling (1994) postulated that hypovirus infection led to recovery of many stands of *C. sativa*. Widespread recovery has not occurred in the U.S. on *C. dentata* despite 45 years of effort. Several factors have been proposed to explain this failure, including higher diversity of vegetative compatibility groups in the US (Anagnostakis *et al* 1986), the extreme susceptibility of *C. dentata* to blight, strong competition from other tree species in the US (MacDonald & Fulbright 1991; Heiniger & Rigling 1994), and different silvicultural practices (Mittempergher 1978; Griffin *et al* 2005).

Michigan State University (MSU; Jarosz) found that hypovirus infection of *C. parasitica* led to recovery of some *C. dentata* stands (Davelos & Jarosz 2004). However, further modeling suggested that equilibrium has not been attained (Davelos-Baines *et al*, 2014). Further, the frequency of hypovirus infection can change over time, leading to the decline of large trees (Springer *et al* , 2013). Chestnut blight cankers are complex communities of interacting cohabitants (vertebrates, arthropods, microbes) that may modulate canker severity. Interactions can be further complicated by infection of *C. parasitica* by hypoviruses, other viruses and microbes, and by host resistance. We can predict the fate of a canker only at the extremes of these interactions. Recent data suggest that microbial invaders of cankers may play an important role in canker severity. Invader frequency increases over time in cankers that do not kill the distal stem. In addition, the prevalence of virulent *C. parasitica* declines steadily over time while hypovirulent *C. parasitica* remain at a moderate level (~25%) (Double *et al,* 2018). We hypothesize that these invading microbes play an important role in canker severity and longevity.

MSU (Sakalidis) and CAES (Kë[…]rio) have also considered population genomics of *C. parasitica* to understand pathogenicity and host responses. As a tripartite model system, interactions among the pathogen (*C. parasitica*), the host (*Castanea* species) and the virus (*Hypoviridae* family) of chestnut blight disease have been intensively studied in Europe and North America. Despite substantial research of *C. parasitica* populations in Europe, there is very limited recent research of *C. parasitica* populations in North America. Additionally, none of these studies have used genome resequencing. The most recent study in North America focused on four locations using microsatellite analysis (Dutech et al. 2012). The high diversity of VC groups in the US has led to failure of biocontrol through hypovirulence. To overcome restrictions caused by vegetative incompatibility, a super mycovirus donor (SD82+SD382/CHV1-EP713) that can donate the mycovirus to any VC group has been engineered (Zhang and Nuss 2016). However, there is limited data as to how this super donor strain will success in the field, and in the presence of other mycoviruses. In addition, we do not know how the presence of established populations of *C. parastica* on both domesticized chestnut and wild chestnut will affect the reestablishment of American chestnut in US forests.

A critical aspect impacting the success of the American chestnut restoration is whether the achieved resistance in the best backcross hybrids is efficient against multiple strains of the pathogen present in domesticized and wild chestnuts. Currently, majority of the inoculation tests used to screen chestnut progenies for resistance utilize only the EP155 and SG2-3 strains of *C. parasitica*. The highly virulent EP155 strain was initially isolated from Bethany, Connecticut (Anagnostakis and Day 1979), and was used to sequence the *C. parasitica* genome (Crouch et al. 2020). However, using these two isolates may not sufficiently capture the host resistance to naturalized and potentially recombining strains of the pathogen. Genetic analysis of 230 isolates of *C. parasitica* strains mainly from Europe indicate that a highly invasive European lineage of the pathogen arose through recombination of European isolates (Stauber et al. 2021). In this context, the combination of inoculation experiments and population genomic analyses can prove as a successful strategy to identify virulence genes (Tabima et al. 2019). It is also of importance to test the resistance of the trees carrying the wheat oxalate oxidase (*OxO*) transgene (Newhouse et al. 2014) to a wider selection of pathogen isolates. Knowledge of the genetic diversity of *C. parasitica* in North America will facilitate the selection of American chestnut with resistance to a broad selection of *C. parasitica* genotypes. Additionally, this creates the opportunity to identify candidates to be used as superdonors for more effective hypovirulence transmission.

Rutgers University. Like other regions of the Northeast, New Jersey has stands of *C. dentata* trees that are stable with large mature trees recovering from blight. Hypovirulent strains of *C. parasitica* have been isolated from these blighted trees over the course of this project. A wide array of viruses have been isolated from these strains and have been shown to have a measurable effect on fungal growth and virulence. (Hillman and Suzuki, 2004; Eusebio-Cope et al., 2015; Hillman et al., 2018 for reviews). The Hillman lab at Rutgers continues to characterize new viruses from *C. parasitica* isolates from northeast forests, particularly local New Jersey forests, to examine their roles in control of blight in natural settings, and to examine the interplay among viruses in mixed infections of *C. parasitica* and virus/transposon interactions in the fungus. Recently, a hypovirulence-associated reovirus characterized in *C. parasitica* was shown to require the presence of a virus from a different family, the *Hypoviridae* family, for stable infection (Aulia et al., 2019; Aulia et al., 2021). Interplay between two closely related members of the *Hypoviridae,* CHV1 and CHV2, in coinfected fungal isolates has shown the opposite effect, with one of the viruses, with CHV2 being lost in coinfection (Hillman et al., unpublished). The role of such coinfections in other in natural *C. parasitica* infections is unknown.

West Virginia University. With few exceptions (Yu *et al*, 2013), mycoviruses have evolved exclusive intracellular lifestyles (Buck, 1986), limiting their transmission to intracellular mechanisms via conidia or anastomosis. Vegetative incompatibility (*Vic*) systems restrict mycovirus transmission (Boland, 2004; Caten, 1972; Hall *et al*, 2011; Biella *et al*, 2002) due to apoptosis triggered when *vic* incompatible individuals anastomose (Saupe, 2000; Jacobson *et al*, 1989; Glass *et al* , 2000). Through a combination of systematic gene disruption and classical genetics, Zhang and Nuss (2016) developed the SD328/82 super donor (SD) strain, containing gene disruptions at *vic1*, *vic3*, *vic6* and *vic7,* and both alleles of *vic2*. Under laboratory conditions, SD328/82 was able to transmit hypoviruses to uninfected strains heteroallelic at any *vic* locus. Preliminary testing for their efficacy in controlling blight on *C. dentata* in forest environments has demonstrated the feasibility of the approach with increased natural dissemination of hypovirus (Stauder et al., 2019), although improvement in limiting disease spread may be enhanced with use of a less debilitating hypovirus isolate.

In 2022, the **University of Maryland** (**UMD**) demonstrated that CRISPR/Cas9-mediated genetic transformation of *C. parasitica* is more efficient than the prevailing homologous gene replacement (HGR) method. UMD developed a strain of *C. parasitica* strain, DC9, with the *Streptococcus pyogenes* Cas-9 gene inserted into it and driven by a fungal promoter, and then compared the results of transformations using HGR and HGR plus guide RNA targeting the gene of interest. Guide RNA is necessary to direct the Cas-9 endonuclease to make double stranded breaks in the DNA at the intended locus. The experiment was carried out twice targeting the CpSec66 gene, the deletion of which produces a visible change in phenotype as well as reduced virulence against chestnut. In both cases HGR plus guide RNA resulted in an equal or greater number of transformed colonies. More importantly, about half of the transformed colonies produced with HGR plus guide RNA contained only the mutant locus. With HGR alone, transformed colonies always contain both the wild-type and transformed versions of the target locus, requiring an additional step to separate transformed and untransformed spores or hyphae. Attempts to transform DC9 spheroplasts with guide RNA alone did not produce visibly recognizable mutants, but whether there were invisible mutations remains to be determined by amplicon sequencing of pooled DNA from treated colonies.

Mississippi State University (MissSU) has prepared a new, experimentally validated, annotation of the *C. parasitica* genome sequence (Ren and Dawe, in preparation). This significantly improves both gene identification and putative functional assignment. ARV-1 is a predicted gene in *C. parasitica* that shares similarity with genes that code for proteins with important roles in sterol homeostasis in other organisms. The knockout of ARV-1, serendipitously made when investigating LysM proteins during the NE-1333 project period, is avirulent and has a heavily impaired vegetative growth phenotype. **MissSU** has developed and verified a quantitative assay for ergosterol production in *C. parasitica* by modifying published protocols and using a GC/MS system in collaboration with the lab of Todd Mlsna in the Department of Chemistry at Mississippi State. Using derivatization techniques to tag the appropriate class of compounds show that ergosterol accumulation is much reduced in the ARV-1 mutant, as expected. When tested, however, the hypovirus infected strain EP713 reproducibly shows a reduction of ergosterol accumulation similar to that of the mutant, suggestion that a component of the membrane alterations induced by the hypovirus may be due to altered ergosterol presence.

A putative orthologue of *Neurospora crassa vib-1* was found in *C. parasitica. vib-1 is* part of the transcription cascade leading to apoptosis (Dementhon et al., 2006). We knocked out *Cpvib-1* to assess its role. The knockout did not change vegetative incompatibility in strain Ep155 but did in strain EU1. EU1 differs from Ep155 at *vic4*, yet the strains with a *Cpvib-1* knockout were vegetatively compatible. Thus, we concluded that *Cpvib-1* plays a crucial role in the incompatibility reaction modulated by *vic4*. By tagging the VIB-1 protein with an epitope it was possible to demonstrate DNA binding and complete a ChIP-Seq analysis to identify a recognition sequence. Intriguingly, when tested from cultures stimulated by using rapamycin to mimic the vegetative incompatibility response, the recognition sequence was altered indicating that VIB-1 protein is a transcription factor that is responsive to cues from the vegetative incompatibility signaling pathways.

TACF uses two strains of *C. parasitica*, Ep155 and SG2-3, to screen for blight resistance. They are near the top and bottom, respectively, of pathogenicity for virulent strains. The two strains were crossed, and progeny tested, revealing significant variation in pathogenicity. DNA was extracted from 92 progeny and sequenced. Subsequent genetic mapping has shown that there are deficiencies in the some of the data leading to parts of the map that do not resolve properly. There are indications of potential QTL locations on chromosomes 1 and 2 but the size of the regions indicated means that identifying individual genes is not feasible at this stage. **MissSU** will address this with long-read (MinION) sequencing to improve the genetic map and increase resolution. Once identified those genes of interest can be subjected to knockout to assess the role of their protein products. Knockout approaches will be able to leverage advances in this technology by **UMD** (above).

Objective 3

USDA Forest Service, Delaware, OH, in collaboration with the **University of Tennessee** and other partners, is evaluating silvicultural methods for reintroducing improved *C. dentata* to the northern parts of its range. Several studies established in Pennsylvania (2015-2017), track backcross chestnuts planted across a gradient of soil moisture availability, (study 1), with and without protection from deer herbivory (study 2), and across several silvicultural treatments that create varying levels of light availability and competition from woody species (study 3). Early results demonstrate the plasticity of American chestnut; planted chestnuts can survive across site quality and light availability gradients (Pinchot et al., 2017. Pinchot et al., 2020a). Sites with higher resource availability; soil moisture or light; encourage rapid growth of competing woody species and may require competition control to ensure the chestnuts attain dominant or co-dominant canopy positions (Pinchot et al., 2020a, Pinchot et al. 2020b). Fencing or other means of protection from herbivory will be necessary to reintroduce chestnut to forests with moderate to high deer densities (Pinchot et al., 2022). The Forest Service and partners will continue to monitor these plantings to study the competitive dynamics of the planted chestnuts and naturally occurring woody vegetation.

The **USDA Forest Service**, Southern Research Station is also cooperating with The**University of Tennessee** and other partners like **TACF**, **Mississippi State University**, and **Clemson University** to evaluate silvicultural test plantings in NC, TN, and VA. Ten plantings are still being monitored that were established between 2009 and 2015. Chestnut blight disease has led to large-scale mortality across plantings more than 10 years of age, resulting in a 34% survival rate for the most advanced hybrid seedlings (BC3F3). Disease resistance rankings were consistent with orchard inoculation tests from TACF. Nursery studies were also established that determined nut size/weight and genetics affected seedling quality. Soil fungi was not a reliable predictor of seedling planting performance in chestnut and in co-planted white oak, even though chestnut species were associated with different fungi before being planting as bareroot seedlings. These multi-disciplinary projects have yielded a number of publications reporting on novel research findings (Brown et al., 2022; Case et al., 2016; Clark et al., 2010, 2012, 2014, 2016, 2019a, 2019b, 2020; Coughlin et al., 2020; Knapp et al., 2014; Reazin et al., 2019

In collaboration with **TACF**, **Virginia Tech** is experimenting with the use of unmanned aerial and ground-based remote sensing to quantitatively characterize tree growth and architecture, stress, and apparent blight resistance. These data collected with Lidar (Light Detection and Ranging) and multispectral sensors are to be analyzed in relation to ancestry genotype and phenotype data via machine learning techniques to evaluate potential for high-throughput tree phenotyping. **Virginia Department of Forestry** will be joining the collaboration by contributing phenotype/genotype data and access for remote sensing to backcross and single-ancestry orchards at Lesesne State Forest.

TACF and **Virginia Tech** will also be exploring several questions related to plant-soil interactions in chestnut orchards and restoration plots. Chief among these will be soil mesocosm trials at Meadowview Research Farms to evaluate the impacts of varying soil textures on chestnut rhizosphere, biomass, and blight resistance while controlling for climate and other spatially variable effects.

TACF is embarking on the establishment of permanent common garden infrastructure spanning the historic range of the American chestnut, as well as point north potentially encompassing areas due to become suitable with continuing climate change. Apart from evaluating all current and future breeding projects across climates, this will allow researchers to evaluate apparent local adaptation and phenotypic plasticity of populations from across the range through reciprocal transplant. This, in turn, will drive decision=making on provenance selection for geographically diverse reintroduction and restoration plantings.

With support and input from **SUNY ESF**, **TACF** is beginning a side-by-side evaluation of backcross bred, transgenic American, and backcross X transgenic 'Stacked Resistance' trees in nursery, orchard, and forest settings. This will provide highly valuable and actionable data that will – by the end of this decade – help researchers and conservationists make decisions about relative returns on investment in pursuing traditional backcross breeding, OxO-based transgenic, both, or a hybrid of these approaches toward restoration into forest systems. Trees will be challenged with *C. parasitica* at the seedling stage (small stem assays) and sapling stage (orchard and forest settings), while monitoring blight resistance, overall growth rate and form, as well as response to competition. Ultimately, fecundity will be estimated as trees enter reproductive age.

Penn State University has a suite of silvicultural trials, installed using wild-type American chestnuts, which will be used to ascertain and improve best management practices for reintroduction of disease-resistant American chestnuts. In addition, and in collaboration with **William and Mary** and **Wilkes University**, ongoing research will continue investigating the metrics which will determine American chestnut forest regeneration. Several stands of naturally regenerating American chestnuts found in Maine, Vermont, Indiana, and Wisconsin have been studies for 15+ years. Some data have been published regarding the founder effects (Rogstad and Pelikan 2014) and dispersal mechanisms at these sites (Elwood et al 2018). Genomic data collected on these sites within the past year will be evaluated to determine whether calculations established decades ago are currently applicable to reforestation initiatives. These data will then be applied as recommendations for reintroduction plots of disease-resistant American chestnuts.

TACF, **Penn State University**, and **Villanova University** are collaborating on the creation of broad and fine-scale habitat suitability models which can be made publicly available and in a usable format for landowner use. Multiple models have been developed on various local and regional bases (Noah et al 2021). The organizations above aim to collate and merge them into a single on-line tool which can be update over time to reflect changing climate and analogous seed zone recommendations.

Objectives

1. Develop and evaluate disease-resistant chestnuts for food and fiber through traditional and molecular approaches that incorporate knowledge of the chestnut genome.
2. Evaluate biological approaches for controlling chestnut blight from the ecological to the molecular level by utilizing knowledge of the fungal and hypovirus genomes to investigate the mechanisms that regulate virulence and hypovirulence in C. parasitica.
3. Investigate chestnut conservation and reestablishment in orchard and forest settings with special consideration of the current and historical knowledge of the species and its interaction with other pests and pathogens.

Methods

Objective 1

Genotyping by sequencing was performed on over 5,500 trees in TACF's breeding program. A description of the genotyping and bioinfomatic pipeline can be found in Westbrook et al. (2020).

Blight resistance phenotype data were taken on ~5000 backcross and large surviving American chestnut trees from 88 orchard locations in 20 states. All phenotyped trees had been inoculated at least two years beforehand. Data included whether the inoculated stem was dead or alive, whether the canker exceeded 15 cm vertically, presence/absence of sporulation and exposed wood in the canker, presence/absence of basal sprouting, percentage of the original canopy that was alive, and diameter-at-breast height (1.4 m above ground) of the largest trunk. Phenotype data were scaled such that high values were representative of blight resistance.

A genetic-based blight resistance index was created from the sum of estimated additive genetic values for this suite of traits. Additive genetic values for blight resistance traits were estimated with the following generalized linear mixed model in ASReml-R v. 4.1 (Butler et al., 2018). Genetic liabilities for presence/absence traits were estimated with a binomial model and percent canopy survival was modeled as a continuously distributed gaussian trait. The effect of tree age was treated as a fixed covariate. Genotype effects were assumed to be normally distributed and were estimated with a blend of pedigree and genomic relationships (VanRaden 2008; Aguilar et al., 2010) estimated with the R package 'AGHmatrix' (Amadeu et al. 2016). To construct the genetic-based blight resistance index, genetic values were scaled 0 to 1 and these scaled values were multiplied by trait heritability ($h^2 = s^2_{genotype} / s^2_{genotype} + s^2_{error}$). Blight resistance index values were then scaled from 0 = mean for susceptible AM chestnut and 100 = mean for resistant CH chestnut controls.

Reference genome assembly methods for one C. dentata individual and two C. mollissima individuals are described on the Phytozome website (https://phytozome-next.jgi.doe.gov/).

A time-course experiment was performed to quantify differences in gene expression and metabolic responses of Chinese chestnut, American chestnut, and F1 hybrids of these species in response to chestnut blight infection at an early stage of infection (3 days) and a later stage (10 days). Comparison of allele-specific expression in F1s to differentially expressed genes in Chinese and American will provide insights into whether gene expression differences between resistant and susceptible species are primarily regulated in *cis* or *trans*. The experiment included seven genotypes: (2 blight resistant Chinese trees, 2 F1s, 1 Large surviving American, and 2 susceptible American chestnuts), 3 time points: (just before inoculation, 3 days and 10 days post inoculation), three treatments (unwounded/uninoculated, wounded only, wounded and inoculated), and three biological replicates (grafted clones) per genotype, treatment and timepoint.

Small stem assays will be used to compare the blight resistance of progeny of D58 x wild type American chestnut, best x best backcross trees, and intercrosses between large surviving American chestnuts. The cut stem tips of 500+ progeny from each of these cross types and their combinations (e.g., D58 x backcross, D58 x LSA, LSA x backcross) will be inoculated with a virulent strain of *C. parasitica* following methods developed by Cipollini *et al.* 2021. Canker length, rating, and presence/absence of sporulation will be assessed 60 d and 90 d post inoculation. Mean canker severity for each cross type will be compared with ANOVA followed by multiple test correction using Tukey tests.

A high throughput genotyping platform will be developed to assess 1) species ancestry, 2) provenance from the wild *C. dentata* population, and 3) genome inheritance from the D58 founder tree. Whole genome sequencing on 20+ individuals of each *Castanea* species has been performed to discover SNP markers that are informative for species ancestry (Sandercock et al., 2022). A subset of markers spaced at approximately one centimorgan intervals with fixed differences between *C. dentata* and *C. mollissima*, *C. dentata* and *C. sativa*, and *C. dentata* and *C. pumila* varieties will be selected. Markers associated with climatic variation in *C. dentata* and with Fst values > 0.25 between subpopulations will be selected to determine provenance.

Markers that are homozygous in the D58 founder but variable in the wild-type American chestnut population (minor allele frequency > 0.25) will be selected to detect and select against the D58 founder's genome in progeny.

The **Connecticut Agricultural Experiment Station (CAES)** will conduct work that aims to identify mechanisms underlying the high resistance of Chinese chestnuts to *C. parasitica*. A unique resource available at the CAES Lockwood experimental farm is a 20-year-old full-sib progeny of two *C. mollissima* trees, 'Mahogany' and 'Nanking', which both have high blight resistance (Steiner et al. 2017). The progeny has approximately 150 trees which have observable differences in the occurrence of natural blight infections. **Susanna Keriö at CAES** will utilize this resource to identify chemical, molecular, and genetic components associated with the high blight resistance in Chinese chestnuts and the hybrids. Potential avenues to explore this include pathogen plate assays to identify antifungal molecules, studying the expression of candidate resistance genes (Westbrook et al. 2020; Barakat et al. 2009) in naturally infected trees, and cell death assays (Kim et al. 2008; Vijayaraghavareddy et al. 2017 Majtnerová and Roušar 2018).

Objective 2

The CAES will contribute to the population genomic analysis of *C. parasitica* by collecting pathogen isolates from different chestnut species and hybrids available on **CAES** farms and established field plots. First stage of the population genomic analysis is to collect the pathogen isolates. The **CAES** has a diverse collection of pure chestnut species and chestnut hybrids with varying degrees of blight infection. The **CAES** has also established several field plots with hybrid trees which have natural blight infection. Additionally, the CT chapter of **TACF** maintains several germplasm conservation orchards where some of the trees have natural blight infection. This offers the opportunity to study the diversity and virulence of the pathogen isolates colonizing hosts with varying genetic backgrounds and varying resistance. The second stage is to characterize the vegetative incompatibility of these isolates by agar plate tests and PCR assays (Short et al., 2015). In the third stage, the **CAES** will pursue funding to conduct resequencing of these isolates in collaboration with the participants of the NE1833 Chestnut Multistate Project consortium. Finally, in collaboration with **TACF** and the participants of the NE1833 Chestnut Multistate Project consortium, **CAES** will facilitate controlled inoculation experiments (small stem assays) on selected American chestnut genotypes, other pure species or hybrids, or on American chestnut seedlings carrying the *OxO* gene.

Michigan State University will focus on the population genomic analysis of *C. parasitica* aiming to 1. characterize the NA populations of *C. parasitica* using a genome resequencing approach and VC typing; 2. Fine level characterization of *C. parasitica* in MI orchards and remnant American chestnut stands including population genomics and VC typing; 3. epidemiological study focused on spore release timing; 4. comparison of the efficacy of the natural CHV3-GH2 hypovirus with the super hypovirus donor (SD82+SD382/CHV1-EP713) to control the spread of *C. parasitica.* The CHV3-GH2 hypovirus is naturally found in Michigan and data is already available on the diversity of VC groups in Michigan *C. parasitica* populations (Springer et al., 2013). The Sakalidis lab maintains a collection of Michigan *C. parasitica* isolates and the CHV3-GH2 hypovirus. With the presence of domesticated chestnut varieties in an active and expanding chestnut fruit production system, naturalized American chestnut, populations of *C. parasitica* and a native hypovirus used in chestnut blight control Michigan is uniquely suited to studies focused in this tripartite model system of the host-pathogen-virus interaction in the environment. Michigan is unique in that it contains populations of *C. parasitica* in forest settings and a large commercial orchard industry (the largest in the US). This setting provides a unique environment to characterize gene flow between *C. parasitica* populations in domesticized chestnut and wild chestnut.

Rutgers will continue to identify and characterize new viruses found in isolates of *C. parasitica* from New Jersey forests. Our focus is especially on complex virus/fungus interactions that may result from multiple virus infections in a single fungal isolate or complex, multi-segmented viruses. Virus-transposon interactions may later be examined. For that project, single ascospore progeny have been selected from genetic crosses of a strain bearing complete, active copies of a hAT-like element (Linder-Basso et al., 2001) and a strain bearing a helitron copy that is predicted to be complete and possibly autonomous (Du et al., unpublished).

Biological characterization of several viruses identified from *C. parasitica* strains from northern and eastern New Jersey are being performed and will continue. Molecular characterization of viruses from isolates showing hypovirulent phenotype is proceeding following double-stranded RNA analysis, small RNA isolation and sequencing. Small RNA libraries from hypovirulent, virus-infected isolates and from their isogenic, virus-free counterparts are being sequenced commercially. Data returned to us are run through a pipeline that subtracts *C. parasitica* genomic sequence and identifies putative viral sequences. Through this methodology, we have recently identified 10 RNA viruses from the turfgrass dollar spot pathogen *Clarireedia* (Cohen et al., in prep).

A fruitful collaboration with Dr. Nobuhiro Suzuki continues. Hypovirulent *C. parasitica* isolate GH2 from Michigan contains a complex virus, CHV3-GH2, which comprises a genomic RNA segment and two accessory RNAs: a defective RNA segment, and two satellite RNA segments (Smart et al., 1999; Hillman et al., 2000; Yuan and Hillman, 2001). The roles of satellite and defective RNAs are well-studied in plant and animal virus systems, where they may have profound effects on virus pathogenesis, but are poorly understood in fungal viruses. We are now investigating the roles of the accessory segments in CHV3 biology. During a study examining the behavior of several *C. parasitica* isolates in chestnut trees, Suzuki et al. (2021) noted that the CHV3-GH2 isolate used in the study was more virulent than had been previously reported. Subsequent work showed that the isolate contained only CHV3 genomic RNA and had lost the accessory satellite and defective RNAs. Through thorough examination of single conidial isolates and transmission experiments, we are initiating studies to define the specific roles of the defective and satellite RNAs in CHV3-GH2. A full-length cDNA clone of CHV3-GH2 RNA is already available. We will later generate and use infectious cDNA clones of CHV3-GH2 genomic RNA and the accessory RNAs to explore the roles of accessory RNAs in detail.

WVU has completed preliminary testing and analysis of methods of application and the behavior, stability and duration of a hypovirus superdonor strain in the forest (Stauder *et al*., 2019). While the work was a success and demonstrated the increased penetration of the hypovirus into the local *C. parasitica* population, there may be efficiency and effectiveness gains to be realized by using a different hypovirus. CHV1-EP713 is regarded as severe, in that the phenotype it causes in the fungal host is significantly debilitating and this may affect the ability of those infected strains to persist and spread. The related hypovirus CHV1-Euro7 has a reduced effect on the fungal host (Chen and Nuss, 1999) and may represent a more effective choice in the compromise between debilitating the host to minimize plant damage yet retaining sufficiently vigorous viability to promote hypoviral spread using the SD328/82 formulation. Additionally, B_3-F_3s being developed by TACF are predicted to have more resistance than *C. dentata* but less than *C. mollissima.* WVU hopes to test a superdonor strain with B_3-F_3s planted in the forest.

UMD will explore other CRISPR/Cas-9 editing methods that leave no footprint in the fungal genome except the intended mutation, such as through the use of transiently expressed plasmids containing both the Cas-9 and guide RNA sequences, or through the use of commercially prepared ribonucleoproteins. Second **UMD** will work to further characterize the function of the CpSec66 gene by reinserting it with fluorescent tags into one of the *CpSec66* knockout strains. This will demonstrate whether the complementation restores the normal fungal phenotype, as well as to help confirm where the CpSec66 protein accumulates in hyphae.

MissSU project goals fall into two categories:

Fungal Pathogenicity - Improve the available genome sequence data using long-read technology (MinION) and leverage this advance to identify sequences associated with the trait differences between strains SG2-3 and EP155Vegetative

Incompatibility – pathways and targets. Using the CpVIB-1-target recognition DNA sequences we plan to identify specific targets of the vegetative incompatibility signaling pathway triggered through VIB-1.

For all projects, the basic experimental methods to be employed will be similar and focused on removing target sequences from the genome to test hypotheses about the role of gene products in fungal biology. Gene knockouts will be made in the EP155 strain. The deletion constructs will utilize the more efficient **UMD** technology where possible but can be prepared using the method of Colot et al (2006) in which the flanking regions were assembled with the Hyg^r marker using a yeast-based recombination system, if necessary. Transformants will then be single-spored for nuclear homogeneity and verified by Southern blot and PCR. Analysis for phenotypic consequences will include chestnut pathogenicity assays and vegetative incompatibility testing.

Objective 3

USFS Delaware and partners will continue to monitor collaborative chestnut plantings to study the long-term success of the chestnuts in forested settings, in particular competitive dynamics among the planted chestnuts and naturally occurring woody vegetation.

Virginia Tech (Hession lab) has collected at TACF Meadowview and begun analyzing Lidar data with the help of J. Resop (University of Maryland). Preliminary results show few coarse scale correlations between genotype and size and growth dimensions phenotype. Further analysis is underway to derive high resolution point cloud-based metrics of branching patterns and percent canopy die-off with the help of additional computational packages. In addition, this group has begun to compare sensing methods which will allow to pinpoint the best timing to use specific sensors. Early results favor leaf-off season for aerial sensing for branching pattern calculation, however ground-based sensing during leaf-on has shown promising results in assessing proportion canopy die-off due its much higher resolution. In upcoming work at VDOF's Lesesne State Forest, Hession's group will be joined by Abhilash Chandel for the purpose of coupling Lidar and multi-spectral sensors – the latter of which will be used to search for foliar and stem spectral signatures that may differ among tree ancestries as well as tree stress by disease or abiotic factors.

Following small stem assays in 2023, TACF will be establishing a series of permanent common garden sites to evaluate various breeding and transgenic lines with parentage from across the historic range of C. dentata. By setting up each garden along the latitudinal gradient as a replicate reciprocal transplant, TACF will be able to gauge relative performance (i.e., blight response, growth form, competitive ability, fecundity) of various families, but also assess degrees of local adaptation, plasticity, and combinations of the two based on family origin. At the range boundaries (and potentially beyond, in the North), this reciprocal transplant will shed light on potential fitness trade-offs that could indicate limitations range movement.

With other collaborators, Penn State University (PSU) will with installation of common garden and provenance trial installation and measurement. Affiliates of TACF, PSU, and USFS will continue to update best management practices (BMPs) for private forest landowners, ensuring increased success in outplanting and reintroduction of disease-resistant populations. The results of outplantings established between 10-30 years ago offer ongoing data and results which are regularly incorporated into fact sheets, manuals, and other extension-like documentation made widely available to the public.

Regarding improvement of recommendations for appropriate site selection and habitat suitability of American chestnut, TACF and Villanova University will lead the creation of a publicly accessible on-line tool for forest land-owners. By first combining over one dozen models of habitat suitability for American chestnut, researchers will then contract with web-developers to publish to results in an easy-to-use and interactive format.

To further improve recommendations for reintroduction and reforestation populations which will lead to long-term success and ecosystem integration (Pierson et al 2007), researchers at Penn State and William and Mary combine genomic findings with population spread measured at three naturally regenerating sites of American chestnut.

For over 15 years, naturally regenerating American chestnut stands in Indiana, Vermont, and Maine have been thoroughly studied with over 5,000 individual trees tagged, mapped, and measured semi-annually.

In the summer of 2022, leaf-tissue from a small selection of those trees at all three sites was collected for DNA extraction. Of highest importance for collection were the "founder" trees, the first parents of each of trees that eventually carpeted the sites.

These three sites were specifically chosen because of their small Founder populations: anywhere from 3-5 individuals are thought to have derived these sites. DNA was extracted from those collected samples through the fall of 2022 and, in the winter of 2022-2023, the samples are being sent out for GBS, genotype-by-sequencing.

The data which are then retrieved from the GBS will require extensive reconstruction by a molecular genetics statistician. The result of this research has significant implications for American chestnut restoration. Planting hardwood trees is expensive and can be very difficult; therefore, practitioners will be seeking to plant the fewest trees possible while still ensuring long-term viability of any given population being planted in the forest.

Measurement of Progress and Results

Outputs

- The grand output would be restoration of the American Chestnut Tree to millions of hectares of forest in the Appalachian Mountains and environs. This might be accomplished by traditional breeding, genetic engineering, biocontrol or some mixture of the three. This output will not be achieved for 100 years or more. More immediate outputs during the proposal period are below.
- Public release of genetically diverse populations of blight-resistant chestnuts
- Combining of OxO blight resistance with PRR and blight resistance from Chinese chestnut
- Development of blight resistant chinquapin trees for reforestation
- Release of high-quality, chromosome-scale reference genomes for the Chinese chestnut and American chestnut
- Mapping of QTL for blight and PRR resistance in American chestnut backcross populations
- Development of a genetic marker panel to select for maximal inheritance of C. dentata or C. pumila ancestry, infer locally adapted provenance in C. dentata samples, select against the D58 founder genome, reconstruct pedigrees, and perform genomic selection for blight and PRR resistance.
- Assessment of the relative level of blight resistance in large surviving American chestnuts and elucidation of potential mechanisms of blight resistance in American chestnut
- Identification of biota invading chestnut cankers over time
- Development of individual based models (IBMs) of changes in chestnut canker severity over time
- Testing of the biocontrol potential of biota from chestnut cankers
- Further elucidation of the reasons for survival of LSAs
- A list of fungi growing within healthy tissue of American, Chinese, and hybrid chestnut trees
- Continued evaluation of the superdonor strain of C. parasitica for efficacy in controlling blight in in the forest
- Further characterization of transposons in C. parasitica and their effects
- Characterization of the targets of VIB-1 in triggering apoptosis during anastomosis of vegetatively incompatible colonies of C. parasitica
- Fine map QTL for pathogenicity in C. parasitica.
- Determination of tree establishment and growth at outplantings of blight-resistant chestnut in eastern forests
- Identification of factors impacting establishment of chestnut after planting
- Determination of blight incidence and severity at outplantings of blight-resistant chestnut in managed forests
- Continued assessment of the forest competitiveness of backcross hybrid chestnut as blight progresses at forest planting sites
- Corroboration and/or improvement of recommendations for installation of reintroduction plots of disease-resistant populations based on maximizing within-plot allelic diversity and minimizing founder effect.
- Establishment and ongoing maintenance of on-line landowner site-selection tool
- Assessment of relative performance of Chinese-American backcross, American-x-transgenic, and backcross-x-transgenic genotypes over nearly a decade in randomized controlled trials. This will include inoculation assays in the first year, and well as at 3 and 6 years of age. This will demonstrate whether or not short or longer terms gains are apparent from the combination of Chinese and transgenic source resistance.
- Identify technically, logistically, and economically feasible forms of high throughput remote sensing of tree growth form and/or ancestry and/or abiotic stress and/or disease stress. Allows for future precise and objective tree evaluation across sites, as well as elucidation of any potential growth/competition penalties to Chinese genome introgression in American backcross restoration populations.

Outcomes or Projected Impacts

- Greater and more consistent amounts of mast for animal and human consumption; for wildlife, this results in larger populations (Diamond et al., 2000, Lutts 2004).
- Substantial increase (up to 100%) in rates of timber growth and faster harvest rotations on currently unproductive mountain land (Jaynes and Graves 1963; Kuhlman 1978; Smith 2000).
- Incorporation of PRR resistance will broaden the niche of blight-resistant trees to include areas where PRR eliminated American chestnut prior to blight.
- A broad representation of genetic diversity from the wild population of C. dentata is crucial for adaptation to a large geographic range and a changing climate.
- Planting of millions of American chestnut trees will lead to modest increases in the carbon sequestration potential of hardwood forests in the Eastern U.S. (Gustafson et al., 2017)
- High quality annotated reference genome of C. dentata and C. mollissima will be crucial to better understand the biology of blight and PRR resistance and climate adaptation.
- Prediction of future blight severity in recovering stands of chestnut will identify stands where intervention is indicated.
- Identification and culture of biota in chestnut may provide materials to help control blight.
- Comparison of individual-based models (IBMs) and stands with differing predictions of future recovery may help identify promising biocontrol agents.
- Correlations of biota present in cankers differing in severity may identify promising biocontrol agents.
- Superdonor strains of C. parasitica hold promise for effecting biocontrol of blight on American chestnut similar to that observed on European chestnut because the barrier of multiple vc groups to spread of hypoviruses will have been minimized.
- Superdonors plus blight-resistant stock may yield better blight control than either alone and may help elucidate the relative effect of resistance and vc group on control.
- Transposons in C. parasitica may be important components of their evolution and may be helpful to biocontrol efforts.
- Fine mapping of QTL for pathogenicity in C. parasitica should identify genes for pathogenicity that can be tested by gene knockout. Knowledge of genes for pathogenicity and resistance in the pathogen and host, respectively may help elucidate their roles and lead to better disease control through resistance and pathogenicity modification.
- Characterization of VIB-1 and its gene targets will lead to improved understanding of the vegetative incompatibility process, in particular the molecular signals that lead to apoptosis and interrupt hypovirus transfer.
- Comparison of data from different forest plantings will uncover commonalities and patterns across studies, e.g., linking performance of planted seedlings under similar silvicultural treatments in different regions. Collectively, these plantings should lead to improved guidance for optimal seedling quality and planting conditions, pre- and post-planting management, and performance of hybrid chestnut generations. Tracking regional sources of American parents will help determine possible seed zones and the impacts of adaptation.
- Side-by-side evaluation of Ch-Am backcross, AmxTG, and backcross x TG genotypes will allow researchers and conservationists to make data-driven decisions in implementing single- or multiple-mechanism blight resistance in future work. This will determine the direction of multiple science programs for decades and the accompanying allocation of resources.
- The adoption of remote sensing and other techniques from precision agriculture for chestnut cultivation and evaluation is a matter of time. The studies proposed above are the first steps in calibrating these technologies to chestnut systems for the benefit of both conservation and agriculture alike.
- Federal regulatory approval of transgenic blight-tolerant American chestnuts will facilitate the future use of this and similar technologies to address pressing conservation needs.

Milestones

(**2023**):Receive federal approval from the USDA, EPA, and FDA for public release of the Darling 58 blight tolerant variety of American chestnut

(**2023**):Prepare publications on high quality chromosomal American and Chinese chestnut genome sequences. Include results on discovery of candidate genes for resistance to chestnut blight and phytophthora root rot through a combination of QTL mapping, RNA seq, and signatures of natural selection.

(**2023**):Develop a high throughput and cost-effective genotyping platform to assess species ancestry, perform genomic prediction for resistance, and minimize founder bottlenecks on genetic diversity in outcrosses between transgenic and wild type American chestnuts.

(**2023**):Directly compare the blight resistance of progeny of D58, best x best backcross trees, and large surviving American chestnuts using small stem assays for blight resistance.

(**2023**):Complete results on how much climate adaptive diversity from the wild population of C. dentata that TACF has represented in the breeding program.

(**2023**):Complete assays in order to determine whether endophytes increase resistance to C. parasitica in young American chestnut trees

(**2023**):Evaluate experiments on biota likely to affect chestnut canker severity, including on LSAs

(**2023**):Finish evaluating success or failure of superdonor strain first deployed into the forest in 2016

(**2023**):Complete long-read sequencing of C. parasitica strains EP155 and SG2-3 and begin pathogenicity determinant identification

(**2023**):Release TACF backcross hybrids to the public and publish

(**2023**):Analyze Chinese-source vs. TG-source vs. Chinese/TG-source blight response via small stem assays. Analyze Chinese-source vs. TG-source vs. Chinese/TG-source vs. LSA vs. American blight response in larger small stem assays.

(**2023**):Use machine learning and other advance computation to derive 3-dimensional and multi-spectral reflectance signatures of tree ancestry Am/Ch/hybrid and/or tree abiotic stress and/or tree blight stress.

(**2023**):Create on-line landowner site selection tool with ongoing improvements from baseline habitat suitability models

(**2024**):Collect C. parasitica isolates from a diverse selection of chestnut species and hybrids from orchards, wild stands, and restoration sites.

(**2024**):Analyze study designed to evaluate effect of site quality on chestnut competitive ability est. 2015 and publish

(**2024**):Analyze inbreeding quotient and founder effect at a minimum of three sites with naturally regenerating American chestnuts. Use those data to create reintroduction population recommendations.

(**2025**):Assess Chinese-source vs. TG-source vs. Chinese/TG-source blight response via inoculation at 3 years of age.

(**2025**):Characterize C. parasitica vegetative incompatibility in collected diverse isolates.

(**2025**):Between 2020-2025, breed D58 progeny with a range wide sample of 200 + wild type and backcrossed American chestnuts to diversify the blight tolerant population. Establish seed orchards composed of 5000+ trees that collectively have the capacity to produce 1 M open pollinated seed by age 10 years.

(**2025**):Between 2020-2025, breed D58 with PRR resistant backcross trees and establish ‘dual resistance’ orchards at sites where P. cinnamomi is present in the soil.

(**2025**):Between 2020-2025, conserve 200 + additional American chestnut genotypes through collection of seed and grafting of scion from wild trees

(**2025**):Between 2020-2025, induce grafted American chestnuts to flower by growing seedlings in long day conditions. Cryostore the pollen for future breeding to diversify blight resistant transgenic varieties of American chestnut.

(**2026**):Prepare collected diverse C. parasitica isolates for resequencing.

(**2027**):Assess Chinese-source vs. TG-source vs. Chinese/TG-source blight response via inoculation at 6 years of age.

(**2027**):Conduct experiments to compare the efficacy of the natural CHV3-GH2 hypovirus with the super hypovirus donor (SD82+SD382/CHV1-EP713) to control the spread of C. parasitica against on a diverse set of C. parasitica isolates.

(**2027**):Conduct virulence screening of several C. parasitica isolates against highly resistant chestnut germplasm.

(**2028**):Between 2024-2028 and starting from models trained in 2022-23, apply remote sensing and machine learning techniques to larger scale projects including semi-automated seedling screening, remote ‘uncontacted’ LSA detection, and wholesale orchard assessment.

(**2028**):Report findings of the mechanisms underlying the high blight resistance of Chinese chestnuts.

(**2028**):Report findings of the efficacy of hypovirus efficacy against a diverse set of C. parasitica isolates.

(**2030**):Compare Chinese-source vs. TG-source vs. Chinese/TG-source blight response in orchards and blight + competition response + fecundity in forest setting to help determine direction of future work. In the process, assess longer-term predictive capacity of seedling small stem assays

(**2030**):Between 2024-2030, generate 3 to 5 additional transgenic founder trees that contain the oxalate oxidase gene. Confirm expression, copy number and insert location. Induce pollen production through high light treatment and breed with wild type trees. Conduct blight resistance screening on the progeny using small stem assays. Apply for federal regulatory approval for founder lines with sufficient resistance.

(**2030**):Between 2024-2030, refine existing genetic transformation systems for American chestnut other than zygotic embryos, adaptable to diverse genotypes.

Outreach Plan

Since 2014, TACF annually publishes a summary of each NE meeting to showcase high-level advancements from the group into a public forum. In addition, detailed minutes, reports, and recordings from previous meeting are made available via a TACF/Penn State led site (PSU ESM Undated). The easy availability of these proceedings allow students, early-career scientists, as well as citizen-scientists access to a breadth of background information and knowledge otherwise unavailable (Double 2014).

Collaborations between NE-1833 project participants, NGOs, non-profits, and citizen scientists has led not only to research publications and applicable standards for on-the-ground forest tree conservation and restoration, but also strong good-will regarding this project as well a advancement via inclusion of a variety of outside perspectives.

While there are many more to be cited, we cover three major outcomes that have been enjoyed over the course of the NE-1833 project due to the strong collaborations and transparency of scientists participating in this project.

First, because of the major involvement of outside groups and major geographic expansion of American chestnut research ouplantings, the specialist chestnut bee, *Andrena rehni*, once thought extinct, was found on American chestnuts in Maryland (USGS 2019), Connecticut, and Massachusetts.

Second, the TACF and Virginia Tech-led genomics projects described here were made possible by the efforts of dozens of citizen-scientists participating in finding and documenting wild trees, preserving them *ex situ*, and then submitting samples for analysis. As described in the previous project, the TreeSnap program developed USFS, University of Tennessee, and University of Kentucky collaborators allowed tremendous expansion of that program. A small snapshot of citizen-scientist involvement was captured in a publication by Fitzsimmons and Alcorn (2022).

Finally, and regarding the process for requesting non-regulated status of the transgenic Darling 58, TACF and ESF embarked on a major campaign to encourage supporters to comment positively during the August 2020 and November 2022 public comment periods (PCP) opened by USDA. The stories, passion, optimism and hope for the future were consistently evident in the positive comments to USDA. The chestnut community is committed to the mission and demonstrated their support by the sheer number of comments and the quality of many unique responses.

Between the August 2020 Public Comment Period (PCP) and the recently closed PCP regarding the draft EIS and PPRA, over 100 supporting agencies and organizations have submitted supportive comments. Especially noteworthy is the quality of the relationship with Federal and state agencies, particularly with the US Forest Service. In addition, organizations which previously had or still have formalized policies antagonistic toward genetically-modified organisms in general, have since submitted extremely supportive comments toward deregulation of Darling 58.

- The Sierra Club: https://www.regulations.gov/comment/APHIS-2020-0030-4145
- The Nature Conservancy:
    - https://www.regulations.gov/comment/APHIS-2020-0030-3813
    - https://www.regulations.gov/comment/APHIS-2020-0030-11539

A large number of professional scientists, foresters, and naturalists have commented. Of particular note is the outpouring of support and connection to family and place, often incredibly eloquent and heart-warming, that comes from the general public. In the August 2020 Public Comment Period (PCP), the support from unique, individual commenters was strongly supportive (62%), a trend that continued in the most recent period (86% of unique comments are supportive; 56% of comments were supportive when including submitted form letters).

Several common themes have become apparent as ESF and TACF staff and volunteers review comments in the current PCP:

1. Many commenters note a strong connection to both past and future family members, and that they wish to see deregulation of D58 to ensure a legacy or dream can come true for loved ones already lost or yet to come.

    1. In honor of my father who fought in WWII and brought back a love of chestnuts
    2. I want my grandchildren to see what my father wanted but didn't live to see
    3. I am 88 and remember my father talking about the loss of the American chestnut
    4. In honor of Sherret Chase

2. Many hikers, hunters, and other lovers of the natural world note their connection to the forests in which they regularly recreate, seeing the infected stems of dead and dying American chestnuts, and wanting to see them once again a vibrant part of the forest. (https://www.regulations.gov/comment/APHIS-2020-0030-11928)
3. Because humans spread the disease more rapidly than would have otherwise happened, humans need to solve the problem with all technology available. (https://www.regulations.gov/comment/APHIS-2020-0030-11459)
4. Many embrace the cause as a rare positive environmental story for the future, noting the technology should be used to save other species. (https://www.regulations.gov/comment/APHIS-2020-0030-8623)
5. Dozens of commenters note that, while they are typically skeptical of GMOs, the application of the technology to such an important project as American chestnut restoration, and especially one not directly tied to corporate profiteering, sways them to offer rare support:

(https://www.regulations.gov/document/APHIS-2020-0030-14439

https://www.regulations.gov/document/APHIS-2020-0030-13449

https://www.regulations.gov/document/APHIS-2020-0030-15600

6. Finally, and analogous to the daily calls received by staff persons at The American Chestnut Foundation, several hundred commenters note that they have land on which they want to plant D58 trees

(https://www.regulations.gov/comment/APHIS-2020-0030-8594)

These three anecdotes are only a small snippet if outreach results which have occurred because of the wide-reaching effects made possible by the decades-long scientific collaborative established via the NE chestnut group. Finding once-thought extinct species, engaging the public outdoors, and swaying public sentiment to embrace biotechnological tools for forest health have and will continue to pay dividends for conservation and restoration projects beyond this one focused solely on the American chestnut.

For the next iteration of this program, researchers intend to continue similar outreach and engagement with outside collaborators as well as the general public. The inclusion of stakeholders not within the immediate NE-program researchers has and will continue to be essential not only for research success and progress, but also the ongoing support enjoyed for the related programs.

Outplantings.

Penn State has several silvicultural field trials, established with wild-type American chestnuts, examining various methods of installation and maintenance. Established in 1997 and 2005, a majority of these trials are now mostly removed by blight, but have provided a wealth of information regarding best establishment methods for seeds, seedlings, site preparation, long-term maintenance, herbivory control, etc. Results from these trials have been collated with findings from USFS partners to create two BMPs, updated in 2022, and made available to the public for review in anticipation of wide-scale American chestnut reforestation efforts.

TACF volunteers, some who are also Master Naturalists or Master Gardeners, are beginning to collect serious data on numerous plantings. With proper guidance, they could be a great resource and a wonderful opportunity to involve citizens in study of numerous aspects our natural world

In addition to Clark, Nelson and Pinchot, USDA Forest Service scientists contributing and being recruited to help install chestnut plantings in the forest include Labonte and Warwell. University personnel installing plantings include Brian McCarthy (Ohio University), Hill Craddock (UTC), Doug Jacobs (Purdue), Harmony Dalgleish (William and Mary), Michael Saunders (Purdue), Marty Cippolini (Berry College), Sunshine Brosi (Frostburg State University), Brian Roth (University of Maine), Tom Klak (University of New England) and Heather Griscom (James Madison University). TACF personnel include Jared Westbrook, Sara Fitzsimmons, Kendra Collins, Jamie Van Clief and Vasiliy Lakoba. Michael French is doing chestnut installations, primarily on minelands and with GFW (Green Forests Work).

Publicity. Members of the project will take advantage of the public outreach specialists at land-grant and non land-grant institutions, especially extension personnel, of whom we have a participant from PSU. Technical publications also are a form of outreach. Interested members of the public, aka stakeholders, were permitted to and have attended group meetings during past iterations of the project and we expect that to continue. A key component of the productivity of the group is the productive working relationship with the American Chestnut Foundation. With several members pf the group directly affiliated with the organization or with projects supported by it, layperson outreach is effectively handled through TACF and their staff in their publications and meetings.

Organization/Governance

The organization of the regional research project was established in accordance with the format suggested in the "Manual for Cooperative Regional Research". One person at each participating agency is designated, with approval of the agency director, as the voting member of the Technical Committee. Other agency individuals and interested parties are encouraged to participate as non-voting members of the committee. Each year, members elect a Chair-elect, whose duties begin the following year as Chair.

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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**

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2333: Biological Improvement of Chestnut through Technologies that Address Management of the Species and its Pathogens and Pests

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
Multistate project NE_TEMP2333 builds on a solid foundation of interdisciplinary and cross-institutional collaborative efforts for advancing our understanding of chesnut blight biology and developing improved disease management strategies with the restoration of chesnut as the ultimate goal. The research and outreach project combines expertise in pathology, breeding, genetics, genomics, horticulture, ecology, biotechnology, and bioinformatics to address three major research objectives, and disseminate the research findings to various stakeholders. The project is sound, the methodologies are appropriate, and the goals are achievable based on previous and current accomplishments. Therefore, the continuation of multistate NE_TEMP2333 is highly recommended.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2333: Biological Improvement of Chestnut through Technologies that Address Management of the Species and its Pathogens and Pests

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
The scope of importance of this proposed project is impressive. I find the objectives well conceived and the supporting narrative and approach comprehensive and complete. The involvement of the multiple agencies and organizations provide an effective collaboration between sophisticated laboratories to practical approaches to establishing chestnuts in forests. Their appears to be a good feedback mechanism so that tree-fungal responses in the field are reported back to the labs. As stated in the narrative, the tree-fungal-forest relationships are complex, and its critical that the simplified systems that are necessary for lab work are critically evaluated when introduced into the complexities of the forest environment.
I have two comments for consideration. In the introduction of Objective 1 is the following statement: "To date, the most promising approach to enhance blight resistance has been the transgenic ....." I agree that this is a promising approach, but the remaining narrative includes much about the back-crossing approach used by TACF. I think both approaches are promising and are both described and utilized effectively in the remaining narrative. I think both approaches deserve emphasis in the introduction.
My second comment is public acceptance of transgenic organisms in the forest environment. The narrative makes effective use of public comments submitted to the government, but this is not a representative sample of the public that will eventually be confronted with proposed introductions of transgenic organisms into the surrounding forests. Like the forest, society is complex, and understanding that complexity and how it will impact the future goals of the project should not be solely based on public comments at government hearings or belief that providing information will sway public opinion. A Pew poll (https://www.pewresearch.org/short-reads/2020/11/11/many-publics-around-world-doubt-safety-of-genetically-modified-foods/) showed 38% of people thought genetically modified foods were unsafe and 27% thought they were safe. There should be some measure as to how much of the public has concerns about the use of transgenic organisms in nearby forests and if the provided information is having any impact on the acceptance. A human dimensions component that monitors public reaction to proposed use of transgenic organisms could prove helpful to achieving the goals of the project and help avoid negative reactions. The above is only a suggestion for consideration and is no reason to hold-back approval of this excellent proposal.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2333: Biological Improvement of Chestnut through Technologies that Address Management of the Species and its Pathogens and Pests

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
Well, that was a fun read. This is not my scientific area of research but the project seems thorough and well thought out. I wish that I could give the authors feedback to improve the proposal but from my perspective it is flawless. There might be some issue with whether the level of work proposed matches the funding levels associated with this program, but that is a quibble. It is fantastic that NIMSS is supporting this critically important work.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2333: Biological Improvement of Chestnut through Technologies that Address Management of the Species and its Pathogens and Pests

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
Multistate project NE_TEMP2333 aims to continue broad multi-institutional efforts to expand the restoration of chesnut across the native range through advancing disease management strategies. This work builds on existing strengths in continues to expand a strong foundation of transdisciplinary activities combining expertise in pathology, genomics, horticulture, ecology, and biotechnology along with stakeholder engagement, to address three primary research objectives. These objectives are ambitious and will contribute to significant advances in chesnut biology, disease manangement and sustainability as well as impacting the field more generally. The assembled team has an outstanding track record and progress toward the stated goals will clearly be achieved. The approaches are sound, the timeline and benchmarks for making progress are clearly outlined and achievable. The continuation of this project is strongly recommended.
Your Recommendation:
Approve/continue project

NE-TEMP2333: Response to reviewers.

All review comments were extremely supportive for the continuation of the project with unanimous ratings of “Excellent” in every category. We are extremely grateful to the reviewers for the positive feedback and encouragement.

Just two comments were provided in the second review that required a response. Changes are denoted by underlined text in the revised document. In the first, the reviewer has accurately highlighted a simple misstep that inadvertently framed our argument around the notion of transgenic approaches being the most important. The text now reads: “To date, the most promising transgenic approach to enhance blight resistance has been the insertion of the oxalate oxidase (OxO) gene…”. Thus, the ensuing descriptions are framed more accurately and even-handed as originally intended.

In the second comment, the reviewer notes potential pitfalls with public opinion relating to a genetically modified organism and the possibility for its environmental release. The reviewer is correct that considerable public skepticism does exist with regard to GM foods, although the experience of this team with the public comment periods mandated by the deregulation process for the D58 genetically modified chestnut has demonstrated overwhelming support for the aims of the project. We consider developing a tool for measuring the changing opinions with regard to GMO’s writ large would be very worthwhile, but an undertaking worthy of a project on its own and not within the remit of this current proposal. Nonetheless, as the reviewer mentions, there is an intersection of the outcomes of this project and these public perceptions that we should be cognizant of. To address these points, we have included two paragraphs summarizing public comment reactions in the third page of the narrative in the Statement of Issues and Justification section. In addition, we have added the knowledge gained from this approach to tree conservation, with implications for other applications, to our list of Outcomes and Projected Impacts. These modifications emphasize the long term and ongoing public interest in the project and commitment of the team to public education, outreach and transparency.

We hope that these changes will be sufficient for continued support of this project through the Multistate system.

On behalf of the NE-1833 technical committee renewal writing team,

Angus L. Dawe, Ph.D.
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Biological Sciences
Mississippi State University

Stacy L. Clark, USDA Forest Service, Southern Research Station

Hill Craddock, University of Tennessee-Chattanooga

Angus Dawe, Biological Sciences, Mississippi State University

Sara Fitzsimmons, Forest Resources Lab, Pennsylvania State University, and TACF

Jill Hamilton, Ecosystem Science and Management, Pennsylvania State University

Fred Hebard, Virginia Chapter, The American Chestnut Foundation

Bradley Hillman, Plant Biology, Rutgers University

Jason Holliday, Forest Resources and Environmental Conservation, Virginia Tech

Steven N. Jeffers, Plant & Environmental Sciences, Clemson University

Matthew Kasson, Plant and Soil Sciences, West Virginia University

Susanna Kerio, Connecticut Agricultural Experiment Station, New Haven

Thomas Klak, School of Marine and Environmental Programs, University of New England

Scott Merkle, Forestry and Natural Resources, University of Georgia

Dana Nelson, USDA Forest Service, University of Kentucky

Andy Newhouse, SUNY-ESF, New York

Leila Pinchot, USDA Forest Service, Northern Research Station

Bill Powell, SUNY-ESF, New York

Charles Ray, Ecosystem Science and Management, Pennsylvania State University

Monique Sakalidis, Plant, Soil and Microbial Sciences, Michigan State University

Jared Westbrook, The American Chestnut Foundation, Asheville, NC

Tetyana Zhebentyayeva, USDA Forest Service, University of Kentucky

NE_TEMP2334: Genetic Bases for Resistance and Immunity to Avian Diseases

Status: Submitted As Final

Duration	10/01/2023 to 09/30/2028
Admin Advisors:	[Robert Taylor]
NIFA Reps:	

Statement of Issues and Justification

The Ongoing Need for this Work, as indicated by stakeholders

Poultry consumption has increased worldwide at a steady rate since 1960. Estimates for American per capita consumption of poultry are 113 pounds for 2022 with an expected increase to 114.5 pounds in 2023 (National Chicken Council, 2022). In addition, per capita poultry (chickens, turkeys) consumption in the US equals the combined per capita consumption of beef and pork. United Nations (UN) forecasters project the 2050 world population to be 9.7 billion people, increased from the 2022 population of 8.0 billion people [United Nations, 2022]. Producing protein to feed this number of people will require economical systems that have environmental sustainability. Poultry fit both criteria because of high feed efficiency. In order to meet this ever-increasing food demand for the growing human population, poultry breeders, scientists and producers seek to provide consumers with a safe, wholesome product with higher production efficiency, lower production cost combined with high welfare standards.

Disease remains a significant issue for the poultry industry. Morbidity, poor performance, and mortality cause significant economic losses combined with the human health threat attributable to bacterial and viral zoonotic pathogens. The impact of diseases is one major impediment for sustained productivity despite advances in poultry pathogen control. Economic impacts of disease in the poultry sector can be divided into production and prevention. Poultry production impact includes losses due to mortality, decreased meat and egg production, and condemnations at processing, Prevention impact encompasses the increased costs in vaccinations, biosecurity, and eradication programs for exotic diseases. Maintaining sustainable production by understanding and optimizing immune function has reached critical importance due to increased focus on antibiotic free (ABF) or organic production as well as the re-emergence of previously controlled pathogens.

The project goal of improving poultry health will reduce the need for antibiotics and other drugs which aligns with the producers' goal of judicious antibiotic use to produce poultry meat and eggs. Animal production, the environment, and poultry wellbeing are challenged by the use of pharmacological agents (e.g., antibiotics) to treat disease. For organic production, the project aligns with the goal of healthier animals, reducing disease incidence and ameliorating welfare issues.

Meat and egg producers place high priority on protecting poultry flocks against endemic and exotic diseases. Two U.S. outbreaks of highly pathogenic avian influenza (HPAI) in 2015 and the current one in 2021-2022 reminds us of system vulnerabilities. Avian influenza has been detected in commercial and backyard chickens across 39/50 states during the current outbreak. A major outbreak is expected for the 2022-2023 season due to the amount of virus in wild birds. In addition, the risk for exotic disease introduction is also elevated by consumer preferences driving commercial production systems to be more open (free range and cage free). The U.S. Poultry and Egg Association (USPEA) defines multiple research priorities for controlling disease and ensuring food safety in poultry. These priorities seek to reduce antibiotic, pesticide and anti-parasitic drug use through a focus on disease prevention, genetics, welfare, nutrition and hatchery management.

This project addresses the genetic bases of disease resistance and immunity in poultry. The work also seeks mechanistic understanding of innate and adaptive immune processes; issues having fundamental importance. Poultry breeding, vaccine, and allied animal health industries are the most immediate users of these data, reagents, and tools generated from project research. These primary stakeholders are frequent participants through attendance at annual technical committee meetings as well as their many collaborative research efforts with the members. Interaction as described signifies the high value that stakeholders ascribe to this project.

The importance of the work

A sustainable poultry industry which can increase production to feed a growing world population needs disease prevention and control strategies. Natural selection produces genetic variability in populations. This multistate project fills this crucial need by enhancing stakeholders' knowledge of the genetics of resistance and immunity to poultry diseases. Stakeholders can improve their stocks by applying this knowledge of genetic variability and disease resistance to augment desired traits while eliminating unwanted characters. Genetic responses to disease may be controlled by one or a few genes. The major histocompatibility complex (MHC) is the primary example. Responses may be more complex because many genes contribute to the overall response. Low heritability, a consequence of multigene control makes improvement through traditional selection.

Knowledge gaps about polymorphic loci and their interactions persist, despite completion of multiple chicken genome sequences. This project effort will facilitate understanding of immune responses to common poultry pathogens. The knowledge gained will enhance development of targeted responses providing protection.

Both large-scale and small-scale poultry production face multiple limiting factors. Disease impacts in both systems have been exacerbated by efforts to reduce antibiotic use plus managing birds in less-controlled environments. Production has been disrupted by recurrence of diseases that once seemed to be controlled along with emerging pathogens. Without this project's work to study the important issues of environmental and physiologic factors that impact immune system development, optimal immune function and disease resistance, disease will increase, production efficiency will decrease, food safety will be greatly compromised, and export markets will be closed to products.

Unlike model species in biomedical research, few poultry research reagents are available through commercial sources. In addition, genetic resources, such as stocks that enable experiments to answer critical questions, are limited. The multistate objective focuses on resource development (methods, reagents, specialized genetic stocks) that will facilitate immune system assessment. Morbidity and mortality could increase if immune development is not monitored to prevent deterioration of immune responses to vaccines and disease organisms. Poultry breeding companies will have direct application for methods to address genetic and environmental factors that impact the immune system. Production companies will realize lower production costs benefit through improved health and effective responses to vaccination.

The Technical Feasibility of This Project

Technical committee members for NE-1834 cross multiple disciplines including genetics, genomics, immunology, infectious diseases, microbiology, virology, poultry medicine, physiology, kinomics, nutrition, biochemistry, and molecular biology. The committee's expertise enables individual and collaborative research which is essential for the future of poultry production. Methods that have demonstrated success in other species, i.e. humans, have been adapted to poultry work. Development of other sophisticated methods have enabled examination of gene expression and interaction that may affect disease resistance. Defined genetic stocks can be studied with techniques such as next generation sequencing and gene editing to understand responses against disease. Project participants are leaders in their respective specialties. The work is technically feasible, and the necessary infrastructure exists for members' success in completing the work. A final necessary component is adequate financial support for the proposed effort.

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

Project members conduct studies on genetic resistance to disease across multiple disciplines. Operating through the multistate system offers the advantage of shared resources that synergize to address critical scientific questions. No one station or participating laboratory has the range of expertise, facilities, equipment and biological resources to investigate the crucial scientific questions for the project. The effort is enhanced by the unique skills of the project members. Some stations have specialized genetic stocks or reagents (antibodies, cell lines, pathogen stocks), that are shared with the scientific community. Genetic stocks (inbred lines, congenic lines, selected lines) have facilitated many experiments that have answered key questions on disease resistance and the immune response. The value of these resources cannot be overstated. The regular interaction through the multistate committee enhances research opportunities for each participant. Networking has brought about multiple collaborations.

The current NE-1834 group of scientists each addressing complementary aspects of the genetics of resistance and immunity to avian diseases includes 23 independent laboratories representing 13 institutions across 12 U.S. states [**AL**, **AR**, **CA** (2), **DE** (3), **GA** (2), **IL**, **IA**, **MA**, **MD** (2), **NC**, **OH** (2), **WU**, and **WV** (2)]. Two USDA scientists (**ADOL, FFSRU**) and one international researcher in the Netherlands (**NL**) collaborate on the project. Conducting this work as a multistate effort allows for the greatest efficiency of resource use among these scientists.

Participating stations and scientists have authored **165** refereed publications and **203** abstracts, a very productive effort for the project period. Forty-three publications have joint-authorship illustrating the truly essential, cooperative, multidisciplinary nature among the NE-1834 project members. Technical committee members and collaborators from NE-1834 contributed to reference works such as Avian Immunology (Kaspers, et al, 2022) co-edited by a project member plus authorship in 7 of the 22 chapters and one of the two appendices.

In addition, five project members hold endowed faculty positions indicting the prestigious nature of their research. The Poultry Science Association (PSA) and the American Association of Avian Pathologists, scientific organizations tied most closely to the project, have awarded their highest honors to project scientists. Five project members are Fellows of the Poultry Science Association. Other awards presented to project members include the Bayer Snoeyenbos New Investigator Award. Embrex Fundamental Science Award, Evonik Degussa Award for Achievement in Poultry Science, Hy-Line International Poultry Science Research Award, Novus International, Inc. Teaching Award, PSA Early Achievement Award for Research, and two US Poultry Distinguished Poultry Industry career awards.

Synergies exist with other multistate projects. The NE-1834 project members cooperate with relevant multistate projects including NC1170: Advanced Technologies for the Genetic Improvement of Poultry and NC1180: Control of Endemic, Emerging and Re-emerging Poultry Respiratory Diseases in the United States. Multiple NE-1834 technical committee members also participate in either NC1170, NC1180 or both. One participant has been active in the NE-1834 and NC1170 technical committees for more than 34 years. Excellent communication and coordination are the primary benefits of their active participation in both projects. Duplication across projects is avoided thereby enhancing efficient resource use.

Outcomes and Impacts of this Project

Greater fundamental understanding of how the avian immune system functions, improved comprehension of immune responses to specific antigens, higher efficacy of pharmaceutical agents including vaccines; advanced vaccine programs for controlling existing as well as emerging diseases; increased knowledge of genetic selection consequences on poultry health and production; insight into polymorphic loci effects; and breeding strategies that enhance disease resistance and robustness in poultry are among the impacts expected for this project. Production efficiency, animal health and welfare will increase through improved disease resistance and better prevention strategies. These advances will produce immediate as well as long-term benefit. Consumers will favor improved poultry product safety and reduced antibiotic use. Valuable resources for stakeholders and project participants will be generated as scientific knowledge, both basic and translational, combined with new or modified tools and reagents.

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Related, Current and Previous Work

Objective 1. *To identify and characterize genes and their relationship to disease resistance in poultry with emphasis on the major histocompatibility complex as well as other genes encoding alloantigens, communication molecules, and their receptors and other candidate systems.*

CA – Indigenous ecotypes in Africa were challenged with Newcastle disease virus and genome-wide association studies were used to identify genetic markers associated with increased immunity, reduced viral load, and enhanced survival time.

IA – At Iowa State University, the splenic transcriptome of birds infected with avian pathogenic*E. coli* (APEC) revealed innate immune pathways associated with response to APEC. Differentially expressed genes were identified in the splenic transcriptome of birds after infection with Newcastle disease virus (NDV) at IA. Targeted gene expression assays and gene knock-down studies at IA demonstrated the involvement of the eukaryotic translation initiation factor (eIF2) gene family and the oligoadenylate synthase-like (OAS) gene in response to NDV.

IL – Illinois studies host genetics in horizontal transmission of Marek's disease virus (MDV). Preliminary studies in chicken lines at UI showed significant differences in disease incidence and transmission between three chicken lines. Our work has focused on identifying cellular proteins that interact with MDV proteins and the allelic variations in these host factors.

MD – Maryland explored some statistical methods in epigenomic and systems biology in poultry. We inoculated inbred lines of White Leghorn chickens with infectious bursal disease virus (IBDV) and evaluated clinical signs, gross and histopathology, viral replication, and gene expression in the bursa of Fabricius (BF) by RNASeq. We determined that birds with more severe disease had more inflammation that correlated with an increased number of macrophages/monocytes in the BF.

WV – DNA sequences and SNP analyses of samples from chickens with defined alloantigen genotypes to identify candidate genes for chicken alloantigens. Linked alloantigens *A* and *E* on chromosome 26 were *C4BPM* (complement component 4 binding protein membrane) and *FCAMR*, Fc fragment of IgA and IgM receptor, respectively. Alloantigen*D*, found on chromosome 1, was revealed to be *CD99,* part of the Xg blood system. Chromosome 23 contained RHCE (human: Rh blood group CcEe antigens), the candidate gene for alloantigen *I*. Two independent analyses showed a chromosome 4 region associated with alloantigen *L*. A gene has yet to be identified in this area. Allele frequencies for alloantigens*A*, *E*, *B*, and *D* differed significantly between Wageningen University and Virginia Tech high antibody (HA, HAS) lines versus their corresponding low antibody (LA, LAS) lines. The distribution for alloantigen *I* differed between the Virginia Tech HAS vs LAS lines but not the Wageningen lines.

WU – Western University investigated genes involved in differential disease resistance of B2/B2 and B19/B19 MHC haplotypes. RNA sequencing has shown that genes are largely dysregulated in B19/B19 during differentiation and after stimulation, leading to decreased immune function. T-cells did not have as much influence as macrophages. Focusing on specific genes such as Cluster Homolog of Immunoglobulin-like Receptors (CHIR), we completed phylogenetic analysis and re-annotation of CHIR, which was submitted to NCBI. CHIR-B silencing affects haplotypes differentially. Single-cell sequencing of the reproductive tract show differences in cellular populations and CHIRs in B2 and B19 haplotypes.

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

The group has made significant accomplishments in characterization of innate and adaptive immune responses, factors that influence these activities, and mechanisms of dysfunction and pathology. Research approaches included use of genetic and divergently selected lines, whole animal experiments, novel cellular and molecular analyses, including analyses at the transcriptome, proteome, kinome, and metabolome level.

Using genetic lines that spontaneously develop multifactorial, non-communicable diseases, **AR** further defined the cellular and molecular mechanisms involved in the immunopathology associated with development of vitiligo in Smyth chickens, demonstrated autoimmune memory in Smyth line vitiligo, and identified suppressive activities in tolerance maintenance in vitiligo-susceptible, pigmented parental Brown line controls. Additionally, **AR** demonstrated aberrant innate responses in scleroderma-prone UCD200/206 chickens, especially heightened TGF-β, IL-17 and IFN-γ responses following intradermal injection of killed *Mycobacterium butyricum*. *In vivo* studies by **AR** on the inflammatory response to various microbial components revealed different leukocyte recruitment profiles to products of Gram-negative versus Gram-positive bacteria, reduced local and systemic inflammatory responses with heat-stress, and heterophil dominated responses to autogenous *Salmonella* bacterin vaccines in pullets.

NC increased the characterization of the adaptive immune functions and the influence of genetic and environmental factors contributing to these processes by studying High Antibody Selected (HAS) and Low Antibody Selected (LAS) White Leghorn lines that continuously have been divergently selected for 5-day post-injection antibody titer to injection with sheep red blood cells (SRBC) for nearly 50 years. Differential gene expression was analyzed combining traditional statistics and machine learning to obtain signature gene lists for functional analysis, which revealed differences in energy production and cellular processes between lines and with SRBC injection.

DE compared immune responses of ACRB and modern broilers. This work revealed that the modern broilers displayed greater inflammatory response to CpG administration and showed continuous response to Cocci vaccination, while ACRB show increased inflammation and apoptosis to challenge.

Through detailed examination of blood cell films from various poultry species and environmental/health conditions,**MA** demonstrated heterogeneity among plasmacyte (PC) series, cells known for antibody secretion. The capacity to recognize differences among primitive PC and derived types is an important step in understanding the complexities of immune reactions and factors influencing these activities. Atypical plasmacytes include Mott cells recognized by the presence of retained as unsecreted antibodies (Russell bodies) and Dutcher bodies (nuclear vacuoles). Recognition of Türk cells as members of the avian proplasmacyte cell series adds to basic immune function. These developmental cells are typically large with deep basophilic cytoplasm and sometimes a nucleolus. Many retain a capacity for division. They are not widely known in the veterinary immunity or pathology community but are found in chickens, ducks, and turkeys either as components of the circulation or in the bone marrow. **MA** also continued the study of reactive thrombocytes and their relation to stress.

DE studied the immunometabolic response in the broiler chicken to pathogens and immune modulatory agents. Their results showed that postbiotic fermentates are anti-inflammatory and improve outcomes to a necrotic enteritis challenge. **DE** also demonstrated that administration of essential oils and antioxidants resulted in reduction in inflammatory state beneficial for growth during early stress by activating pathways via FOXO, and that butyrate displays pro-inflammation activities in the context of *Salmonella* infection, which may be the mechanism butyrate aids in clearance. Furthermore,*Salmonella* studies by **DE** showed that calmodulin signaling is an important pathway of response, whereby signaling is through TLR, NOD, cytokine and chemokine PRRs and Jak-STAT pathways. Additionally, **DE** observed a greater inflammatory response in chickens with early post-hatch *Salmonella* inoculation.

NC expanded our understanding of how diet, environment, and the microbiome influence the host's immune response and how this impacts the colonization of pathogens, like *Salmonella*. **NC**'s work demonstrated that the microbiome regulates the phenotype of *Salmonella* and the immune system, and the microbiome is controlled through diet and environmental conditions.

CA found perturbation in metabolic pathways related to arginine and proline metabolism associated with*Salmonella* infection, and that the TCA cycle contributed to *Salmonella* persistence in chickens. Furthermore, chickens from a knockout line (KO) lacking B lymphocytes were examined by **CA** for their resistance to a challenge with*Salmonella enterica*. When challenged, the KO chickens lost less body weight than the controls, had similar mortality, but higher indices of intestinal inflammation.

VA/**GA** investigated necrotic enteritis (NE) which continues to present critical challenges to the poultry industry. They revealed key microbiome signatures associated with an attenuated immune response and a modification of tight junction proteins, which would promote better gut integrity. **VA**/**GA** explored of the host response during NE identified new markers of disease progression, including inflammasome and autophagy markers that were differentially expressed in NE birds at 3 weeks of age (time of NE outbreak in commercial birds). **VA**/**GA** also found signature host responses to two NE-inducing isolates of *Clostridium perfringens*, which is aiding in elucidating their pathogenicity.

OH showed age-related effects on the role of pioneer colonization of the GI-tract in neonatal birds, especially with regards to immune tolerance and innate immune function. Generally, Gram-negative bacteria decreased ability of birds to respond to inflammatory events and lactic acid bacteria promoted colonization with segmented filamentous bacteria, thought to support beneficial innate immune function. **OH** demonstrated that early inoculation with Gram-negative bacteria increased gut permeability and susceptibility to necrotic enteritis. Gram-negative inoculation promoted dendritic cell migration to gut tissue, decreased HNF1-alpha, decrease pathways associated with D-glucose, and $F_c\gamma$ receptor dependent phagocytosis. Conversely, lactic acid bacteria promoted gluconeogenesis, B cell receptor signaling, Class I MHC antigen processing and IL-1, while downregulating heterophil degranulation and MHC Class II antigen presentation. **MD** evaluated the structure and movement of the intracellular replication complexes of infectious bursal disease virus (IBDV) and how they form.

DE examined the molecular pathogenesis of Marek's disease virus (MDV), resulted in obtaining and analyzing transcriptomes and proteomes of exosomes purified from the serum of vaccinated and protected chickens and tumor-bearing chickens. **DE** also patterned macrophage cell line HD11 to become mature macrophages, dendritic cells and polarized dendritic cells. **DE** also found that key drivers of the MDV evolution of virulence are at the level of innate immune selection of the oncoprotein Meq and mutations that affect specific Meq-binding partners.

Research conducted by **IL** on identification of cellular proteins important for horizontal transmission of MDV, included cloning of the putative chicken complement receptors (CR) 1 (CR1), CR2, and CR1-like (CR1L) from established chicken cell lines and two chicken lines. Variations of protein coding sequences were identified.

The emergence of antibiotic resistant pathogens from food animal production has restricted the application of antimicrobial compounds in the poultry industry. Removing antimicrobial compound application has increased the incidences of poultry gut pathogen infections, namely coccidiosis, necrotic enteritis, salmonellosis and campylobacteriosis. **GA** has identified that probiotics stimulate the immune responses, competitively exclude pathogenic microbes, stimulate digestive enzymes, and produce antibacterial substances. We have synthesized and characterized Salmonella and Clostridium perfringens nanoparticles for cytotoxicity, morphology, protein loading efficiency and pH stability. The synthesized vaccines successfully decreased the enteric pathogen loads in poultry intestine.

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*

To increase disease resistance and protect poultry from disease, our working hypothesis is that the ideal model system and powerful tools can help facilitate excellent scientific research to enhance poultry health and welfare. The avian immune community has put tremendous efforts into developing and employing new genetic stocks, methods, reagents, and advanced tools to assess basic immune function, characterizing immune evolutionary processes, guiding genetic selection, and providing valued information for continued research on the genetic bases of resistance and immunity to avian health and welfare. Multiple stations (**AR, CA, GA, IL, IA, OH, MD, NC, WU, WVU**) have developed, maintained, characterized, and made available unique genetic resources to the NE-1834 project members and the entirety of the poultry research community.

IA maintained, characterized, and shared eight unique genetic lines of chickens and their biological products (fertile eggs, chicks, DNA, RNA, tissues, blood, etc.) for research on genetics and genomics of immune response and disease resistance. They studied the expression levels over time of host-defense peptide (HDP) genes in fibroblasts and bone-marrow derived cells from Fayoumi (resistant) and Leghorn (susceptible) lines, after exposure to immune stimulants, and demonstrated innate and post-exposure differences in HDP expression between the chicken lines and demonstrated differences between the ISU chicken genetic lines in MHC class I or beta2 microglobulin cell-surface proteins. Their research will determine the genetics associated with response to infections with APEC and NDV.

Histomoniasis (blackhead disease) is a perennial problem in the poultry industry, particularly in turkey poults, where it inflicts substantial losses but is also problematic in broiler breeders. **GA** recently developed a lateral transmission model of the parasite *Histomonas meleagridis* in poults raised in floor pens. This research model closely resembles commercial field conditions and affords a much-needed platform for conducting detailed research on histomoniasis. The newly established lateral transmission model in floor pens is a key system to study this disease, its progression, and potential mitigation strategies. In mechanisms, **GA** conducted a study to identify the effects of *Salmonella enterica* ser. Enteritidis and Heidelberg on host $CD4^+CD25^+$ regulatory T cell suppress immune responses in chickens. Besides, **GA** also recognized if a *Salmonella* chitosan nanoparticle vaccine administration is protective against *S.* Enteritidis in broiler birds. Chitosan nanoparticle-vaccinated birds had 0.9 Log10 CFU/g decreased SE cecal loads compared to the control ($P<0.05$). The vaccine under study did not adversely affect the bird's BWG and FCR or the IL-1β, IL-10, IFN-γ, or iNOS mRNA expression levels. They have found that the CNP vaccine, either as a first dose or as a booster vaccination, is an alternative vaccine against *Salmonella* in poultry.

In genetics and epigenetic marks, **MD** characterized copy number variations (CNV) and epigenome modifications in the host genome in the resistance to Marek's disease. Most importantly, **MD** also developed a novel reverse genetics rescue system and a novel neutralization assay using only chicken B cells to rescue molecular clones of IBDV and quantify antibody responses against them. They then used these methods to engineer a panel of viruses containing hypervariable regions (HVRs) belonging to diverse genogroups and screened serum from vaccinated birds to evaluate the breadth of antibody responses. Finally, the group at **IL** examined complement and complement receptors in chickens. They have developed reagents monoclonal antibodies (mAbs) against chicken CR1L and have cloned chicken CR1, CR2, C3, and C4 to produce mAbs against these proteins, which will provide tools to address resistance to MDV infection.

DE has cloned for expression chicken innate immune genes, as well as other cellular gene products that interact with MDV proteins. The work focused on forms of the Meq oncoprotein of MDV (pathotypic point mutations and splice variants). The collection of cloned chicken genes in expression vectors has been made available to all participants in this project and is accessible in a shared Google folder.

Objectives

1. To determine how genetics, epigenetics and gene regulation influences innate and acquired immune functions in poultry.
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

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Methods

NE1834 Objective 1. *To determine how genetics, epigenetics and gene regulation influences innate and acquired immune functions in poultry.*

CA – RNA-seq, whole-genome sequencing, ChIP-seq and ATAC-seq will be utilized by University of California-Davis to identify genetic variants affecting immunity and disease resistance to poultry pathogens in diverse genetic lines in chickens.

MD – Maryland will characterize epigenetic modifications and copy number variations (CNV) within the host genome in lines that are resistant or susceptible to Marek's disease. Tools developed, such as monoclonal antibodies and a novel reverse genetics rescue system will be available for community use.

IL – The University of Illinois will continue exploring host genetics in horizontal transmission of Marek's disease virus (MDV). We will identify cellular proteins important for MDV transmission and examine allelic variations within these proteins by cloning and sequencing the putative genes.

IA – Iowa State University will complete analyses and dissemination of studies on genetics associated with response to avian pathogenic E. coli and avian influenza virus. IA will collaborate with CA on studies of genomics of response of African ecotypes of chickens to Newcastle disease virus.

WV – DNA sequences from chickens with defined alloantigen genotypes will be examined to identify a chromosomal region associated with alloantigens L and C. Individual and pooled samples from matings with segregating L and C alleles will be analyzed for SNP associations with alloantigen type. Bioinformatic analyses will focus on genes that are expressed on cell surfaces where SNP in exons alter the protein product (WV). Chickens segregating for different alloantigen alleles will be tested for responses against sheep red blood cells. Alloantigen type will be tested for effect on responses against pathogens: *Eimeria tenella* and Marek's disease.

WU – WU will continue genome-wide annotation of cis-regulatory elements in the chicken: Characterization of cis-regulatory elements (such as enhancers and others) by performing several genomic and epigenomic assays for chicken tissues and cells. Generation of data and bioinformatic analysis. We will investigate epigenetic regulation in coronavirus infection in different haplotypes, using RNA-sequencing and ATAC-sequencing. We will identify the role of CHIRs in infectious disease through using small interfering RNA and detecting aberrant changes in immune response in disease susceptible and resistant line (B2/B2 and B19/B19). The project will evaluate for differences in cellular activation and inflammation markers expression. We will characterize the tissue distribution of CHIRs in the reproductive tract in disease susceptible and resistant lines (B2/B2 and B19/B19) to provide the genetic biomarkers for selective breeding of chickens with high production traits and a robust immune system.

NE1834 Objective 2 *To identify factors and agents affecting poultry immune development, function, dysfunction and pathology*

A variety of approaches will be used to address this objective. **AR** will use chicken lines that spontaneously develop multifactorial, non-communicable diseases, such as scleroderma/systemic sclerosis (a fibrotic disease), autoimmune-vitiligo, and -hypothyroidism to study mechanisms of immune dysfunction, immunopathology, and chronic inflammation.

MA is working on further expanding diagnostic insights that can be gained by microscopic examination of blood cells when studying poultry immune system function and pathology. Specifically, **MA** is working on a three-tiered system or strategy for examining stained blood films from avian samples. Tiers are cellularity, given as the percentage of leukocytes occupying space in a microscopic field; cytology, as the sorting scheme used to differentiate leukocytes as lymphocytes, granulocytes, and others; and, behavior, the third tier, includes both intracellular and intercellular facets.

Intercellular behavior may also include leukocyte/erythrocyte interactions and interactions between blood cells and microorganisms such as phagocytosis and other cell-microbe relations. **AR, DE, NC, OH, and GA** will focus on studying immune system function and development in the context of nutritional, environmental, microbiome, genetic selection, and/or microbial infections. **AR** will use minimally invasive *in vivo* procedures and molecular/cellular *ex vivo* analyses to conduct longitudinal studies of cellular innate and adaptive immune responses in chickens reared in different nutritional and environmental conditions and/or divergently selected for genetic traits, like water efficiency.

DE will characterize at a molecular and proteomic level, chicken-disease pathogenesis and potential feed treatments and therapeutics. Recently developed techniques such as qPCR, kinome peptide arrays, Seahorse metabolic flux analysis and *in vitro* and *in vivo* trials will be employed. **DE** is working to further develop applications of digital PCR to poultry health, including detection of host-effects from subclinical bacterial infection in broilers. Other projects planned by **DE** include comparisons of gut immunometabolic responses of ACRB and modern broilers to a variety of pathogens and interventions; *in vivo* and *in vitro* infections of chickens with *Salmonella* with and without treatment and antibiotics to determine mechanism of action and disease pathogenesis; and, research towards refining and characterizing alternatives to antibiotics, including probiotics and eukaryotic cell fractions.

NC will use next generation sequencing, metabolomics, and data analytics to establish mechanistic links between the influences of diet, environment, and the microbiome on the host's immune response and colonization of pathogens, like *Salmonella*. Additionally, research by **NC** will focus on determining metabolites produced by the microbiome and how they modulate immune cell activity, identify the key members of the microbiome producing these metabolites, and develop novel ways to use these mechanisms to better control immunity of production birds.

OH will investigate the molecular and cellular mechanisms of intestinal immune responses to enteric diseases (coccidiosis and necrotic enteritis) in chickens, as well as the metabolic role of intraepithelial lymphocytes in progression of wooden breast syndrome in broilers. Cell population analysis by flow cytometry will be used to identify immune cells that confer resistance to diseases, particularly in the intestinal mucosa. This will be followed RNA-seq and/or scRNA-seq on sorted immune cells that confer protection to the target diseases. **OH** also plans to continue studying the role of neonatal exposure to bacteria on gut function and immune development, especially how early exposure to Enterobacteriaceae affects susceptibility to opportunistic diseases like necrotic enteritis, *Salmonella* and avian pathogenic *E. coli*.

GA will expand their investigations on the impact of *in ovo* and *in vivo* delivered supplements (e.g., postbiotics) on the gut microbiota and the development of the immune response in poultry (broilers) during progression of critical diseases (e.g., coccidiosis, necrotic enteritis), and examine their impact on response and resistance to such enteric challenges. **GA** also will conduct research on detailed immune responses of heritage and modern breeds to better characterize their responsiveness to such challenging enteric diseases in order to better design mitigation strategies.

AL will assess how infections with coccidia and avian reoviruses (ARV) interact with each other and the immune system. Coccidia are among the most important intestinal pathogens in chickens. ARV are one of the possible causes of runting-stunting syndrome. ARV also are known to be immunosuppressive. There are indications that co-infections with coccidia and ARV act synergistically. **AL** seeks to explore how these two parasites interact and how better prevention against one pathogen, e.g., by improved management or vaccination strategies, can mitigate the damage done by the other pathogen. Towards this goal, **AL** will first establish a model for oral infections of chickens with ARV and characterize the expression of a panel of immune genes and lesions in various organs. Based on these data, ARV isolate, infection dose, time points and target genes will be selected, and birds will be co-infected with coccidia before, with and after the ARV infection to test for synergism of the pathogens.

Research towards a better understanding of immune system function and dysfunction in protection and transmission of Marek's disease virus (MDV) and infectious bursal disease virus (IBDV) will be carried out at **DE** and **IL,** and **MD**, respectively. **DE** plans to further investigate serum exosomes as these relate to the patterning of innate immune cells (monocyte, macrophages, dendritic cells and polarized dendritic cells). In the case of vaccine-associated exosomes, **DE** plans to determine their effects on eliciting anti-MDV vaccine responses through the treatment of dendritic cells with exosomes and co-cultivation with MHC-matched peripheral blood mononuclear cells from vaccinated chickens. Furthermore, **DE** plans to examine the effects of exosome treatment on innate and acquired immune responses using kinomic, proteomic and targeted transcriptomic analysis in cell culture and on vaccine responses *in vivo.* **IL** will focus efforts on identification of cellular proteins important for horizontal transmission of MDV. They will develop reagents to test potential cellular receptors for MDV infection using protein-protein interaction studies.

MD will define the processes that occur in the intracellular IBDV replication complexes and evaluate whether these structures play a role in antagonizing antiviral immune responses, for example through the sequestration of proteins involved in innate immune pathways. **MD** will use the novel IBDV rescue system and neutralization assay to assess the individual contribution mutations in the HVR make to immune escape to determine which HVR mutations should be included in vaccines to ensure optimal disease control.

GA will apply commercially available nutritional supplements or nanoparticle vaccine to control coccidiosis, necrotic enteritis, Salmonella, Campylobacter infections of poultry. In vivo experiments will be conducted to study the efficacy of probiotics, synbiotics, and vaccines to decrease the loads of gut pathogens in poultry. Production performances, gut integrity indices and immune parameters will be measured. Nanoparticle vaccines will be designed to deliver antigens against intestinal pathogens can deliver vaccine payload through an oral route and will ideally elicit protective mucosal immunity against gut pathogens. Serum, cloacal swabs, and bile samples from vaccinated and unvaccinated chicks will be analyzed of anti-CP specific IgA and IgG antibodies. Splenocytes will be analyzed for recall response, and liver and cecal tonsils will be analyzed for cytokines.

NE1834 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*

AR continues to maintain autoimmune disease models such as the Smyth (SL), UCD 200/206, and the Obese Strain (OS) lines of chicken as models for human disease as well as for multifactorial non-communicable diseases in poultry and to study immune system development and function of the avian immune system in health and disease. Interestingly, AR has updated the growing feather as an "in-vivo test tube and window into cellular/tissue responses *in vivo* to intradermally injected test materials. The particular focus is on the valuable techniques for refining the method and demonstrating its uses. **WVU** also maintains two inbred lines, four congenic lines, and four line crosses typed at the MHC. Stocks typed for other alloantigen systems are maintained for station research and collaboration.

CA holds twelve lines with different MHC types. Eight of these lines are congenic to each other on the background of inbred line UCD 003. The station also maintains inbred Red Jungle fowl Line UCD 001, the reference line for the original chicken genome sequence. Multiple lines having single gene developmental mutants are also available.

Unique genetic stocks are preserved at **IA**. These stocks include six lines which have been inbred between highly inbred between 80 to 110 generations while maintaining defined MHC types. In addition to these egg-type lines, other stocks are Fayoumi, Spanish and broiler type. An advanced intercross line (AIL) from the cross of a single outbred broiler male with Fayoumi females is produced annually.

WU continues functional annotation of the chicken genome on 20 tissues/cells. They have completed DNA methylome analysis for reproductive and intestinal tissues/ peripheral immune cells and transcriptome with RNA-seq for all tissues and peripheral blood cells. In the future, **WU** will annotate CHIR receptors in various haplotypes and employ spatial sequencing to further locate tissue and cell distribution. Meanwhile, OH uses RNA-seq to discover and characterize new immune cell populations in intestinal mucosa that confer resistance to enteric diseases in chickens, specifically in intraepithelial-lymphocyte subpopulations.

DE will continue to provide access to the cloned chicken genes collected as well as determine the relevance of specific protein-binding to Meq isoforms, in terms of cellular localization and changes in function.

The knowledge of associations of biomarkers with specific immune traits, MHC haplotypes, and segregating alloantigen alleles will allow genetic selection to enhance innate disease resistance in poultry, thus improving bird health and production. Enhance poultry health by identifying physiological and immune response markers key to improving host resistance to pathogens. This information will facilitate the development of intervention and mitigation strategies. Identifying cellular factors will allow better techniques to prevent and control infectious diseases and provide marker-assisted selection and breeding.

Measurement of Progress and Results

Outputs

- Genetic variants associated with immune response, virus infection in diverse chicken population were investigated. Peer-reviewed abstracts and papers were published. Participating regional, national and international scientific conferences, workshops, symposiums and disseminating gap knowledge.
- Identification of cellular factors important for transmission of MDV and assessment of allelic variations in these genes. This project will clone specific immune-related cellular genes important for MDV infection in chickens.
- This project will determine the genetics associated with response to infections with APEC and NDV and disseminate the results in peer-reviewed journal publications and at scientific conferences.
- Evaluate the effect of alloantigen allelic variation on response against bacterial, viral or parasite pathogens or other immune stimuli. Disseminate information to stakeholders and the public through refereed publications, symposia, invited lectures and informal discussions at regional, national and international workshops and meetings.
- Apply genetic, epigenetic and immunological approaches to define the role of individual genes on disease resistance or susceptibility in different B-haplotypes. Results will be disseminated via publications and conferences.
- New knowledge on temporal, qualitative, and quantitative aspects of innate and adaptive immune responses to microbial components and vaccine antigens in genetic lines and commercial poultry.
- Disseminate our findings to stakeholders at regional (NECAD), national (AAAP) and international (Marek’s disease Symposium) meetings, via publications, and through our undergraduate outreach program (UD Envision).
- Characterize the immunometabolic interaction of various gastrointestinal pathogens with the broiler chicken.
- Study putative treatment/therapeutic compounds against gastrointestinal pathogens and their mechanism of action in broiler chickens.
- Solutions to replace antimicrobial products in poultry production will be provided through refereed publications, symposia, lectures and informal discussions at regional, national and international meetings.
- Evaluate the role microbiome associated metabolites have on avian immune function using transcriptomics and metabolic analysis, along with in vitro and in vivo assays.
- Continue microscopic studies of the plasmacyte series by describing variant cells with comparisons to classic types that should allow for a better interpretation of complex hemograms and their relation to disease stress
- Elucidate the protective role of T-cell subpopulations in the intestinal mucosa against Eimeria infection

Outcomes or Projected Impacts

- Identification of genes that are associated with resistance to heat stress and Newcastle disease virus and can be used to genetic enhancement of disease resistance of chicken in adaption to hot climate.
- Identification of cellular factors important for transmission of MDV will allow better strategies to prevent and control MDV in poultry houses, as well as provide marker-assisted selection and breeding.
- Knowledge of associations of biomarkers with specific immune traits will allow genetic selection to enhance innate disease resistance in poultry, thus improving bird health and production.
- Identification of alloantigen genes that improve the immune response to vaccine and pathogens, facilitating breeding for improved immune response in poultry and other species.
- Characterization of genes, and their regulation, in disease susceptible and resistant lines that will provide genetic biomarkers for selective breeding of chickens with high production traits and a robust immune system.
- Assessing the impact of microbial components and vaccine antigens on the direction and quality of the immune response, for application to modulate and optimize immune system function.
- Classifying fundamental mechanisms of virulence evolution of Marek's disease virus via exosome studies that are essential to the understanding how these vesicles affect systemic immunity.
- Interpreting the obtained results to better determine how disease occurs, identify the most effective means of prevention or mitigation at the tissue or cellular level and to develop targeted intervention.
- Establish mechanistic links between the microbiome, its metabolites and immune function to facilitate new ways of controlling the immune response of production birds
- Identifying the mechanisms, magnitude, dose and duration of probiotics, acidifiers, and vaccine schedules to decrease incidence of important enteric or foodborne pathogens of poultry
- Designing an effective vaccine against coccidiosis using T data for -cell subpopulations and their functions during coccidia infection in chickens
- Publishing refereed papers, symposia, invited lectures and informal discussions at regional, national and international workshops and meetings to disseminate information to stakeholders and the public.

Milestones

(**2023**):Publication of four peer-reviewed journal papers disseminating the results of studies on genetics associated with response to pathogens. Develop tools to address MDV transmission in chickens. Identify genetic variations between chicken lines related to MDV transmission. Establish genetic lines with altered immune system function to produce experimental animals for collaborative work with multiple stations. Determine expression changes elicited by serum exosomes. Identify pathogenesis mechanisms and compounds related to disease in vivo. Identify the dose and duration of probiotics, acidifiers, and vaccine schedules decrease incidence of enteric pathogens of poultry Prepare and submit papers for publication Establish a list of 10 metabolites most likely involved in regulating immune function

(**2024**):Detection of distinct alloantigen genotypes and their interactions that affect immune function. Completion of functional annotation of chicken genome in selected cells and tissues. Establishing immune response profiles to microbial components for phenotyping innate immune response capabilities in poultry populations. Determining how mutations in Meq increase MDV virulence. Establishing an in vitro system for evaluating identified metabolites' ability to regulate function of individual immune cells. Measuring the dPCR response after infection. Identifying the mechanism through which probiotics, acidifiers, and vaccine schedules decrease incidence of enteric pathogens of poultry Submitting papers for publication.

(**2025**):Examining data to better comprehend the range of factors and agents affecting the poultry immune system. Establishing a definition of the CHIR role in infectious disease and reproduction. Modeling key effectors of MDV virulence in vitro and in vivo. Characterizing markers of immune response Developing an in vivo system for validating the impact of identified metabolites on immune system regulation. Identifying the magnitude of protection of probiotics, acidifiers, and vaccine schedules against enteric pathogens of poultry Publishing papers in peer-reviewed journals

(**2026**):Characterizing gene regulation in infectious disease, differentiation of immune cells and reproduction, specifically enhancers. Developing strategies to block MDV spread in poultry houses. Targeting key immune responses with feed interventions Characterizing the immunometabolism of negative responses. Submitting papers for publication.

Outreach Plan

The Technical Committee invites industry stakeholders to attend the annual project meetings. The frequent attendance of these stakeholders facilitates information exchange. Project members learn of the emerging problems which can be examined through experiments. Poultry breeding companies can learn of genetic impacts in the immune system. The information exchange often leads to successful collaborations. Additional extension personnel and other potential collaborators will be invited. The project scientists will disseminate their new data using refereed publications, symposia, invited lectures, informal discussions and online data bases of genetic lines and genome/transcriptome information. Participants will work to develop a symposium at a national meeting. The cooperative effort among project members and other researchers will include sharing scientific expertise and genetic resources held at numerous project stations will continue. This cooperation has enabled members to make significant scientific contributions to the improvement of poultry immune responses as well as the genetics of disease resistance.

Project research will assess the effects of genetic variation on responses to immune stimuli including specific pathogens. Multiple stations will examine the major histocompatibility complex (MHC) which has significant impact on immune responses. Other stations study alloantigens or various molecules that modulate immunity via enhancement or suppression. All these approaches seek to improve poultry health in production environments that employ limited medications.

Poultry immune responses are influenced by many genetic, environmental, nutritional, physiological, management, and microbial factor. Technical committee members of NE-1834 are leaders in research to understand basic mechanisms as well as the unique features of the avian immune response. Furthermore, members have worked to develop effective means to promote poultry health and production. Project participants have published **165** refereed publications and **203** abstracts. Grants have been awarded from multiple agencies and businesses. This productive research focused on genetic factors, agents that affect immunity and toll development, to increase our knowledge and understanding of the immune response.

Collaboration and group interaction remain hallmarks of the group. The diversity of expertise among the project members contribute to project success. Resources are shared among project members. Genetic stocks including highly inbred chicken and turkey lines, sets of MHC-congenic lines, random breed lines and lines with distinct phenotypes combined with research techniques such as SNP analysis, qPCR, and next generation sequencing allow the assessment and selection of functional genetic elements that are related to immune function. Continuous communication and collaboration will occur through 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.

Organization/Governance

The Multistate Technical Committee has the responsibility for planning and supervision of the Multistate Research Project. Committee membership of this 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 prior to the annual meeting. The Technical Committee will meet annually.

At the annual meeting, the Technical Committee will 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 every other year. 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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* = Collaborative publication among multiple NE-1834 participants

43 journal articles (26%) are collaborative

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Technical Reports: 10

Abstracts: = **203**

MS Theses: 5

PhD Dissertations: 14

Undergraduate Honors Theses: 3

Land Grant Participating States/Institutions

Non Land Grant Participating States/Institutions

Participation

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

Combined Participation

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

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

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2334: Genetic Bases for Resistance and Immunity to Avian Diseases

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
Understanding the underlying causes and developing strategies for enhanced resistance to avian diseases will require a multidisciplinary approach involving genetics, genomics, immunology, cell and molecular biology, infectious disease, microbiology, nutrition, physiology and poultry medicine. During disease outbreaks (e.g., HPAI) and changing consumer demands (e.g., antibiotic-free), a better basic understanding is needed to develop novel strategies to address these challenges. The members of the proposed multistate project have expertise in a variety of complementary disciplines and have a documented record of collaboration not only within but also outside of the multistate project. Significant scientific contributions to our fundamental understanding of avian diseases will result from this multistate project.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2334: Genetic Bases for Resistance and Immunity to Avian Diseases

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
Multi-hurdle approaches are urgently needed to address the interdisciplinary nature of avian diseases that continue to pose significant threat to both public and economic health. The team that comprises this multi-state project has shown strong collaborative efforts that successfully leverage team-member individual expertise into effective multidisciplinary approaches that maximize understanding of avian diseases. This multi-state project has demonstrated significant progress in addressing several such avian diseases, and the continuation of this research project is highly likely to result in novel solutions that solve important remaining challenges.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2334: Genetic Bases for Resistance and Immunity to Avian Diseases

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
Comments for “Genetic Bases for Resistance and Immunity to Avian Diseases”

The first objective to be addressed will be to understand how genetics, epigenetics and gene regulation influences innate and adaptive immune responses. The work will include well characterized infection models to identify genes important in viral transmission, and delineate the immune pathways activated in response to bacterial infection. Work will also continue with well defined disease resistant and susceptible chicken lines to evaluate the role of regulatory sequences in the genome in response to infection. Defined genetic lines will also be used to identify genes encoding chicken alloantigens.

The second objective will examine the influence of nutrition, the microbiome and postbiotic supplements on the development of innate and adaptive immunity in poultry. Immune dysregulation will be evaluated with viral and parasite infection models, and with chickens that develop autoimmune disease. These studies will further clarify how systemic inflammation contributes to immune disfunction.

The third objective seeks to assess basic immune function in poultry using lines selected for resistance and susceptibility to economically important diseases and with animal models of autoimmune disease. Use of these valuable genetic resources in characterization of immune defense mechanisms in the responses to viral and bacterial infections will help identify genes controlling immune responses in poultry.

This research is essential to understand basic mechanisms of innate and adaptive immune responses in poultry. The collaborative effort involves investigators with integrated expertise that were very productive in the last 5-year period with an impressive 165 peer reviewed publications, 203 abstracts submitted, and 10 book chapters published.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2334: Genetic Bases for Resistance and Immunity to Avian Diseases

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project with revision
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Good
5. Overall technical merit:
Excellent
Comments
Well written proposal from a productive group. Projects planned should meet goals and enable excellent research to be conducted and published. Some suggestions for improvement of the proposal are as follows:

Consider revising Objective 1 to include “poultry” as the area of focus (i.e., To determine how genetics, epigenetics and gene regulation influences innate and acquired immune Functions in poultry). As written, the current objective is quite broad, which could lead to new members thinking they can contribute efforts focused on non-poultry immune function. It is unlikely that this was the intent of the current multistate project members.

Portions of the Methods contain limited descriptions, making it challenging to evaluate the technical approach. For instance, for Objective 1, “…will explore some methods and tools in poultry health, making them available for community use” is not particularly informative or insightful. Consider revising to contain more explicit details about methodology. These concerns notwithstanding, in general, most of the methods do contain sufficient detail, should be feasible, and will provide valuable information.

In the Outcomes or Projected Impacts and Milestones sections some further editing is needed for consistency in the bullet points. For instance, the first 3 bullets (Identification of…, Identification of…, Knowledge of…) read like outcomes or impacts. The next 2 bullets (Enhance poultry…, Characterizing genes…) do not. Suggest revising the latter, and others like them to be consistent. For instance, “Identification of alloantigen genes that improve the immune response to vaccine and pathogens, facilitating breeding for improved immune response in poultry and other species.” “Characterization of genes, and their regulation, in disease susceptible and resistant lines that will provide genetic biomarkers for selective breeding of chickens with high production traits and a robust immune system.”

Similar issues exist in Milestones. Milestone 2023 is fine. The others should be edited for consistency. For instance, (2024) Detection of distinct alloantigen genotypes…. For (2025) A defined role for CHIR in infectious disease and reproduction in poultry. And so on….

There is a growing trend for multistate groups to develop symposia at regional or national meetings where extension educators will be present to aid outreach efforts. It is unclear if the current Outreach Plan in which the technical committee invites industry stakeholders to attend also includes extension educators, but should be considered to help enhance the outreach goals.

Your Recommendation:
Approve/continue project with revision

WestVirginiaUniversity.
DAVIS COLLEGE OF AGRICULTURE, NATURAL RESOURCES AND DESIGN
DIVISION OF ANIMAL AND NUTRITIONAL SCIENCES

M E M O R A N D U M

TO: Richard Rhodes, PhD
Executive Director
NERA

FROM: Robert L. Taylor, Jr., PhD
Administrative Advisor, NE-1834

DATE: April 25, 2023

RE: Project Renewal NE-1834 (NE_TEMP2334) *Genetic Bases for Resistance and Immunity to Avian Diseases*

The renewal for the NE-1834 project renewal *Genetic Bases for Resistance and Immunity to Avian Diseases* has been reviewed by four external reviewers. The document has been revised in accordance with the reviewers' suggestions. Below please find the responses to these comments and the action taken. The renewal committee and I hope that these changes will be satisfactory. Three reviewers recommended *Approve/continue project* whereas the fourth reviewer recommended *Approve/continue project with revision.*

Thank you for the opportunity to revise and renew this project.

Reviewer 1 (received 04/05/23)

Comments: *Understanding the underlying causes and developing strategies for enhanced resistance to avian diseases will require a multidisciplinary approach involving genetics, genomics, immunology, cell and molecular biology, infectious disease, microbiology, nutrition, physiology and poultry medicine. During disease outbreaks (e.g., HPAI) and changing consumer demands (e.g., antibiotic-free), a better basic understanding is needed to develop novel strategies to address these challenges. The members of the proposed multistate project have expertise in a variety of complementary disciplines and have a documented record of collaboration not only within but also outside of the multistate project. Significant scientific contributions to our fundamental understanding of avian diseases will result from this multistate project.*

Your Recommendation: *Approve/continue project*

Action: **none required**

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304.293.1983 304.293.2232

Reviewer 2 (received 04/10/23)

Comments: *Multi-hurdle approaches are urgently needed to address the interdisciplinary nature of avian diseases that continue to pose significant threat to both public and economic health. The team that comprises this multi-state project has shown strong collaborative efforts that successfully leverage team-member individual expertise into effective multidisciplinary approaches that maximize understanding of avian diseases. This multi-state project has demonstrated significant progress in addressing several such avian diseases, and the continuation of this research project is highly likely to result in novel solutions that solve important remaining challenges.*

Your Recommendation: *Approve/continue project*

Action: **none required**

Reviewer 3 (received 04/11/23)

Comments: *The first objective to be addressed will be to understand how genetics, epigenetics and gene regulation influences innate and adaptive immune responses. The work will include well characterized infection models to identify genes important in viral transmission, and delineate the immune pathways activated in response to bacterial infection. Work will also continue with well defined disease resistant and susceptible chicken lines to evaluate the role of regulatory sequences in the genome in response to infection. Defined genetic lines will also be used to identify genes encoding chicken alloantigens.*

The second objective will examine the influence of nutrition, the microbiome and postbiotic supplements on the development of innate and adaptive immunity in poultry. Immune dysregulation will be evaluated with viral and parasite infection models, and with chickens that develop autoimmune disease. These studies will further clarify how systemic inflammation contributes to immune disfunction.

The third objective seeks to assess basic immune function in poultry using lines selected for resistance and susceptibility to economically important diseases and with animal models of autoimmune disease. Use of these valuable genetic resources in characterization of immune defense mechanisms in the responses to viral and bacterial infections will help identify genes controlling immune responses in poultry.

This research is essential to understand basic mechanisms of innate and adaptive immune responses in poultry. The collaborative effort involves investigators with integrated expertise that were very productive in the last 5-year period with an impressive 165 peer reviewed publications, 203 abstracts submitted, and 10 book chapters published.

Your Recommendation: *Approve/continue project*

Action: **none required**

Reviewer 4 (received 04/21/23)

Comments: *Well written proposal from a productive group. Projects planned should meet goals and enable excellent research to be conducted and published. Some suggestions for improvement of the proposal are as follows:*

Consider revising Objective 1 to include "poultry" as the area of focus (i.e., To determine how genetics, epigenetics and gene regulation influences innate and acquired immune functions in poultry). As written, the current objective is quite broad, which could lead to new members thinking they can contribute efforts focused on non-poultry immune function. It is unlikely that this was the intent of the current multistate project members.

Action: **Edited as requested**

Objective 1. To determine how genetics, epigenetics and gene regulation influences innate and acquired immune functions in poultry.

Portions of the Methods contain limited descriptions, making it challenging to evaluate the technical approach. For instance, for Objective 1, "…will explore some methods and tools in poultry health, making them available for community use" is not particularly informative or insightful. Consider revising to contain more explicit details about methodology. These concerns notwithstanding, in general, most of the methods do contain sufficient detail, should be feasible, and will provide valuable information.

Action: **Edited as requested**

Objective 1. To determine how genetics, epigenetics and gene regulation influences innate and acquired immune functions in poultry.

In the Outcomes or Projected Impacts and Milestones sections some further editing is needed for consistency in the bullet points. For instance, the first 3 bullets (Identification of…, Identification of…, Knowledge of…) read like outcomes or impacts. The next 2 bullets (Enhance poultry…, Characterizing genes…) do not. Suggest revising the latter, and others like them to be consistent. For instance, "Identification of alloantigen genes that improve the immune response to vaccine and pathogens, facilitating breeding for improved immune response in poultry and other species." "Characterization of genes, and their regulation, in disease susceptible and resistant lines that will provide genetic biomarkers for selective breeding of chickens with high production traits and a robust immune system."

Action: **Edited as requested (edits underlined)**

Identification of genes that are associated with resistance to heat stress and Newcastle disease virus and can be used to genetic enhancement of disease resistance of chicken in adaption to hot climate.

Identification of cellular factors important for transmission of MDV will allow better strategies to prevent and control MDV in poultry houses, as well as provide marker-assisted

selection and breeding.

Knowledge of associations of biomarkers with specific immune traits will allow genetic selection to enhance innate disease resistance in poultry, thus improving bird health and production.

Identification of alloantigen genes that improve the immune response to vaccine and pathogens, facilitating breeding for improved immune response in poultry and other species.

Characterization of genes, and their regulation, in disease susceptible and resistant lines that will provide genetic biomarkers for selective breeding of chickens with high production traits and a robust immune system.

Assessing the impact of microbial components and vaccine antigens on the direction and quality of the immune response, for application to modulate and optimize immune system function.

Classifying fundamental mechanisms of virulence evolution of Marek's disease virus via exosome studies that are essential to the understanding how these vesicles affect systemic immunity.

Interpreting the obtained results to better determine how disease occurs, identify the most effective means of prevention or mitigation at the tissue or cellular level and to develop targeted intervention.

Establishing mechanistic links between the microbiome, its metabolites and immune function to facilitate new ways of controlling the immune response of production birds

Identifying the mechanisms, magnitude, dose and duration of probiotics, acidifiers, and vaccine schedules to decrease incidence of important enteric or foodborne pathogens of poultry

Designing an effective vaccine against coccidiosis using T data for -cell subpopulations and their functions during coccidia infection in chickens

Publishing refereed papers, symposia, invited lectures and informal discussions at regional, national and international workshops and meetings to disseminate information to stakeholders and the public.

Similar issues exist in Milestones. Milestone 2023 is fine. The others should be edited for consistency. For instance, (2024) Detection of distinct alloantigen genotypes…. For (2025) A defined role for CHIR in infectious disease and reproduction in poultry. And so on….

Action: **Edited as requested (edits underlined)**

Milestones 2024

Detection of distinct alloantigen genotypes and their interactions that affect immune function.

Completion of functional annotation of chicken genome in selected cells and tissues.

Establishing immune response profiles to microbial components for phenotyping innate immune response capabilities in poultry populations.

Determining how mutations in Meq increase MDV virulence.

Establishing an in vitro system for evaluating identified metabolites' ability to regulate function of individual immune cells.

Measuring the dPCR response after infection.

Identifying the mechanism through which probiotics, acidifiers, and vaccine schedules decrease incidence of enteric pathogens of poultry

Submitting papers for publication

Milestones 2025

Examining data to better comprehend the range of factors and agents affecting the poultry immune system.

Establishing a definition of the CHIR role in infectious disease and reproduction.

Modeling key effectors of MDV virulence in vitro and in vivo.

Characterizing markers of immune response

Developing an in vivo system for validating the impact of identified metabolites on immune system regulation.

Identifying the magnitude of protection of probiotics, acidifiers, and vaccine schedules against enteric pathogens of poultry

Publishing papers in peer-reviewed journals

Milestones 2026

Characterizing gene regulation in infectious disease, differentiation of immune cells and reproduction, specifically enhancers.

Developing strategies to block MDV spread in poultry houses.

Targeting key immune responses with feed interventions

Characterizing the immunometabolism of negative responses.

Submitting papers for publication.

There is a growing trend for multistate groups to develop symposia at regional or national meetings where extension educators will be present to aid outreach efforts. It is unclear if the current Outreach Plan in which the technical committee invites industry stakeholders to attend also includes extension educators, but should be considered to help enhance the outreach goals.

Action: **Edited by adding two sentences (underlined) to paragraph 1 of the outreach plan**
The Technical Committee invites industry stakeholders to attend the annual project meetings. The frequent attendance of these stakeholders facilitates information exchange. Project members learn of the emerging problems which can be examined through experiments. Poultry breeding companies can learn of genetic impacts in the immune system. The information exchange often leads to successful collaborations. Additional extension personnel and other potential collaborators will be invited. The project scientists will disseminate their new data using refereed publications, symposia, invited lectures, informal discussions and online data bases of genetic lines and genome/transcriptome information. Participants will work to develop a symposium at a national meeting. The cooperative effort among project members and other researchers will include sharing scientific expertise and genetic resources held at numerous project stations will continue. This cooperation has enabled members to make significant scientific contributions to the improvement of poultry immune responses as well as the genetics of disease resistance.

Your Recommendation: Approve/continue project with revision

NE-1834 Genetic Bases for Resistance and Immunity to Avian Diseases

Technical Committee Members

Station	Abbreviation	NE-1834 project scientists (n)	New project scientists (n)
Auburn University	**AL**	1	1
University of Arkansas	**AR**	1	1
University of California-Davis	**CA**	1	2
University of Delaware	**DE**	3	3
University of Georgia	**GA**	1	2
University of Illinois	**IL**	1	1
Iowa State University	**IA**	1	1
Beckman Research Institute, City of Hope	**BRI**	1	0
University of Maryland	**MD**	2	2
Cotter Laboratory	**MA**	1	1
North Carolina State University	**NC**	2	1
Ohio State University	**OH**	2	2
University of Prince Edward Island	**PEI**	1	0
Virginia Tech	**VA**	1	0
West Virginia University	**WV**	1	2
Western University of Health Sciences	**WU**	1	1
Collaborators			
Wageningen University	**NL**	1	1
USDA Avian Disease & Oncology Laboratory	**ADOL**	1	1
USDA Food & Feed Safety Research Unit	**FFSR**	1	1
TOTAL		**24**	**23**

NE_TEMP2335: Resource Optimization in Controlled Environment Agriculture

Status: Submitted As Final

Duration	10/01/2023 to 09/30/2028
Admin Advisors:	[Puneet Srivastava]
NIFA Reps:	

Statement of Issues and Justification

Participants

The team members represent multi-institutional and interdisciplinary collaborations. Our members include the following representatives for three main objectives:

Administrative Advisor: Adel Shirmohammadi

Objective 1. To optimize environmental management and control and reduce energy use for high-quality greenhouse and indoor crop production.

- *Specific objective 1.1.* Develop crop-specific guidelines for light quantity and quality in both supplemental and sole-source lighting applications.
- *Specific objective 1.2.* Investigate the conversion efficiency of electric light sources used for controlled environment crop production.
- *Specific objective 1.3.* Investigate environmental control strategies that incorporate artificial intelligence techniques.
- *Specific objective 1.4.* Investigate wavelength selective greenhouse coverings and CEAgrivoltaics applications for environmental controls and reduced resource use.
- *Specific objective 1.5.* Co-optimization of environmental variables and enhancing resource use efficiency in indoor crop production.

Qingwu Meng (University of Delaware)

Shuyang Zhen (Texas A&M University)

Jennifer Boldt (USDA-ARS)

Shamim Ahamed (UC Davis)

Ying Zhang (University of Florida)

Neil Mattson (Cornell University)

Genhua Niu (Texas A&M AgriLife Research)

Kellie Walters (University of Tennessee)

Meriam Karlsson (University of Alaska Fairbanks)

A.J. Both (Rutgers University)

Roberto Lopez (Michigan State University)

Murat Kacira (University of Arizona)

Objective 2. To improve root-zone management of biotic and abiotic factors for high-quality greenhouse and indoor crop production.

- *Specific objective 2.1.* Select new crops that may be grown all year round in soilless substrates and water culture or using novel production techniques.
- *Specific objective 2.2.* Improve the efficacy of organic fertilizer for hydroponic crop production using beneficial microorganisms and controlling rootzone environments (e.g., temperature, dissolved oxygen, and pH).
- *Specific objective 2.3.* Develop aquaponic production strategies that optimize plant productivity while improving nutrient use efficiency (e.g., decoupled aquaponics and aerobic/anaerobic digestion of fish waste solids).

Stephanie Burnett (University of Maine)

Yujin Park (Arizona State University)

Genhua Niu (Texas A&M AgriLife Research)

Youping Sun (Utah State University)

Neil Mattson (Cornell University)

Kellie Walters (University of Tennessee)

Roberto Lopez (Michigan State University)

Murat Kacira (University of Arizona

Objective 3. To train growers and students on new controlled-environment production and engineering knowledge.

- *Specific objective 3.1.* Develop and offer an online class in scouting for insects and diseases in controlled environment agriculture.
- *Specific objective 3.2.* Develop and share curricula for undergraduate and graduate courses in hydroponics and soilless crop production and controlled environment engineering applications for a new program in Agricultural and Environment Technology at UC Davis.
- *Specific objective 3.3.* Develop Scholarship of Teaching and Learning (SoTL) projects in CEA with university undergraduate students.
- *Specific objective 3.4.* Develop a hydroponics textbook that can be used for CEA industry members and for classroom use with contributions by many other team members.
- *Specific objective 3.5.* Develop a hydroponic training course for growers and organize an annual conference on urban agriculture - controlled environment.

Qingwu Meng (University of Delaware)

Stephanie Burnett (University of Maine)

Shuyang Zhen (Texas A&M University)

Shamim Ahamed (UC Davis)

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Statement of Issues

Objective 1.

Centralized open-field vegetable production suffers from low productivity, foodborne pathogens, extreme weather patterns, and seasonal disruptions. As an alternative, greenhouse and indoor vertical farming is emerging to meet consumers' demand for safe, local, fresh, and nutritious vegetables all year round. However, this industry is limited by its high energy use, which is among the highest input costs for controlled environment agriculture (CEA, including greenhouses and vertical farms). Energy use for plant lighting, temperature control, and dehumidification is also associated with the largest share of carbon emissions from indoor farms. A range of energy-efficient technologies are available (LED lighting and smart climate control); however, successful adoption requires greater knowledge of complex plant interactions with the growing environment.

Objective 2.

Hydroponic production, either nutrient solution or soilless substrate-based, is a preferred method for crop production under controlled environment like greenhouses and indoor farms. Hydroponics provides an opportunity to control the physical, chemical, and biological environment of the root zone. Physical and chemical environmental factors of root zone include nutrient composition (recipe) and concentration (electrical conductivity, EC), pH, temperature, and dissolved oxygen concentration. The biological factors are types of microorganisms (that is, microbiome) and their population. Under controlled environment in hydroponics, the microbiome in the root zone is completely different from that in soil rhizosphere.

Root zone temperature can influence plant growth and development. Greenhouse producers have been using root zone heating for decades by regulating media temperature during propagation and production of annual bedding plants and vegetable transplants. For solution-based hydroponics, root zone temperatures can be controlled through chilling and heating the nutrient reservoirs (Hooks et al., 2022; Miller et al., 2020; Sakamoto et al., 2015). The effect of root zone cooling and heating depends on air temperature and crops. However, available research-based information is limited for high-value leafy greens and culinary herbs.

As the demand for organic produce increases, interest in growing organic food crops under controlled environment using hydroponics is increasing as well. Nutrient management is more challenging with organic nutrient sources than inorganic fertilizers since organic nutrient sources often have imbalanced nutrient contents, high salinity and can introduce toxic pollutants and infectious agents and can decrease dissolved oxygen (Bergstrand et al., 2020; Kano et al., 2011; Williams, 2014). In addition, in organic fertilizer, many nutrients bound to organic substances are not immediately available for plant uptake and require microbially mediated mineralization processes (Bergstrand et al., 2020; Williams, 2014). However, current hydroponic rootzone environments are optimized principally for using inorganic fertilizer, and a significant knowledge gap exists regarding how biotic and abiotic factors of rootzone environment affect the efficacy of organic fertilizer for hydroponic crop production.

In recent years, application of bioproducts or plant biostimulants (PB) has gained recognition as a sustainable approach to boost plant growth and development under normal or stressed conditions (Askari-Khorasgani et al., 2019; Del Buono, 2021; Massa et al., 2017). PBs can be derived from a wide variety of materials: beneficial fungi such as arbuscular mycorrhizal fungi (AMF), beneficial bacteria such as plant growth-promoting rhizobacteria (PGPR), protein hydrolysate, humic substances, seaweed extract, and others (Del Buono, 2021; Shahrajabian et al., 2021). Thus, the effect of PBs largely depends on the type of PB. Few studies have been conducted to assess the efficacy of various PBs for (organic) crop production under controlled environments.

Aquaponics is a combination of aquaculture and hydroponics where fish waste is used as plant fertilizer and plants filter the water for fish. These production methods are land-use efficient and lend themselves well to urban production and to school science and agriculture classes. In the U.S., there are 5,350 aquaculture farms producing $1.8 billion wholesale annually (USDA, 2017). Aquaponics is an emerging industry, especially for urban agriculture.

Objective 3.

Controlled Environment Agriculture (CEA) is a rapidly changing field with high levels of technology. Growers and students working in CEA must understand topics including greenhouse engineering, irrigation and fertilization, business management and economics. Just in the past five to ten years, the tools and technology used in CEA have expanded to include an increased focus on LED lighting and other energy efficient technologies, hydroponic production of food, and improved sustainability of irrigation and fertilization practices. CEA technology will continue to change and evolve, making it critical to provide up to date, research-based training for growers. Highly skilled undergraduate and graduate students are needed to work in CEA; there are often more positions for graduates in this field than students to fill those positions. Our group is well positioned to train the next generation of growers and engineers as well as to connect students and the industry.

Since technology changes rapidly in this area, there is a strong need for research-based review articles and books on this topic. Currently, there are no student or grower-oriented books or review articles on the topic of hydroponics, which is a rapidly growing area of CEA. A hydroponics book would support the development of undergraduate and graduate hydroponic classes, which many members of our group either have developed or are planning to develop.

Justification

Objective 1.

The photon spectrum and intensity influence photosynthesis, plant shape, and accumulation of mineral nutrients. Light use efficiency of indoor crops also depends on other environmental factors (e.g., temperature, humidity, and carbon dioxide concentration) and cultural factors (e.g., nutrient solution concentration and composition). Therefore, optimizing the light environment based on key environmental and cultural factors has the potential to improve crop growth and nutritional value while saving electrical costs.

Optimal control of the environment in plant production facilities is a complex task due to the multiple interactions of the parameters involved. Traditional environment controls rely on sensor feedback from aerial or rootzone environment without information and data from crop growth as well as an approach considering entire production system in decision making. Integration of artificial intelligence (AI) can assist growers in making more logical, data-driven, site-specific management decisions which influence crop productivity, quality, as well as use of labor and other resources. An AI framework consists of models, controllers, and real-time data, that combined with domain knowledge can optimize decisions for selected outcomes (e.g., profitability, resource use efficiency).

Objective 2.

Traditional food crops grown in greenhouses include tomatoes, peppers, eggplant, strawberries, leafy greens, and culinary herbs. Additional novel food crops will be explored to find the high-yielding and profitable crops in greenhouse and indoor hydroponic systems to justify the high crop production cost in indoors.

Realizing year-round production under controlled environment is economically important to provide constant supply of fresh produce and increase the efficiency of the facility use. Greenhouse hydroponic crop production is energy intensive. In hot summers in southern region, cooling the air temperature of a greenhouse to optimal temperatures for many cool-season leafy greens and herbs is challenging. In cold winters, heating the entire greenhouse to the optimal level is costly. To reduce energy cost, root zone temperature control under suboptimal air temperature may be a solution. Quantifying the interaction between air and nutrient solution (root zone) temperatures is crucial to optimize crop yield, nutritional quality, and post-harvest longevity. The lack of this information limits the full utilization of the major advantage of controlled environments, which is the ability to manipulate the production environment. Consequently, there are significant gaps in economic feasibility, and the potential to provide high-quality, flavorful food to people from all socioeconomic backgrounds is diminished.

Greenhouse gas emissions associated with chemical fertilizer production, the global phosphorus shortage, and soil and water pollution caused by over application and mismanagement have all been recognized as severe threats to sustainable food production (Nosheen et al., 2021; Oelkers and Valsami-Jones, 2008). In addition, recent inorganic fertilizer shortage raised fears of a global food crisis. The use of organic materials as fertilizers has multiple advantages, such as recycling nutrients, supplying beneficial organic biostimulants, and decreasing the demand for mined minerals (Bergstrand et al., 2020).

Organic farming is one of the fastest growing segments in the U.S. agriculture. Increasing organically grown fresh produce such as fruiting vegetables, leafy greens and herbs under controlled environment is essential. Therefore, information on how to manage organic fertilizers in soilless substrate and organic hydroponics is urgently needed. In addition, the demand for organic seedlings for open field production far exceeds the supply. Controlled environment is an ideal facility for producing organic seedlings and transplants. Research-based information on how to best utilize available organic fertilizers amended with PBs to produce quality transplants will reduce transplant shock, and thus economic losses, and increase transplant tolerance to biotic and abiotic stresses after transplanting. Therefore, it is imperative to evaluate the effectiveness of PB products in tandem with organic fertilizers with optimal application rates and timings to organic farmers/stakeholders.

Aquaponics is one of the most popular systems for urban agriculture and for science classes in high schools and agriculture colleges. Although aquaponics concept is not new, there are many areas that need more research work. Cornell University research will address issues faced by aquaculture and aquaponics operators: the large volumes of solid waste that must be frequently removed and cleaned from their systems.

Objective 3.

Our group includes greenhouse engineers, horticulturists, and economists working in CEA. We are well suited to provide education and training on a broad range of topics. Many members of our team provide education in CEA through undergraduate instruction, mentoring of undergraduate and/or graduate students working on research projects, or Extension programming with CEA growers. The collaborative nature of our group allows us to broaden our programming so that our individual efforts coalesce for greater regional and national impact.

Related, Current and Previous Work

Related, Current, and Previous Work

Objective 1.

Previously, researchers at Michigan State University and Iowa State University have quantified the effects of air temperature on growth and development of 16 species and cultivars of culinary herbs determining the base and optimum temperatures for node appearance and fresh mass accumulation (Walters and Currey, 2019; Walters and Lopez, 2021). Additionally, the influence of air temperature was quantified on basil volatile compound concentration and consumer preference. For example, increasing air temperature from 23 to 36 °C increased basil volatile compounds but did not influence consumer sensory preference (Walters and Lopez, 2022).

Previous research by team members has determined the energy efficacy of horticulture lighting fixtures (NJ) and plant responses to light intensity (OH) and spectrum for specific plants and applications (DE and NY). Adjusting the lighting strategy at the end of the crop cycle can also impact growth and nutritional quality (TX), though more information is needed on specific crop systems, nutritional assays, and consumer sensory evaluation. Team members have also begun to explore the interaction between environmental factors such as light and carbon dioxide (NY) and temperature (TX).

Previous work by team members has sought to develop efficient control strategies for airflow, light, carbon dioxide, and dehumidification (AZ, NY, CA), however current control strategies often focus on discrete actions (e.g., lighting control, irrigation control), without being able to determine an optimized strategy for the entire production system. New approaches including artificial intelligence are required to develop integrated and optimal control strategies.

Objective 2.

Researchers at Arizona State University evaluated fish-based organic fertilizer and liquid food waste anaerobic digestate for soilless cultivation of lettuce and tomato transplants compared to commercially available chemical fertilizer. At the same total nitrogen concentration, lettuce ‘Cherokee’ seedlings had 75% less shoot fresh weight and 64% less dry weight under organic fertilizers, regardless of organic fertilizer types, than chemical fertilizer. Similarly, tomato ‘Red Robins’ seedlings grown with organic fertilizers had one fewer leaf, 36% smaller stem diameter, 40% shorter stem length, and 75% or 67% less shoot fresh or dry weight, respectively, compared to seedlings grown with chemical fertilizer. In another study, we investigated if using microbial biostimulants and supplementing dissolved oxygen, can improve nutrient availability, plant nutrient uptake, and thus, plant growth under organic fertilization. We identified applying arbuscular mycorrhizal fungi *Rhizophagus intraradices* every two days increases shoot fresh weight of lettuce ‘Cherokee’ by 30-98% and ‘Rex’ by 5-85% compared to un-inoculated controls under organic fertilization. In addition, supplementing oxygen to the hydroponic nutrient solution made with an organic fertilizer increased dissolved oxygen from 1.0 ppm to 5.5 ppm and promoted both shoot and root growth in lettuce ‘Cherokee’ and ‘Rex’.

Currently,an ongoing multi-state project (TX, UT) determines the efficacy of different kinds of commercially available PB products on onion and watermelon seedlings, the optimal application rates and methods, and the carry-over effects of PBs applied during seedling stage on the subsequent growth in the field. In addition, TX team is conducting research on organic fertilizers and management and determining the efficacy of various biostimulants on organic seedling production under controlled environment conditions.

Cornell University has an ongoing project in aquaponics research and extension to address issues faced by aquaculture and aquaponics operators. In preliminary research, we have developed and tested a low-cost aerobic digestion method to turn most of the solid waste into a liquid organic fertilizer. More work is needed to optimize the digestion system and we will determine the impact of temperature, residency time, and waste source on the resulting fertilizer and crop performance.

Objective 3.

Over the years, members of this project have collaborated formally and informally in many educational programs. Many of our members have partial extension appointments and organize annual greenhouse workshops and short courses in AZ, CT, IA, KS, NJ, NY, and OH. In all of these instances, we invite members from other stations to participate as speakers. We also have several members who teach controlled environment agriculture, hydroponics, sensors, controls, and/or greenhouse management to undergraduate and graduate students in CA, CO, KS, ME, TX, FL, NJ, AZ. Our collaboration makes it easier to provide up-to-date information on new topics as well as new approaches to instruction and learning.

One goal of our group is to provide research-based publications on current topics in CEA. For example, in 2019, our group collaborated on a literature review of food production in controlled environment agriculture in an urban setting (Gómez et al., 2019; CT, FL, IA, IN, NJ, NC, MI). This manuscript reviews critical aspects of CEA production including the production environment, lighting, carbon dioxide enrichment, and specialty production systems such as hydroponics. It has already been cited 58 times since publication.

Various group members organize webinars or participated in webinar series focused on greenhouse and indoor vertical farm based controlled environment crop production. For instance, AZ organized ISHS VerticalFarming Talks webinar series with 13 presentations with speakers from around the world with topics including lighting, environmental control, co-optimization of environment variables, economics, life cycle assessment, advances in vertical farming, and reached more than 5000 viewers.

To improve the ease of collaboration, OH created a directory of contact information for Extension Specialists throughout the United States in 2022 to make it easier for CEA researchers, educators, and Extension personnel to connect. Faculty at land-grant universities in nearly every state are members of this group along with several USDA-ARS research scientists who work in CEA. This contact group is relatively new, but it has already served to share information about upcoming Extension programs and assistantships available for new MS or PhD students. OH is working with each member of this group to develop a list of greenhouses and vertical farms throughout the US.

Objectives

1. To optimize environmental management and control and reduce energy use for high-quality greenhouse and indoor crop production.
   Comments: • Specific objective 1.1. Develop crop-specific guidelines for light quantity and quality in both supplemental and sole-source lighting applications. • Specific objective 1.2. Investigate the conversion efficiency of electric light sources used for controlled environment crop production. • Specific objective 1.3. Investigate environmental control strategies that incorporate artificial intelligence techniques. • Specific objective 1.4. Investigate wavelength selective greenhouse coverings and CEAgrivoltaics applications for environmental controls and reduced resource use. • Specific objective 1.5. Co-optimization of environmental variables and enhancing resource use efficiency in indoor crop production.
2. To improve root-zone management of biotic and abiotic factors for high-quality greenhouse and indoor crop production.
   Comments: • Specific objective 2.1. Select new crops that may be grown all year round in soilless substrates and water culture or using novel production techniques. • Specific objective 2.2. Improve the efficacy of organic fertilizer for hydroponic crop production using beneficial microorganisms and controlling rootzone environments (e.g., temperature, dissolved oxygen, and pH). • Specific objective 2.3. Develop aquaponic production strategies that optimize plant productivity while improving nutrient use efficiency (e.g., decoupled aquaponics and aerobic/anaerobic digestion of fish waste solids).
3. To train growers and students on new controlled-environment production and engineering knowledge.
   Comments: • Specific objective 3.1. Develop and offer an online class in scouting for insects and diseases in controlled environment agriculture. • Specific objective 3.2. Develop and share curricula for undergraduate and graduate courses in hydroponics and soilless crop production and controlled environment engineering applications for a new program in Agricultural and Environment Technology at UC Davis. • Specific objective 3.3. Develop Scholarship of Teaching and Learning (SoTL) projects in CEA with university undergraduate students. • Specific objective 3.4. Develop a hydroponics textbook that can be used for CEA industry members and for classroom use with contributions by many other team members. • Specific objective 3.5. Develop a hydroponic training course for growers and organize an annual conference on urban agriculture - controlled environment.

Methods

Methods

Objective 1.

Environmental control and management (lighting, temperature, CO_2 enrichment, and nutrient)

We will perform a series of experiments to further understand the interactions between light properties and other environmental and cultural factors. We will focus on balancing energy input and growth and quality attributes of emerging and less studied hydroponic crops, including hot pepper, spinach, and arugula. We will grow plants under sole-source, color-tunable LED fixtures on vertical shelves in a growth room or in reach-in growth chambers with independent environmental control. Over time, we will test various combinations of the photon spectrum, intensity, and duration, aerial environmental factors, and nutrient solution factors. When plants are ready for harvest, we will measure plant shoot and root biomass, morphological traits, pigmentation, mineral nutrient concentrations, and photosynthetic parameters, as well as assess physiological disorders, if present.

We will comprehensively examine plant growth, nutritional quality, morphological and physiological responses to temperature and sole-source electric lighting conditions (spectral quality, intensity, and photoperiod) in growth chamber studies. Multiple leafy green crops will be selected based on their commercial value, suitability for indoor farming, and nutritional values. Results from those experiments will be used to develop integrated lighting and temperature control strategies for improved crop yield and quality, and ultimately to facilitate the development of energy-efficient management approaches for lighting and cooling.

We will conduct a series of studies to characterize the effects of CO_2 enrichment, nitrogen availability, and light conditions on plant growth, quality, transpiration, and water use efficiency; interactive effects among the environmental factors will be examined.

We will perform a series of greenhouse experiments to determine end-of-production lighting strategies to increase culinary herb volatile concentration and flavor while enhancing post-harvest longevity. We will grow plants in a greenhouse and subject plants to altered light intensity and/or quality for different durations at the end of production. We will quantify plant morphology, yield, photosynthesis, volatile concentrations, and consumer preference.

Climate control technologies and algorithms development

We will model the different techniques for dehumidification of various indoor vertical farming settings with various types of crops. Then, we will select some best potential solutions for optimization in indoor farming applications and test the prototype units in terms of their performance for moisture removal and energy consumption.

We will investigate the correlations between canopy microclimate, air distribution, and crop growth with computational fluid dynamics to achieve precision microclimate control for crop production in indoor farming to improve energy use efficiency.

We will use previously developed greenhouse and vertical farm energy models to compare energy use of tomatoes and strawberries in different U.S. climates.

We will conduct greenhouse experiments using a hydroponic system and lettuce as a model crop. A machine learning control algorithm will be integrated into an existing commercial control system. Crop production experiments will be conducted comparing yields and resource consumption for crops grown using either a conventional environmental control approach versus a control approach that incorporates sophisticated models and predictive techniques.

We will co-optimization of environmental variables, alternative air distribution system designs that can help enhancing resource use and reducing energy costs are needed.

We will conduct greenhouse experiments using drip irrigation-based system with tomato as model crop in a greenhouse located in semi-arid climate. Crop production experiments will be conducted comparing yields and resource consumption with water and energy using a conventional environmental control approach versus a control approach that incorporates predictive models and AI integrated controls.

We will conduct experiments to quantify the interactive effects of light, temperature, and CO_2 concentration on crop growth, morphology, yield, and photosynthetic rate. Focus crops will be culinary herbs, young plants (liners), and other specialty-crops. Photosynthetic response measurements will be made using a LI-6800 portable photosynthesis system and modeled.

We will conduct a series of greenhouse experiments to identify suitable species and cultivars for high yield, high resistance to abiotic (heat) and biotic (pests and diseases, bolting and tip burn, if applicable) stresses. We will select promising genotypes for further study to optimize the production protocol, including nutrient management, root zone temperature, and supplemental lighting strategies in winter season. Sensory relevant parameters such as color, chlorophyll content, texture, taste (non-volatiles), and aroma (volatiles) will be quantified. Flavor instrument analysis data will be correlated to consumer test data to discover the key flavor compounds associated with consumer sensory preference. In addition, nutrient compositions such as total polyphenols, individual polyphenols, vitamin C, and mineral profiles will be further used as quality indicators for the leafy greens in the study.

Objective 2.

We will conduct trials with a variety of crops that are not yet widely grown hydroponically using nutrient film technique (NFT) and in soilless substrates. The yield and the overall quality of the crops in both systems will be compared . Previous work has indicated that some crops, such as carrots, have lower visual quality when grown in NFT, however, some cultivars performed better than others (Gichuhi et al., 2009). Crops grown in both systems will be analyzed for texture, sweetness, and flavor through sensory evaluation.

For organic soilless cultivation and hydroponics, we will investigate the effectiveness of beneficial microorganisms and controlling rootzone environments for organic hydroponic crop production. Organic fertilizers require microbially mediated mineralization and nitrification processes, which can be affected by rootzone environment, such as pH, dissolved oxygen concentration, and temperature. Thus, when organic fertilizers are used, the effects of the hydroponic rootzone environment on plant nutrient uptake and growth as well as microbial activities and mineralization and nitrification process should also be considered. Optimization of the environmental and microbial components in the rootzone will improve the efficacy of using organic fertilizer in hydroponic systems.

A series of experiments will be conducted to determine the effectiveness of various plant biostimulants on the promotion of growth and quality of onion and watermelon seedlings and possibly other vegetable seedlings and the carry-over benefits after transplanting. In addition, experiments will be carried out to optimize organic fertility management using representative organic fertilizers for transplant production with or without the use of PBs.

We will determine how nutrient solution and air temperature interact to increase the yield, post-harvest longevity, flavor, and quality of culinary herbs. Plants will be grown at three air temperatures (20 to 40 °C) and five nutrient solution temperatures (15 to 40 °C) depending on the crop. A minimum of 10 plants per treatment per replication will be grown and growth, production time, plant appearance, and yield will be assessed. Photosynthesis (CO_2 assimilation) and key volatile compounds will be quantified for 4 plants per treatment per replication with an LI 6800 portable photosynthesis system and GC-MS, respectively.

The overall research goal for aquaponics is to develop and test aerobic digestion of solid fish waste as a value-added organic fertilizer that can also be divert large scale commercial fish waste solids from the waste stream. Research will be completed by 1) constructing 4 aerobic digesters, 2) testing the digesters as an organic nutrient source in hydroponics with leafy greens and herbs, 3) using the aerobic digestate as a fertilizer source for organic vegetable transplants, and 4) estimating nutrient elements N and P.

Objective 3.

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

We will continue to train graduate students and involve them in this project. We regularly invite graduate students to attend our meeting and present their work. In the past, we have financially incentivized student attendance through a poster and oral competition and providing travel grants.

We will continue to host short courses on hydroponics, energy efficiency, water management, and general controlled environment production practices. Our members with Extension appointments will continue to organize annual greenhouse workshops and short courses in AZ, CT, IA, KS, NJ, NY, OH, TX. During these short courses, we will provide opportunities for graduate students to present about their research and activities to the participants, as part of the facility and technical tour programs. In all of these instances, we will invite members from other stations to participate as speakers. Upcoming events include the Northeast Greenhouse Conference (November 2023 and 2025), the 4th Annual Controlled Environment Conference at Dallas AgriLife Research and Extension Center (December 6-7, 2022), Great Plains Growers' Conference (January 13-14, 2023), 22nd Annual UArizona Greenhouse Engineering and Crop Production Short Course (March 15-17, 2023), and NCERA 101 at UC Davis in April 19-21, 2023. Many of the events we host for members of the industry are offered every year or every other year and our programming focuses on providing the most current information.

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

We will work as a group to develop a curriculum for undergraduate courses in hydroponics to increase the ease of offering courses in this important area. These courses include a new course for engineering students at UC Davis addressing the control and optimization of microclimates, equipment (HVAC, irrigation, control systems) and materials (cover, screen, etc.) selections, and using simulation software for energy calculations. The University of Maine is working with Cornell University and the University of Vermont to develop an online greenhouse scouting course. This course will focus on instruction about the tools and techniques involved in scouting greenhouses for insect, mite, or disease problems. Scouting is a cornerstone of integrated pest management and allows growers to be offered to greenhouse growers and undergraduate students. Participants can receive a certificate in scouting, which would support training of future professional scouts.

Much of our education and outreach efforts will focus on providing instruction to growers and undergraduate students in the area of hydroponics. This has become an important area for education, because hydroponics allows for the production of food crops year-round in a variety of climates with less water and fertilizer. Hydroponic food production in the US has increased rapidly, and our educational efforts will ensure that current and new growers are using best practices.

Other member stations, including Cornell University, Iowa State University, Texas A&M University, Kansas State University, University of Arizona offer undergraduate and graduate level courses in Hydroponics and Soilless Crop Production. The courses focus on the science, management practices, and engineering in controlled environment crop production using hydroponics and will enable students to set up hydroponic systems and cultivate diverse crops such as leafy greens, microgreens, fruiting vegetables and small fruits in a research and commercial production setting. Curriculum development and in-class experiences at KS and TX will help to guide development of additional new hydroponics courses at other member stations developing their CEA curriculum.

We plan to write a hydroponics textbook for undergraduate students and CEA industry members that will support the development of new hydroponics courses or Extension training. The writing and editing of this book will be led by IA, MI, NJ, and NY and will be based on an outline for the book that our group wrote in 2021. Other members will contribute chapters to the book based on their diverse expertise.

Measurement of Progress and Results

Outputs

- Objective 1. Comments: • Develop lighting strategies based on environmental and cultural conditions for efficient indoor production of emerging crops. • Develop integrated lighting and temperature control strategies for improved crop yield and quality, and to facilitate the development of energy-efficient management approaches for lighting and cooling. • Develop efficient thermal environment management guidelines to reduce the carbon footprint of indoor farms, improve crop water use efficiency, and reduce energy consumption. • Add models fit to the data for PhotoSim, which will allow growers to evaluate how altering the growing environment will affect photosynthetic rates, yield, and compare costs for various environmental set points. • Develop an efficient dehumidification system in terms of energy use and humidity control. • Model energy use and carbon emissions of tomatoes and strawberries in greenhouse and vertical farms. • Develop microclimate control algorithms to promote crop quality and energy use efficiency. • Determine the genotypes of leafy greens with high yield, high-stress tolerance for year-round production. • Identify cost-effective strategies such as end-of-production (EOP) regimens to improve the yield, appearance, nutritional content, and post-harvest longevity of leafy greens species and cultivars. • Develop guidelines for cost-effective end-of-production lighting strategies to enhance crop flavor, aroma, and appearance. • The controlled environment industry will be educated about the use of advanced control tools that improve plant quality and reduce resource consumption. • Develop machine learning powered control algorithms for greenhouse environmental control. • Evaluate alternative air distribution system design configurations that can be suited to variety of vertical farming production system designs.
- Objective 2. Comments: • We will select at least two new crops for hydroponic production based on yield, quality, and flavor (ME). • We will develop cultural practice guidelines for using organic fertilizers in hydroponic systems for common hydroponic crops, including leafy vegetables, herbs, and strawberries (AZ, KS, TX). • We will evaluate the effectiveness of selected plant biostimulants (PB) on mitigating drought stress using onion and watermelon as model crops. The optimal application methods and rates of the top performing PBs will be determined. The best organic fertilizers and their application methods and rates will be determined. In addition, the synergistic or interaction of organic fertilizers and PBs will be investigated (TX, UT). • We will develop integrated air and root-zone temperature management guidelines for five culinary herb species that maximize growth, shelf-life, and flavor (TN, TX). • The resulting product from the aquaponics research is a high-value fertilizer which diverts fish waste from waste-streams (NY).
- Objective 3. Comments: • 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 an online hydroponics production book. • We will enhance undergraduate and graduate research training on controlled environment plant production to prepare the students for careers in the field. • We will develop and share curricula related to hydroponic food production in CEA, including controlled environment engineering applications. • We will publish scholarship of teaching and learning that demonstrates and enhances effectiveness of our programming efforts.

Outcomes or Projected Impacts

- Objective 1. • We will improve crop growth and nutritional value while saving electricity and carbon emissions by optimizing growing environments based on key environmental and cultural factors. • We will improve crop production, reduce water use, and reduce energy consumption for dehumidification. • We will improve crop models to help improve grower's decision-making, focusing on culinary herbs, young plants (liners), and other specialty-crops. • We will improve the sustainable production of crops in CEA systems through HVAC system design. • We will inform the research community about the benefits of implementing advanced control approaches/algorithms.
- Objective 2. • More crops suited for greenhouses and indoor farms will be available for producers with production protocols (ME). • The effectiveness of selected commercial plant biostimulants (PB) on promoting the growth and quality of onion and watermelon seedlings under stressed and non-stressed conditions will be determined. The optimal application methods and rates of the top performing PBs will be identified. The best organic fertilizers and their application methods and rates will be determined. In addition, the synergistic effect of organic fertilizers and PBs will be investigated. These results will guide organic producers in selection of organic fertilizers and PBs (TX, UT). • Nearly 90% of NYS's population lives in urban areas and aquaculture, aquaponics, and hydroponics are common urban agriculture practices aa land-efficient systems producing high nutrient density fish and vegetables. These systems are also often used in school science and agriculture programs. Fish farming produces large volumes of solid-waste, and this project will develop low-cost aerobic digestion methods to that can reduce solid waste (and N and P) by more than two-thirds for NY's 105 aquaculture farms. We will develop and test a low-cost fertilizer source which can be utilized by hydroponic and certified organic vegetable transplant growers. Continual interaction with NY stakeholders will ensure the project is rooted in real-world production methods and crops. Dissemination will take place via on-site tours, an annual aquaponics short course, a workshop for educators, and the project website at cea.cals.cornell.edu ensuring that diverse stakeholders can benefit from project findings (NY).
- Objective 3. • The proposed book on hydroponics will help keep growers competitive and aware of the latest research in controlled environment agriculture. • New courses focusing on hydroponics and agricultural engineering will train the next generation of growers.

Milestones

(**0**):Annual recurring milestones AK, AZ, CA, DE, FL, MI, NJ, NY, TN, TX, and USDA-ARS will collaborate to optimize environmental management and control to reduce energy use for high-quality greenhouse and indoor crop production through environmental control and management, climate control technologies and algorithms development, and optimizing crop growth with crop modeling and selection. Teach undergraduate and graduate courses on controlled environment crop production practices and agriculture engineering: CA, DE, IA, KS, ME, FL, NJ, TX, AZ. Communicate new horticultural knowledge with local, regional, and national stakeholders: KS, MN, OH, NJ, NY, TX, AZ.

(**2024**):Objective 1. • Set up new lab spaces and hardware for indoor crop experiments in DE. • Set up a multi-chamber canopy gas exchange system equipped with LEDs lights and capable of temperature control; re-model lab space for indoor crop cultivation/research in TX. • Set up and test the new indoor growth facility in USDA-ARS. • Work for more precise tools for dehumidification demand and simulate the performance of the possible solutions under various settings in CA. • Set up a growth chamber equipped with an engineered air distribution system in FL • Screening 20 leafy greens and determining their suitability for warm climate greenhouse hydroponic production in TX. • Plan and prepare for end-of-production lighting experiments in TN. • Completed growing system installation and control hardware setup and testing for greenhouse experiments in NJ. • Determine the impact of CO2 enrichment with real-time lighting control for hydroponic greenhouse lettuce in NY. • Model energy use and carbon emissions of tomatoes in greenhouse and vertical farms (NY). • Design and construct air distribution system in the vertical farm facility for experiments in AZ. • Completed growing system, sensors and instrumentation installation, control hardware setup and testing for a control approach greenhouse climate control in AZ. Objective 2. • Two to three crops will be grown in NFT (ME). • Conduct research determining which microbial inoculants are beneficial in organic hydroponic production in leafy vegetables and herbs (AZ, TX). • Identify the most effective plant biostimulants; screening organic fertilizers for seedling propagation (TX). • Plan and prepare for root-zone temperature experiments (TN, TX). • Construct 4 aerobic digesters (NY). Objective 3. • Members of the group will edit our existing outline for a book on hydroponics as needed. • Member stations that instruct courses in hydroponics will work together to share curriculum plans. • A new online course on greenhouse scouting will be offered to greenhouse growers and undergraduate students in spring, 2023 and fall, 2024. • Continue offering intensive workshops and short courses to educate public and practitioners of CEA.

(**2025**):Objective 1. • Conduct experiments on light and environment interactions in hot pepper in DE. • Examine the interactive effects of light spectral quality by temperature in vegetable crops in TX. • Conduct lighting and temperature experiments in culinary herbs in USDA-ARS. • Design prototype units as a sustainable solution for dehumidification and testing in small-scale CEA facilities in CA. • Investigate the correlation between air distribution and canopy microclimate in FL • Screening 20 leafy greens and determining their suitability for warm climate greenhouse hydroponic production in TX. • Conduct end-of-production lighting greenhouse experiments in TN. • Completed baseline experiments in NJ. • Determine the impact of CO2 enrichment with real-time lighting control for hydroponic greenhouse tomatoes in NY. • Model energy use and carbon emissions of strawberries in greenhouse and vertical farms (NY). • Conduct experiments to evaluate crop growth, tipburn mitigation, environmental uniformity in indoor farming in AZ. • Completed experiments with data collection on crop yield, greenhouse

environment, and resource use in AZ. Objective 2. • Two to three crops will be grown in NFT (ME). • Conduct research determining which microbial inoculants are beneficial in organic hydroponic production in strawberries (AZ, TX). • Identify the most effective plant biostimulants and their applicate rates (TX). • Conduct root-zone temperature experiments (TN, TX). • Test the digestates as an organic nutrient source in deep-water culture hydroponic systems with leafy greens and herbs (NY). Objective 3. • Chapters will be assigned to members of the group who will contribute to the book on hydroponics. • At least two member stations will offer courses in hydroponics; their experiences will inform the development of the book on hydroponics. • Continue offering intensive workshops and short courses to educate public and practitioners of CEA.

(**2026**):Objective 1. • Conduct experiments on light and cultural factor interactions in hot pepper in DE. • Examine the interactive effects of light spectral quality by temperature in ornamental transplants in TX. • Conduct lighting and CO2 experiments in culinary herbs in USDA-ARS. • Design prototype units as a sustainable solution for dehumidification and testing in small-scale CEA facilities in CA. • Evaluate the techno-economic feasibility of indoor production with some feasible solutions in CA. • Conduct experiments to study plant growth under different air distribution treatments in FL. • Determine the root zone temperatures for cooling and consumer preference of selected promising leafy greens in TX. • Conduct end-of-production lighting greenhouse experiments in TN. • Informed/trained growers about improved control strategies, completed experiments in NJ. • Determine the impact of CO2 enrichment with real-time lighting control for hydroponic greenhouse tomatoes in NY. • Model the energy use and carbon emissions benefits of artificial intelligence in climate control of greenhouse and vertical farm production (NY). • Conduct experiments to evaluate co-optimization of environmental variables with DLI, air temp, CO2 and VPD in AZ. • Completed experiments with data collection on crop yield, greenhouse environment, and resource use in AZ. Objective 2. • At least one additional crop will be grown in NFT; crops will be evaluated using sensory analysis (ME). • Conduct research determining the impacts of controlling rootzone environment in organic hydroponic production in leafy vegetables and herbs (TN, TX). • Identify the most effective plant biostimulants and their applicate rates; determine the interaction and/or synergistic effects of organic fertilizers and PBs (AZ, TX). • Conduct root-zone temperature experiments (TN, TX). • Evaluate aerobic digestates as fertilizer source for organic vegetable transplants (NY). Objective 3. • Members will write and edit chapters for the book on hydroponics. • Continue offering intensive workshops and short courses to educate public and practitioners of CEA.

(**2027**):Objective 1. • Conduct experiments on light and environment interactions in leafy greens in DE. • Characterize the effects of CO2 enrichment and light conditions on plant growth, quality, transpiration, and water use efficiency in TX. • Conduct environmental experiments on young plants in USDA-ARS. • Improve and increase the sustainability of indoor productions in CA. • Conduct experiments to study plant growth under different air distribution treatments in FL. • Determine the root zone temperatures for cooling and consumer preference of selected promising leafy greens in TX. • Complete volatile compound quantification in TN. • Informed/trained growers about improved control strategies, completed experiments in NJ. • Model the potential for green energy and geothermal systems for greenhouses. • Comparisons for effectiveness of air distribution system designs in AZ. Objective 2. • Conduct research determining which microbial inoculants are beneficial in organic hydroponic production in strawberries (AZ). • Determine the interaction and/or synergistic effects of organic fertilizers and PBs (TX) • Complete volatile compound quantification (TN). • Estimate the N and P quantity diverted from waste streams (NY). Objective 3. • Community outreach about advance control strategies with AI integration in AZ. • Members will continue to write and edit chapters for the book on hydroponics. • Continue offering intensive workshops and short courses to educate public and practitioners of CEA.

(**2028**):Objective 1. • Conduct experiments on light and cultural factor interactions in leafy greens in DE. • Characterize the effects of light conditions, CO2, and nutrient availability on plant growth and quality in TX. • Conduct environmental experiments on other specialty crops in USDA-ARS • Adapting the proposed solutions for commercial scale production as energy efficient and sustainable approaches for dehumidifying indoor farming systems in CA. • Develop intelligent environmental control algorithms for indoor farming in FL. • Determine the nutritional quality parameters for the selected promising leafy greens in TX. • Provide recommendations for end-of-production lighting in culinary herbs in TN. • Informed/trained growers about improved control strategies, completed final experiments, published results and developing a plan for the next steps in NJ. • Model the potential for green energy and geothermal systems for vertical farms. • Prepare recommendations for air distribution system alternatives and environmental control strategies in AZ. • Completed experiments, published results, and community outreach with presentations at conferences, workshops, webinars in AZ. Objective 2. • Develop the cultural practice guidelines of using organic fertilizers in hydroponic systems for common hydroponic crops, including leafy vegetables, herbs, and strawberries (ME, AZ, TX, TN, NY). • Determine the optimal PB rates and organic fertilizer rates (AZ, TX). • Provide recommendations for culinary herb air and root-zone temperature (TN). • Extension activities on aquaponics will be followed (NY). Objective 3. • Our book on hydroponics will be published. • Continue offering intensive workshops and short courses to educate public and practitioners of CEA.

Outreach Plan

Outreach Plan

We will work closely with the controlled environment agricultural industry throughout the United States. Several team members have Extension appointments, providing additional connections with grower associations, industry suppliers, and the Land-grant Extension network. We use these connections with the industry to answer 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 journal articles and Extension publications.

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

We plan to publish a book on hydroponic production for greenhouse growers that will include the results of our group's previous work on water and energy savings, ventilation and cooling, and alternative energy use.

We will work closely with 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 and VerticalFarming Daily 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, the use of energy curtains, air distribution system design, environmental controls. 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 provide a valuable contribution to the industry in which practical and research-based solutions are quickly developed and spread in time and space.

Organization/Governance

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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Literature Cited

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Land Grant Participating States/Institutions

NJ,AZ,ND,OH

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Both, A. J.	Yes	New Jersey - Rutgers University	1,3	205	5399	2020	0.10	0.00	0.00	0.1	205
Feng, Iris (Xiaoyu)	Yes	North Dakota - North Dakota State University	1,2	102 133 141 401 402 403 404	110 410 5210 5399 7210 7310 7410	2020 0 0 0 0 0 0	0.40	0.00	0.00	0	0
Giacomelli, Gene		Arizona - University of Arizona	3	402	1499	2020	0.10	0.00	0.00	0.2	402
Kacira, Murat	Yes	Arizona - University of Arizona	1	205 401 402 405	1499 1499 1499 1499	2020 2020 2020 2020	0.10	0.00	0.00	0.2	402
Kubota, Chieri	Yes	Ohio - Ohio State University	1,2,3	205	2410	1020	0.10	0.00	0.00	0.5	205
Ling, Peter P.		Ohio - Ohio State University	1,3	402 404	2410 2410	2020 2020	0.10	0.00	0.00	0.1	205
Owen, Garrett		Ohio - Ohio State University	2,3	205	2199	1020	0.10	0.00	0.00	0.1	205

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
205-5399-2020	0.1	0	0
402-1499-2020	0.1	0	0
205-1499-2020	0.03	0	0
401-1499-2020	0.03	0	0
402-1499-2020	0.03	0	0
405-1499-2020	0.03	0	0
102-110-2020	0.06	0	0
133-410-0	0.06	0	0
141-5210-0	0.06	0	0
401-5399-0	0.06	0	0
402-7210-0	0.06	0	0
403-7310-0	0.06	0	0
404-7410-0	0.06	0	0
205-2410-1020	0.1	0	0
205-2199-1020	0.1	0	0
402-2410-2020	0.05	0	0
404-2410-2020	0.05	0	0
Grand Total:	**1.00**	**0.00**	**0.00**

Program/KA	Total FTE
205	0.03
402	0.07
402	0.07
0	0
205	0.17
205	0.03
205	0.03
Grand FTE Total:	**1.2**

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2335: 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:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
Project has an outstanding team of key players in these areas of research and technology development and implementation. This is a interesting, detailed, and well planned series of tests to gather data on complex plant interactions with controlled environment crop variables such as lighting, temperature of the root zone, microbiome, growth method, aquaponics, etc. The teams approach will be to integrate multiple investigations, test multiple technologies and approaches, and forward the knowledge in this area for the benefit of multiple stakeholders. The addition of modeling, artificial intelligence/machine learning components and guidelines and recommendations for growers will help translate data collected into usable products for researchers and industry. The addition of the personnel training component enables the continued transfer of information. There are a few typographical and grammatical challenges with the writing, consistent with a proposal being written by multiple contributors, but in general this is an excellent planned project that will have significant return on investment and great benefits for the field of controlled environment agriculture.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2335: 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
Excellent research/extension team and well structured proposal.
The only item that seems out of place is that under objective 2, which is about root zone optimization, objective 2.1 seems more appropriate under objective 1 related to plant responses to the environment. Similarly, the "Related, Current and Previous Work" paragraph on air temperature and herbs at MSU seems more appropriate under objective 1.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2335: Resource Optimization in Controlled Environment Agriculture

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
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:
Excellent
Comments
There is significant overlap with the NCERA-101 regional working group, particularly activities related to Objective 1. The proposal would be strengthened if objectives and methods were more unique to this group and the scope was focused.

Activities read as a compilation of what individuals are doing or are planning to do in other projects, with little evidence of planned research collaboration.

A strength of the project is teaching/education collaboration and development of resource materials for students.

The proposed work is expansive and goals/outcomes seem unrealistically lofty (i.e., aspirational).
Your Recommendation:
Approve/continue project

NE1835: Resource Optimization in Controlled Environment Agriculture

Renewal Proposal (2023)

Responses to Reviewers' Comments

We appreciate the time and feedback from all reviewers and have revised the renewal proposal accordingly. The reviewers' comments are in black text below, whereas our responses and specific changes made are in blue text.

Reviewer 1:

Project has an outstanding team of key players in these areas of research and technology development and implementation. This is a interesting, detailed, and well planned series of tests to gather data on complex plant interactions with controlled environment crop variables such as lighting, temperature of the root zone, microbiome, growth method, aquaponics, etc. The teams approach will be to integrate multiple investigations, test multiple technologies and approaches, and forward the knowledge in this area for the benefit of multiple stakeholders. The addition of modeling, artificial intelligence/machine learning components and guidelines and recommendations for growers will help translate data collected into usable products for researchers and industry. The addition of the personnel training component enables the continued transfer of information. There are a few typographical and grammatical challenges with the writing, consistent with a proposal being written by multiple contributors, but in general this is an excellent planned project that will have significant return on investment and great benefits for the field of controlled environment agriculture.

Response: We appreciate the reviewer's helpful feedback and have made corrections of typographical and grammatical errors throughout the document.

Reviewer 2:

Excellent research/extension team and well structured proposal. The only item that seems out of place is that under objective 2, which is about root zone optimization, objective 2.1 seems more appropriate under objective 1 related to plant responses to the environment. Similarly, the "Related, Current and Previous Work" paragraph on air temperature and herbs at MSU seems more appropriate under objective 1.

Response: We appreciate the reviewer's constructive input.

We understand the confusion of listing Objective 2.1 under Objective 2 and have rephrased Objective 2.1 as "To research new crops that may be grown all year round in soilless substrates and water culture or using novel production techniques." It emphasizes the intended scope of this work to optimize the root-zone environment to grow new crops in soilless culture.

We agree that the related, current, and previous work on how air temperature influences herb growth fits better under Objective 1 and have moved it under Objective 1.

Reviewer 3:

There is significant overlap with the NCERA-101 regional working group, particularly activities related to Objective 1. The proposal would be strengthened if objectives and methods were more unique to this group and the scope was focused. Activities read as a compilation of what individuals are doing or are planning to do in other projects, with little evidence of planned research collaboration. A strength of the project is teaching/education collaboration and development of resource materials for students. The proposed work is expansive and goals/outcomes seem unrealistically lofty (i.e., aspirational).

Response: We thank this reviewer's time and feedback.

We understand that there are seemingly overlapping goals with the NCERA-101 group since both NE-1835 and NCERA-101 revolve around controlled-environment agriculture, and many of our members are actively participating in both groups. However, the proposed objectives of the renewed NE-1835 project are distinctly different from those of NCERA-101. NCERA-101 is more extension and outreach-oriented, with focuses on 1) technology advancement, dissemination, and transfer; 2) development of standards and guidelines for environmental control and monitoring; 3) communication of research and educational materials to stakeholders; 4) instrument calibration; 5) advocacy of sustainable operation of controlled-environment facilities; and 6) support of students' participation and presentations in annual meetings. In contrast, NE-1835 has specifically defined research and educational goals to tackle present and emerging issues in the controlled-environment agriculture industry. In addition, NCERA-101 historically originated from work conducted in growth chambers, especially those in academia and government agencies, and has evolved to include greenhouse and indoor vertical farming systems, whereas NE-1835 is specifically addressing resource use efficiency bottlenecks in the greenhouse and indoor vertical farming industry.

Considering the scope of the funding support for this multistate project, the extent of collaborations is inevitably based more on individual participants' existing projects, collaborations, and interest areas. A multistate group like NE1835 provides a helpful platform for like-minded researchers to meet and exchange information, and to foster and develop new collaborations. Our proposal thus leverages our existing resources and connections, combines our shared interests in new collaborations, and expands into new directions that members of this group believe would advance this field. We are confident that we will be able to achieve the proposed goals.

NE_TEMP2336: Improving Quality and Reducing Losses in Specialty Fruit and Vegetable Crops through Storage Technologies

Status: Submitted As Final

Duration	10/01/2023 to 09/30/2028
Admin Advisors:	[Christopher B. Watkins]
NIFA Reps:	

Statement of Issues and Justification

The need as indicated by stakeholders

US growers produce an abundance of fresh fruits and vegetables, but deterioration of quality, storage disorders, decay and contamination with mycotoxins continue to cause considerable losses. As a result, the fresh fruit and vegetable industries rely on numerous pre- and postharvest practices to ensure minimization of losses. Several key developments in production and storage technologies make this project highly relevant for the fruit and vegetable industries in North America.

Postharvest losses for fresh produce are severe and vary widely, depending upon the crop and handling conditions employed. Gustavsson et al. (2011) noted that global food losses are about 1.3 billion tons annually. The FAO Sustainable Development Goal 12.3 aims, “by 2030, to halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including postharvest losses” (Fabi and English, 2019). Considering factors of climate change, a growing population reaching Malthusian limits, and less agricultural land being used to produce more food, new technologies, crops and decay management strategies are needed to increase food security and help slow food loss and waste.

Apples are a prime example of a commodity that is subject to significant losses during storage and transport. However, pre and postharvest rots caused by *Colletotrichum* (bitter rot) are significant problems for apple, citrus, blueberry and vegetables like tomato (Barad et al., 2017; Jurick II and Cox 2016; Liu et al., 2020). Postharvest rots of fresh fruits and vegetables not only reduce quality, but also limit their availability for consumption, and sometimes processed products can contain mycotoxins that harm human health. While postharvest fungicides are approved for use to control these pathogens on a limited number of these crops, concerns over maximum residue limits, antimicrobial resistance, and environmental impacts necessitate development of new tools and strategies to abate decay. New disorders in apple (skin wrinkling and leather blotch) appear to be more prevalent, as well as continued occurrence of others such as bitter pit and soft scald. The ability to identify conditions that lead to disorder development is dependent on a collaborative effort.

More frequent and deleterious environmental stresses, particularly high heat, have impacted produce quality and caused losses in the storage and supply chain. It is anticipated that this will increase losses due to poor quality and higher incidence of storage disorders. Preharvest growing conditions such as high temperature have increasingly become more stressful for plants, and this can have a deleterious effect on the postharvest outcome. High temperatures during maturation can delay apple coloration and force growers to delay harvest, but with limitations for storage life. Grower use of reflective ground covers to address poor color can impact fruit maturity and postharvest conditions. Unknown growing conditions are involved in the development of storage disorders, such as apple bitter pit. Therefore, a coordinated effort is needed to identify the conditions that lead to greater incidence and our predictive capacity.

Fresh produce items are sold in a wide range of locations from local farmers’ markets and roadside stands to local and regional markets to school lunch programs and to export destinations. These locations place varying demands on the techniques and technologies necessary to extend postharvest quality while reducing losses during this value chain. The fresh produce industry also provides reliable and constant employment to significant numbers of U.S. and foreign workers.

Increased consumer expectations that fruits and vegetables are available year-round and often out of season have created changes in which cultivars are grown and have expanded the regions where fruits are produced. This has been coupled with the development of new cultivars with specific adaptations to regional climates outside traditional production regions. Blueberries, strawberries, cherries and peaches are examples of this phenomenon. New types of these traditional fruits such as crisp-fleshed blueberries and firm-fleshed peaches have expanded the potential for marketing highly perishable fruits. Where once only round and cherry tomatoes were available in supermarkets, nowadays typical produce sections sell a variety of tomato types including on-the-vine (aka cluster), grape, varietal, heirloom, Roma, and hydroponic-grown, by both conventional and by organic methods. A similar phenomenon has occurred with other types of vegetables such as Ripe bell peppers and cucumbers. Consumer interest in new types of fruits such as muscadine grapes and elderberries has increased but with relatively unknown postharvest needs. New cultivars of virtually every type of produce are continually being adopted that may have unknown issues.

New cultivars typically have unknown postharvest needs that make their handling challenging. Small-scale operations oftentimes are at the forefront of testing and marketing new cultivars. However, the postharvest practices differ between large-scale operations with extended storage durations and small-scale operations with rapid marketing and brief periods of storage. Consequently, new cultivars initially tested for small-scale production may be unsuitable for large-scale production with concomitant stringent postharvest expectations of quality. Honeycrisp is a good example of an apple cultivar grown to satisfy demand, but with many storage issues.

Techniques for season extension, most notably high tunnels, have also contributed to expanded production of fruits and vegetables for small-scale local markets, but with unknown impact on quality. In addition, production in protected culture such as high tunnels and greenhouses is increasing in the U.S. with simultaneous changes in cultivars and postharvest needs. Therefore, it is essential that our growers, packers and shippers be provided with the latest postharvest information to thrive in this highly competitive arena. Limited research on the postharvest needs has necessitated the need for multistate research to gain an understanding of how these new cultivars and growing environments contribute to preservation of quality and prevention of decay at the physiological, molecular, genomic and genetic levels.

Severe limitations in the availability of labor have forced the fruit and vegetable industry to investigate greater use of mechanical harvesting and to adopt cultivars that are better adapted to mechanization. Many fruits and vegetables ripen at uneven rates. Thus, to maintain quality during marketing, fruit and vegetables are spot-picked to ensure harvest at the optimum stage of maturity. However, mechanical harvesting often necessitates a once-over harvest that requires the crop to be at a uniform stage of maturity to maximize yield and overall quality. Crops that can be mechanically harvested for the fresh market include blueberries, cranberries, leafy crops and root crops, and for processing markets include grapes, prunes, oranges, peaches, cherries, jalapeno peppers, tomatoes and olives. Research is needed to determine the impact of mechanical harvesting on consumer acceptability and shelf-life.

Continued demand for reduced reliance on chemicals and for organic fruit is accompanied by a requirement for compatible storage technologies. Improvements in the use of nonchemical approaches for preventing storage scald of apples called dynamic controlled atmosphere (DCA) storage comes with risks and additional need for how to apply this method to new cultivars. Highly sophisticated and sometimes risky postharvest storage technologies can be used in place of synthetic chemical controls, but uncertainties of their efficacies for the many different cultivars remain. Consumer demand for reduced Minimum Residue Levels is a significant driving factor to develop novel tools to maintain quality, reduce losses and mitigate toxins. Postharvest chemical applications remain an important method of preventing decay, but new nonchemical approaches have been developed to meet the needs of organic markets, and to minimize losses of fruit during storage and transport. New approaches to preventing losses come with potential risks whereas, generally recognized as safe (GRAS) chemical alternatives that prevent decay are largely untested. Testing of reduced-risk materials such as essential oils has shown effectiveness for controlling decay in some fruits, but large-scale application and impact on eating quality are untested. A better understanding of relationships between postharvest physiology of fruits and their susceptibility to physiological disorders and decay pathogens is essential for developing improved control measures and reducing chemical use.

New postharvest technologies that extend postharvest storage and shelf life need additional testing to confirm efficacy and identify risks for the different cultivars and types of produce. Elevated CO_2 storage for raspberry can extend shelf-life, but research is needed to pinpoint optimum levels for the many cultivars and regions where raspberries are grown. Physiological disorders can be affected positively or negatively by these new storage technologies. For example, superficial scald of apples is inhibited by 1-MCP, DPA, and DCA storage, while other disorders, especially carbon dioxide-related ones tend to be increased by 1-MCP. The adoption of the plant growth regulator 1- MCP has altered the risks of long-term storage. These practices have increased greatly, largely in response to the need to maintain quality during storage and marketing. New application methods for in-carton application of 1-MCP such as sachets and incorporation into films have the potential to improve storage of other types of produce, but with many unanswered questions regarding efficacy.

Growers increasingly rely on predictive tools and rapid tests to determine storage potential and relative susceptibility to postharvest losses. Low-cost, rapid tests that measure postharvest characteristics and predict storage life or potential losses will allow growers to segregate high-risk produce and make informed decisions regarding storage duration. Peel analysis and the “passive” tests for predicting bitter pit in apples are examples of industry use of tests that were developed through collaborative research. Additional research is needed to understand how regional variation will impact application of these predictive tools. Recent advances in understanding how loss of xylem function leads to bitter pit can be combined with these predictive tests to improve grower ability to prevent severe cases of this disorder. Water loss, an important cause for quality loss in several fruits and vegetables can be measured using a low cost, nondestructive sealed chamber. Wide scale testing is needed for its commercial application. Other predictive or rapid tests currently in development include rancidity in walnuts, surface moisture measurement on leafy vegetables for preventing decay and measuring aroma volatiles to predict chilling injury in peaches. Knowing the status of produce going into storage is critical and can extend shelf life by a few days for highly perishable fruits and vegetables such as asparagus. Storage and marketing practices such as “first in first out” logistics lead to losses because they do not consider the physiological maturity or any adverse preharvest and harvest conditions (high temperatures, poor cooling to remove field heat) that compromise shelf-life in different batches of harvested fruit and vegetables.

Members of the NE1836 have collaborated to address the multiple disorders of apple cultivars that create complex storage requirements with the added need for predictive tools. In addition, recent advances in understanding the causes of disorders, mycotoxin abatement, and prevention will require multi-year testing and refinement in the many regions where they are now grown, and a coordinated effort to speed up real-world application of these new tools.

Expanding our knowledge on the harvest and storage of these specialty crops is critical to ongoing success in maintaining product quality of the fresh fruit and vegetable industries. Sharing of knowledge, especially about application of new technologies, increases the probability of successful outcomes. The project involves postharvest scientists in the different geographical regions of the US and Canada, nearly all with extension/outreach responsibilities, thereby providing a powerful platform for development and extension of this knowledge.

Research in this project will focus on these and other needs under the following objectives:

1. Enhance and/or adapt current handling, storage and postharvest practices/technologies to ensure high-quality products to increase their acceptability by consumers.
2. Expand and translate fundamental plant biology to develop new storage technologies and plant materials that will enhance human nutrition and energy-efficient postharvest systems.
3. Advance our fundamental knowledge of host-pathogen-microbe interactions to maintain high-quality fruits and vegetables while reducing food loss and waste.

Importance of the Work

Previous and current versions of this project (NE103, NE1018, NE1036, NE1336, NE1836) have made major contributions to the fresh fruit industry. These include industry adoption of innovative applied methods developed by the group, and basic research on postharvest problems such as bitter pit in apple, and chilling injury (CI) in pome and stone fruits. The efforts of this project have led to more effective control measures and new knowledge of the genetic and biochemical causes of the disorders and diseases. Other environmental impacts during fruit growth, especially elevated temperature and solar irradiation, are major contributors to annual postharvest losses in major production regions. By necessity, our collective work continues to assess the fundamental basis of these losses and to identify novel solutions to mitigate them in a changing regulatory environment. Nonchemical and reduced risk chemical methods of preventing losses have been studied/developed to extend storage life of highly perishable fruits such as berries. Studies on apples have continued to be a major focus with an emphasis on newly emerging problems such as increased CO_2 injury in storage and the ability to predict disorders that cause major losses for producers. 1-Methylcyclopropene (1-MCP), an ethylene action and ripening inhibitor has been used to maintain quality in storage of apple and is now being used for several other fruits and vegetables. New methods of application can potentially expand the use of the compound for the benefit of small-scale producers.

This multistate project was traditionally focused on fruits, but with research on vegetables being conducted concurrently. Research on vegetable storage practices is just as important and will benefit from the collective abilities of the group.

Technical Feasibility of the Research and Advantages for Doing the Work as a Multistate Effort

A goal of this group is to actively collaborate and exchange results from different systems to find solutions and to develop methods for rapid implementation to maintain industry profitability. We can accomplish this by conducting applied research in conjunction with a strong basic program while seeking to understand fruit/vegetable and pathogen physiology and biochemistry, particularly concerning responses to genetic differences among cultivars, and responses to technologies such as 1-MCP and modified/controlled atmosphere storage regimes. Many years have gone into selection of apple cultivars that maintain quality during long storage durations, but this type of coordinated effort is also needed to identify cultivars of other fruits and vegetables that may be better suited to the rigors of storage and marketing. The researchers in this project have diverse skill sets that span several scientific fields and have an established track record for collaboration on projects across North America.

Members of the group have the ability and expertise to study physiological, biochemical, and genetic/genomic basis of quality and development of storage disorders as well as fungal and bacterial pathology. Combined efforts have led to finding the causes of browning disorders in apple, quality loss and decay in blueberry and to predict or prevent such problems. Recent research has combined applied and basic approaches to identify underlying causes and potential solutions for such issues as apple bitter pit disorder, senescence associated genes (SAGS) in broccoli and lettuce, and changes in texture, peel elasticity and weight loss that predict shelf life in 60 blueberry cultivars. Furthermore, research that is based in many regions of the US and Canada contributes to the thorough study of many storage disorders that are impacted by local growing conditions so that cultural practices and storage conditions can be designed for regional needs. The broad geographical distribution of the team in this project provides a unique opportunity by which responses of fruits and vegetables to a wide range of growing conditions can be studied and the difficulties posed by the intrinsic variability of a fruit and vegetable crop can be overcome.

Members of the NE1836 have access to state-of-the-art facilities to conduct sensory and consumer testing. Integration of sensory testing into postharvest research is needed to determine how production and storage practices impact consumer preferences since technical measurements do not encompass the full human experience of quality. The combination of new cultivars and changes in pre- and postharvest practices can influence consumer acceptance.

As in the past, the current NE1836 members work as a research and extension team to solve industry problems and to provide rapid dissemination of research results that is greatly enhanced by the organization of a multistate project. The strong connections with producers that many members possess is a strength that ensures research is relevant and addresses the most pressing issues they face. The multistate project combines the respective strengths of each member for coordinated effort to investigate postharvest problems and to find much needed solutions to the specialty crop producers, both regionally and nationally.

Impact

The accomplishments of the NE1836 project include thorough postharvest evaluation of commercially important and emerging fruit cultivars, development of methodologies that enhance storage life, quality, and flavor and elucidation of mechanisms involved in flavor and storage disorder development in fruits. An example of impact resulting from our continued collaboration is the testing and development of a predictive test for bitter pit disorder for the Honeycrisp apple in various regions of the U.S. and Canada. This and other tests will allow producers to segregate at-risk produce to prevent their loss in storage. The collaborative efforts of the NE1836 program have also generated key information that has provided solutions to storage of apples including the development of storage regimes that address CO_2 sensitivity in CA storage. Continued and wide-scale industry use of previous recommendations has been important for the economic success of Honeycrisp producers in the northeastern US. Successful transfer of information to researchers and industries is well integrated within the project and has been done via peer-reviewed publications, grower meetings, trade publications and websites.

Most members of this project have strong extension/outreach programs associated with their research, and they have been successful at guiding practices of fruit growers. The North American apple industry, for instance, has directly and demonstrably benefitted from our research and extension efforts and now employs a protocol for dynamic CA storage for apples with reduced reliance on postharvest chemicals. NE1836 members have worked with the National Mango Board (NMB) to develop the handling practices such as for harvest maturity, temperature management, ripening and transport that are recommended to that industry. The same is true for many other crops such as stone fruits, strawberries, and tomatoes. The primary goals of our new project are to increase competitiveness for domestic fruit and vegetable production and preserve 'fresh-picked' sensory and nutritional quality, which in turn will increase the availability of locally grown and highly perishable fruit. The large increase in new cultivars has the potential to increase availability of highly perishable produce in the market, but this will depend on research that leads to cultivar selection that is appropriate to commercial harvesting practices and storage. The overall impact of this project will be to improve the long-range health of the American populace via greater consumption of fresh fruits and vegetables, and to increase farm profitability at all levels of scale.

Annual reports are available on the NIMSS project website:NE1836: Improving Quality and Reducing Losses in Specialty Fruit Crops through Storage Technologies – NIMSS

Related, Current and Previous Work

A search of the NIMSS database revealed that, apart from efforts by the NE1836, there is an absence of multistate projects and coordinating committees that focus on the biology and technologies used to preserve the quality and condition of fresh specialty crops. This project is needed to provide coordination and collaboration among scientists to service diverse and valuable fruit and vegetable industries and avoid duplication of effort and inefficient use of resources.

Related Work.

The following projects were found to address some aspects of postharvest preservation of food crops.

S294 - Quality and Safety of Fresh-cut Vegetables and Fruits. This project emphasizes food safety and standardization of microbiological procedures, instrumental and subjective methods for sensory quality analysis, flavor-based shelf-life measurement and emerging treatments and techniques for assuring fresh-cut quality. The focus is on packaged products, not whole perishables.

NE2231 - Collaborative Potato Breeding and Variety Development Activities to Enhance Farm Sustainability in the Eastern US. This project aims to improve yields, fresh market appeal, processing or value-added traits, and pest resistance, but does not focus on postharvest problems per se.

WERA27: Potato Variety Development. This project focuses on potato germplasm development, seed potato evaluation, decay and disease resistance, and storability and processability of germplasm lines. The project acknowledges the need for “postharvest management research” but does not target specific postharvest problems.

NC1023: Engineering for Food Safety and Quality. This project tries to develop a systems-level understanding of food quality and safety development in complex processes, but does not deal with living produce.

NCCC212: Small Fruit and Viticulture Research. The top priority is “breeding and releasing superior, adapted cultivars”. One objective is to evaluate pre- and postharvest fruit quality components, but does not focus on the development or application of technologies to improve storability.

Current and Previous Work.

Over the last several decades, extensive research has been conducted nationally and internationally on maintaining fruit quality after harvest. Despite this long-term effort, the preservation of quality in the postharvest period continues to be a global issue of great importance at all levels of the value chain (Gustavsson et al., 2011). Historically, members of this multistate project have contributed significantly to this effort, albeit primarily on fruits. Previous work also includes fundamental research on processes related to fruit quality, the development of postharvest interventions to suppress quality decline, and evaluation of fruit responses to storage conditions (e.g., disorder incidence, and decay). While the focus of this multistate project has traditionally served the fruit industries, the lack of a counterpart program to focus on vegetable crops makes the inclusion of vegetables in the research portfolio a natural extension of this multistate project. The following sections detail current and previous work as it relates to proposed objectives and the inclusion of vegetables in future efforts.

Storage disorders. The control of storage disorders has been an important focus of postharvest research for fruits and vegetables. Important among these are those related to storage temperature (chilling injury) and atmosphere (high CO_2 and low O_2 injury). Many other storage-related disorders are physiological in nature, but still are influenced by the storage regimen.

Chilling injury (CI) is a significant storage disorder for numerous perishables. The multi-state project for fruit has led to methods of managing chilling injury in apple and stone fruit that have been adopted industry wide. The apple cultivar ‘Honeycrisp’ is particularly sensitive to low temperature storage, and work in this project has led to the development of a preventative treatment (conditioning at 10 to 20 °C for up to seven days followed by storage at 3.5 °C; (Watkins et al., 2004; Watkins et al., 2005). Conditioning treatments developed as part of the NE1836 activities are now standard practices (Beaudry, 2014). Conditioning treatments and the application of the antioxidant diphenylamine also help to suppress CO_2 injury in Honeycrisp (Contreras et al., 2014). Chilling injuries are not limited to apple fruit; many peach cultivars are highly sensitive to low temperature storage (Lurie and Crisosto, 2005). Protocols for the diminution and/or avoidance of chilling in peaches have emerged from efforts of NE1836 team members (Manganaris et al., 2019; Moore and Farcuh, 2020). Early indicators of CI symptoms in fruits (e.g. loss of key volatiles, sugar metabolic shifts) can be used as potential predictors/diagnostic tools of susceptibility and may be helpful in developing management practices.

Bitter pit in apple is another serious disorder occurring in all growing regions in North America and is especially challenging for Honeycrisp and Granny Smith. Current efforts have focused on methods that predict disorders at or before harvest or that reduce risk of bitter pit (NE1836 publication in preparation). This work will allow growers to market high risk fruit before disorders develop and to put low risk orchards in long-term storage with minimal losses. Additional tests such as peel analysis (Baugher et al., 2017) can improve prediction accuracy (Moran, unpublished; Robinson et al. *in* Milkovich, 2023) and be performed by growers at relatively low cost. Conditioning treatments to suppress chilling injury in Honeycrisp enhance bitter pit (Watkins et al., 2004). However, a reduction in the O_2 concentration during the warm temperature conditioning period ("rapid CA") can suppress bitter pit formation (Serban et al., 2019), but the elevated CO_2 during rapid CA sometimes leads to internal browning of this CO_2-sensitive cultivar. As this method is an effective bitter pit/soft scald/soggy breakdown control strategy, determining "safe" combinations of temperature and CO_2 is needed to reduce losses due to CO_2 sensitivity during conditioning.

Sun stress has been evaluated by NE1836 team members as it relates to metabolic and spectral fingerprinting and quality of Granny Smith, Honeycrisp, and other apple cultivars (McTavish et al., 2020). Recently, hyperspectral analysis has identified indices that may be a predictive tool to minimize physiological disorders development, in particular sunscald, and reduce fruit quality variability in the supply chain (Torres, unpublished).

Storage disorders also plague the potato industry. In the U.S., potatoes are usually stored for up to 12 months in bulk storages with piles reaching 16 to 18 feet high (Muthukumarappan et al., 1994). Under such conditions potatoes can develop a defect called flattening or pressure bruise (Castleberry and Jayanty, 2012). The development of this defect is incompletely understood and might be related to multiple factors including cultivar susceptibility, hydration status of tubers, temperature, and ventilation, in addition to static pressure (Jayanty, 2009; Thornton and Bohl, 1998). While the physiological mechanism of this injury is only partially understood (Storey and Davies, 1992), several factors may play a role including membrane permeability (Herremans et al., 2014), mechanical resistance of a rigid cell wall (Castleberry and Jayanty, 2017; Ralet et al., 2016), cell hydration (Singh et al., 2014), and tissue organization and cellular properties (Gancaerz, 2016; Hudson, 1975; Zafeiri et al., 2021).

Tools to modify perishable physiology to maintain sensory and nutritional quality. Common postharvest treatments include placing fruits or vegetables in a modified atmosphere, the application of hormones or chemicals through sprays or dips, irradiation with ultraviolet light, or exposure to reduced or elevated temperatures (Elmnasser, et al., 2007; Garcia, et al., 1996; Lurie and Pedreschi, 2014; Perez, et al., 1999; Yaun, et al., 2004). The suitability of a particular strategy depends on the product, its condition at harvest and the intended market (Gross et al., 2016). Despite a long history of successes with notable contributions from the NE1836 membership, innovation continues. Technologies for applying modified atmospheres for the storage of some fruits and vegetables continue to evolve. For apple, dynamic controlled atmosphere (DCA) storage, used to apply the lowest possible O_2 levels (Mditshwa et al., 2018), is of particular interest for organic apple production. However, DCA can negatively impact fruit aroma (Aubert et al., 2015), so there is some concern regarding the application of this technology. On the other hand, research in the current multistate project has revealed that DCA storage can markedly reduce internal browning disorders in expanding and emerging apple cultivars (DeEll and Lum, 2017). Modified atmosphere packaging (MAP) with or without an ethylene scrubber has potential to improve shipping mangoes at the tree-ripe stage.

1-Methylcyclopropene is a highly effective inhibitor of ethylene action, originally developed by members of the NE1836 team (Sisler and Blankenship, 1996). Despite the expiration of patents for use and formulation, new commercial delivery systems continue to be developed (Beaudry, 2021), some of which need evaluation for the development of commercial recommendations (da Silva et al., 2023). Testing the efficacy of these technologies and, importantly, recovery from 1-MCP for completion of ripening, is essential for providing up-to-date information for the industry. Moreover, many of the newer apple cultivars have very good post-storage quality maintenance, making the use of 1-MCP less critical. Nevertheless, the determining responses of new apple cultivars and other fruits to 1-MCP is a continuing need.

Other growth affecting chemicals with potential for postharvest applications for strawberry include methyl jasmonate (MJ), abscisic acid (ABA), salicylic acid (SA), and $CaCl_2$. MJ treatment (la Pena Moreno et al., 2010) leads to a rapid decline in antioxidant content and the concentration of individual anthocyanins, providing some promise for improved coloration. Interestingly, MJ treatment can also suppress chilling injury in peach (Jin et al., 2009). The treatment can also accelerate ripening (la Pena Moreno et al., 2010), as does $CaCl_2$ or naphthalene acetic acid when applied independently or in combination (Figueroa et al., 2012). In contrast, Garcia et al. (1996) reported that $CaCl_2$ treatment improved strawberry firmness and total soluble solids. Treatment with ABA (Li et al. 2014) did not help maintain firmness, but increased redness (color factor a*) and higher soluble solids and anthocyanin levels. Salicylic acid can enhance vitamin C concentrations and reduce decay (Salari et al., 2013). Interestingly, benzo-thiadiazole-7-carbothioic acid S-methyl ester, a functional analogue of salicylic acid, enhanced total anthocyanin concentrations up to three-fold (Cao et al., 2010).

UV light treatment has the potential to enhance the red coloration and nutritional quality of apple fruit (Onik, 2019). Red coloration in apple fruits is highly desirable; however, poor coloration occurs with hot and humid conditions combined with the poor light environment. Delayed harvest to obtain color can result in harvest at an overripe stage which harms fruit quality, nutrition, safety, and storability. A focus, therefore, is to increase red skin coloration in a way that allows harvesting fruit at the optimal maturity, thus reducing fruit losses, increasing environmental sustainability, grower profitability and consumer acceptability. UV technology is easy to adapt with low cost of implementation, so we foresee it could potentially become a viable technology for ensuring high quality products while increasing consumer acceptability. There is a need to translate this work to diverse apple cultivars and other crops.

Heat and UV treatment, water stress, controlled/modified air, and application of natural compounds, such as jasmonates, chitosan, salicylic acid can impact antioxidant content of perishables (Gonzalez-Aguilar et al., 2010; Papoutsis and Edelenbos, 2021; Romanazzi et al., 2016a; Romanazzi et al., 2016b; Ruiz-García and Gómez-Plaza, 2013). There is a need for further investigation into the impact of these treatments on enzymatic and/or non-enzymatic antioxidant systems of fresh produce as they contribute the maintenance of fruit quality, antioxidant potential and disease resistance.

Edible coatings present a sustainable supplemental or alternative to standard storage methods for fresh produce and have attracted increasing attention due to environmental considerations and consumption trends toward the use of convenience foods (Dhall, 2013). Edible coatings can modify the internal atmosphere of perishables, inhibit metabolic processes like ripening or softening, but can also reduce water loss. Furthermore, edible films can be bioactive molecules (Dharini et al., 2021). Edible films of protein/polysaccharide coatings are quite attractive because of their impressive gas barrier properties and the fact that they can supplement or otherwise enhance the nutritive value of produce. However, the different fruit species behave differently during postharvest storage and handling. In addition, composite edible coatings such as AKORN that combine beneficial properties of a few components provide desired protective effect to the fruits (Mendes-Oliveira et al., 2022).

Tools for pathogen and decay management. Current disease control heavily relies on synthetic chemical input, but the unprecedented rate of emergence of resistance to fungicides is a serious challenge (Fisher et al. 2018). Numerous tools for decay control other than fungicides have been evaluated, but the adoption of new cultivars and technological options can alter efficacy.

CA treatments have been evaluated by several groups with mixed or sometimes contradictory results. For example, one research team concluded that CO_2 enrichment suppressed non-microbial fruit decay (Gil et al., 1997; Holcroft & Kader, 1999), whereas other groups found that CO_2 treatment increased decay (Alsmairat et al., 2011). Enrichment with O_2 to levels of 60 kPa or more resulted in a reduced rate of decay and increased concentration of phenolics (Zheng, et al., 2007). Further investigation into atmosphere modification for decay control is warranted. Alsmairat et al. (2011) demonstrated that blueberry cultivars differ markedly in their tolerance to fungistatic CA, with some cultivars experiencing debilitating internal browning while others were seemingly unaffected.

UV-C treatment inhibits microbial growth in a dose-dependent manner and is not detrimental to quality traits at low doses (Erkan, et al., 2011; Pombo et al., 2011; Shin, et al., 2012). UV-C can stimulate a surge of PAL activity (Pombo et al., 2011), but flavonoid and anthocyanin concentrations were lower in treated than non-treated strawberries (Li et al., 2014). Work by NE1836 team members has revealed that the safer and less damaging UV-B can be used in place of UV-C. Strawberries showed a slight, but significant increase in total soluble phenolic content at the low UV-B dose (Du et al., 2014*)*.

Thermal treatment to suppress decay is common for mango, but there is opportunity to evaluate its use on other crops. Exposure to hot air at 45 ˚C (Dotto et al., 2011) or immersion in hot water at 45 to 60 ˚C (Garcia, et al., 1995; Jing et al., 2010) can reduce decay on strawberries, but no evaluation of their effect on antioxidant or anthocyanin contents has been reported. Since strawberries are sensitive to cooling delay and delays more than 12 hours are detrimental (Kitazawa et al., 2013), strawberries are cooled shortly after harvest and stored under refrigeration. Therefore, it is not unexpected that there are no reports of chilling treatments, such as those done with cherry tomatoes (Gharezi et al., 2013), being used on strawberries.

The use of biocontrol agents to replace and or augment synthetic fungicides has many challenges, and there are untapped opportunities for the discovery of new biocontrol agents. Several products have been developed for postharvest biocontrol (e.g. BioSave, a formulation of *Pseudomonas syringae*), however optimizing antifungal activity has challenges. Additionally, some essential oils significantly reduce fruit decay of strawberry (Leepipattanawit et al, 1997). For instance, in one trial (Taghavi et al., 2021), nonenal had the lowest fungal decay, while clove bud oil had the highest fungal decay after four weeks of storage. The off-flavor shown in a small taste panel prevents the commercial application of the essential oils as sprays or dips. Eugenol had the lowest effect on the flavor and thymol had the most off-flavor.

Fundamental research on the physiology of perishables. Research on practical tools to preserve the quality of perishables and control pathogens is useful, but is also important to understand the biology of the crop to better apply postharvest interventions and to guide the creation and selection of cultivars with improved quality characteristics.

Significant ongoing research among NE1836 project team members uses integrated approaches to characterize fruit quality-related pathways. The climacteric ‘Santa Rosa’ plum and its unique non-climacteric bud-sport ‘Sweet Miriam’ (Minas et al., 2015) have served as tools for understanding the biology of ripening. Key sugar metabolism-related genes that affect fruit sugar composition were identified through integration of transcriptomics and metabolomics data, and subsequent pathway validation through molecular, biochemical and metabolic analysis. Key genes identified in this work could potentially be used as tools for manipulating these aspects in fruits, with direct effects on fruit sugar composition, nutritional value and postharvest storage capacity. Evidence has emerged of distinct hormone accumulation pathways and interactions occurring in fruits with contrasting ripening behaviors.

Ongoing efforts have identified key fruit texture and flavor-related pathways. In melon, phenotyping platforms have been developed to unravel genetic and metabolic linkages to fruit quality, shelf-life capacity and consumer acceptance. In apple, a novel pathway leading to the formation of branched-chain esters has been discovered and described (Sugimoto et al., 2021). The identified gene exists in two forms. One is functional and one is not. Knowledge of the sequence of this gene will help guide the selection of more highly flavored apple varieties. This work compliments earlier findings demonstrating that one of the primary apple ester odorants, hexyl acetate, is largely derived from the action of a single lipoxygenase (Schiller et al., 2015).

Fruit red skin color development is an economically important trait that is influenced by biological and environmental factors. This pathway interacts or is regulated by sugar and hormones, but how this occurs is unknown. In addition, recent results by team members support the notion that ethylene plays a key role in shifting plum fruit flavonoid profiles, which are also associated with changes in fruit sugars (Minas et al, 2015). These changes can directly affect fruit postharvest capacity, its nutritional value, as well as consumer acceptability.

Current work with small fruits is designed to understand the genetic basis of fruit texture, sugars, and organic acids. In blueberry, identifying potential markers for important quality traits will help to deliver higher quality fruit to the consumer. Recently introduced seedless muscadine storage life and fruit composition have been found to be like that of seeded muscadines.

Fundamental research on the physiology of decay organisms.Fitness costs associated with fungicide resistance impact resistance management, but are yet poorly understood (Hawkins and Fraaije 2018). Traditional methods to study fitness costs mainly involve in vitro assays of isolates with different resistance phenotypes (Chen et al. 2016). However, the major drawback is that isolates typically have a different genetic background, making it impossible to exclude potential impact on fitness from many genetic variations other than specific mutations conferring fungicide resistance. One way to overcome this hurdle is to generate transformants carrying mutation(s) or variations of interest only, without leaving footprints via CRISPR/Cas system. Additionally, genetic mechanisms of fungal virulence and those mediating the quiescent stage and transition into pathogenic lifestyle in the bitter rot fungus are largely unexplored, and a system to evaluate candidate genes using CRISPR/Cas would increase our understanding of infection biology.

Species within the genus of *Colletotrichum* often cause yield losses of fruits and vegetables (Dowling et al. 2020). Work by project team members has identified the molecular basis of fungicide resistance in *C. siamense*, a pathogen of blueberry and strawberry fruit, as being linked to E198A mutation in ß-tubulin gene.

Bitter rot, caused mainly by *Colletotrichum* spp, is a major disease of apple fruit (Kou et al., 2013). Phenotypic, metabolomic and biological investigations of wild apple germplasm with resistance to bitter rot are helping to build genomic resources to study sequence variation, determine sources of resistance, and the movement of specific loci and genes for breeders to improve decay caused by *Colletotrichum fioriniae* (Jurick II et al., 2011; Sun et al., 2017; Janisiewicz et al., 2016).Genes, regulators and genetic networks conferring resistance to bitter rot can also be transferred to commercial apple cultivars via the fast-trac breeding system to make decay management less reliant on synthetic pesticides.

Specific compounds identified in decay resistant wild apple germplasm may either play a direct role in abating decay and or prime host defenses against postharvest pathogens in apple fruit, or both (Sun et al., 2016). Such compounds may also impact biosynthetic pathways of the bitter rot fungi. Complementary to the chemical and genetic work, microbiome and microbial ecology have yielded many new bacterial and yeast strains that can be evaluated to combat decay pathogens.

Objectives

1. Adapt or develop harvest, handling and storage technologies to improve fruit and vegetable quality, increase consumption and reduce food waste.
2. Expand and translate fundamental plant biology to develop new storage technologies and plant materials that will enhance human nutrition and energy-efficient postharvest systems.
3. Advance our fundamental knowledge of host-pathogen-microbe interactions to maintain high-quality fruits and vegetables while reducing food loss and waste.

Methods

Methods

Due to size limitations, some details on methodology have been omitted.

Obj. 1. Adapt or develop harvest, handling and storage technologies to improve fruit and vegetable quality, increase consumption and reduce food waste.

1.1 Prediction and prevention of storage disorders and quality loss in 'Honeycrisp' and other apple varieties (MD, ME, MI, NY, ONT, WA).

Prediction tools for chilling injury will be developed for 'Honeycrisp' (ME) using apples placed in a chest freezer set to -0.5 °C. Chilling injury after 3 weeks will be compared with chilling injury after 4 months cold storage. This test can be used to identify potential failures despite conditioning in fruit from high-risk orchards.

To address CO_2 sensitivity during rapid CA, 'Honeycrisp' apples will be harvested from different orchards and at three maturities to account for differing risk of disorders (USDA-WA). Apples will be left unconditioned or conditioned and stored at varying CO_2 and O_2 concentrations. A small subset to be conditioned under the riskiest conditions for CO_2 sensitivity will be treated with diphenylamine or 1-MCP, which controls or exacerbates, respectively, CO_2-related disorders. After 6 months storage, fruit quality and disorders will be evaluated. Peel and flesh metabolites associated with elevated risk CO_2 sensitivity will be measured periodically during storage.

To accelerate red coloration, 'Honeycrisp' apples will be submitted to different postharvest UV irradiation treatments during a 7-day conditioning period, and transferred to cold storage at 3 ºC, along with dark and white light controls (MD). UV treatment is at a preliminary stage with future plans to collaborate with engineers to develop a method for commercial application. This research is to determine the wavelengths that promote color without affecting quality. Evaluations will include measurements of ethylene concentration, fruit physicochemical properties, gene expression as well as metabolite quantification of color-related pathways. We expect to contribute to the identification of optimal dosage application to enhance fruit coloration, and consequently fruit nutritional value.

We will define harvest maturity that limits greasiness of WA 38 apples by studying year to year variation in fruit maturity (WA). We will also investigate the role of ethylene mitigation in greasiness and off-flavor. Fruit maturity will be determined in apples from two commercial blocks in 3 consecutive years. Peel greasiness will be measured starting 3-4 weeks before commercial harvest. Fruit will be stored in air or CA (2.5% O_2, 1.5% CO_2) for 6 months. Fruit quality will be assessed monthly. Off-flavors will be studied using trained panelists and metabolic profiles.

1.2 Determine the storage potential of new fruit varieties (CO, FL, MD, NC, NY, ONT)

Muscadine grapes will be held in large plastic bins (Janny MT) at 2 to 3 °C and 90% RH (NC). One bin will be sealed to increase CO_2 to 10 kPa and the other will be left at ambient CO_2. Oxygen and CO_2 will be monitored in each bin using a Tiempo sensor. Tubing of 2 lengths will be inserted for sampling at different depths. Two flats of clamshells of fruit will be held in the same cooler to follow weight loss, color change, and development of decay or other storage problems.

The University of Florida blueberry, peach and strawberry breeding program evaluates these for eventual production in Florida. Cooling efficacy will continue to be studied for these crops (FL) and also for mango, mandarin, avocado and passionfruit. Tests will include validation of nontraditional cooling methods that show promise to extend postharvest quality by reducing cooling time and increasing cooling uniformity within the load, whether within a carton or within a pallet. Hydrocooling is an excellent, rapid and cost-effective method for cooling crops; of particular interest is to compare efficacy of drench hydrocooling with immersion hydrocooling. Shower hydrocooling may be more efficient and require less water than immersion hydrocooling, a key factor in drought-impacted states. Pulp temperatures of these crops will be measured before, during and after cooling by several methods including hydrocooling, forced-air cooling and vacuum cooling. Resultant crop quality will be assessed at regular intervals.

Quality and incidence of disorders in new apple cultivars will be compared at harvest and after air or CA/DCA storage to identify optimum maturity, temperatures and atmospheres (ME, MD, NY, ONT). Storage operator interest in DCA has increased greatly because of its potential to reduce internal browning. Cultivars will include those from the Midwest Apple Improvement Association (‘Evercrisp’, ‘Rosalee’, ‘Summerset’, ‘Sweet Zinger’) and breeding programs at CU (‘SnapDragon’, ‘RubyFrost’) and WSU (‘Cosmic Crisp’), as well as ‘Ambrosia’ and ‘Triumph’. Apples will be harvested and stored prior to quality evaluation and assessment of disorder susceptibility. The DCA will include multiple stress indicators, including chlorophyl fluorescence, and increases in ethanol and CO_2 production. Each partner contributing to this sub-objective has access to multiple storage rooms and CA equipment. Teams in respective states/provinces will work together on cultivars of common interest.

Fifteen new and standard peach cultivars will be assessed for maturity and quality destructively and using the non-destructive sensors, DA meter and F-750 (CO, ME). We will develop cultivar specific models that can predict peach fruit maturity (index of absorbance difference, I_{AD}) and internal quality (dry matter content and soluble solids). We will validate our developed models with independent groups of fruit under laboratory and field conditions. We will perform storage potential studies on the effect of maturity stage as determined by I_{AD} and internal quality with focus on fruit softening and chilling injury.

1.3 Development of dynamic controlled atmosphere (DCA) storage for apple (MI, NY, ONT, WA)

Pre-and postharvest practices to optimize organic apple quality during and after long-term storage (MI, ONT, NY, WA). We will focus on ‘Gala’ as well as the new cultivars listed in section 1.2. Our goals are, using the same cultivars in different locations wherever possible, to define the effects of fruit maturity, storage temperatures, the pulldown time of O_2, levels of kPa O_2, and the effects of kPa CO_2. The lower O_2 limit for each variety will be determined in each year to account for year-to-year variation. As above, multiple indicators of product stress will be included to address cultivar high CO_2 stress limits.

We will compare postharvest systems and protocols to optimize quality of apples organically grown in warm and cool production sites in WA state to incorporate climatic variability as an influencing factor (WA). Honeycrisp and Gala apples will be stored in RA, CA and DCA using respiratory quotient based on initial O_2 level at 1°C or 3 °C depending on the cultivar. Dynamic control atmosphere (DCA) system will be managed using respiratory quotient (LabPods, Storage Control System, MI, USA). Fruit quality and physiological disorders will be evaluated after 3, 6, and 9 months of storage. The impact of DCA in combination with 1-MCP on aroma recovery will be documented for Honeycrisp, Gala, Evercrisp, Jonagold, Red Delicious, Fuji, and Golden Delicious (MI). The lower oxygen limit for each variety will be determined.

*1.4 Determine efficacy of edible, water soluble corn zein films for shelf-life extension of fruit (CO)*AKORN coating technology and other commercially available coatings and waxes will be evaluated in the following fruit species and cultivars: Gala and Honeycrisp apples, Bartlett and D’Anjou pears, Santa Rosa and Angeleno plums, May Glo and Honey Blaze nectarines, and Rojo Brillande persimmons. Coatings and waxes will be sprayed on half of the fruit (300 fruit per fruit species per treatment) using a system that has been developed by AKORN Technologies and will be compared to an untreated control. Ripening behavior and postharvest performance will be assessed at receiving and every 2 days for up to 10 days at 20^{o}C following treatment.

1.5. Determining the storage potential of vegetables (CA, FL, MI).

Leafy greens and herbs will be stored under different temperatures and atmospheres to determine the best conditions to extend postharvest life (CA). The impact of chilling temperatures on the sensory quality of leafy herbs will be studied to determine the conditions after harvest that best maintain condition and sensory quality. Cooling applications will be tested for minor vegetables with potential for increased production in Florida, such as Asian vegetables, specialty potatoes, bamboo shoots, broccoli, Brussels sprouts, artichoke, and sweet potatoes (FL). The new in-package delivery methods for 1-MCP also show promise for several vegetables such as summer squash, cucumbers and leafy crops. The ability of CA storage (10 kPa CO_2 and 11 kPa O_2) on asparagus to help manage the glut during the peak production period will be evaluated. The effect of harvest date and cutting or snapping of the spear base will be determined (MI).

1.6. Evaluate the release kinetics of 1-MCP from various 1-MCP delivery systems (MI).

Several new delivery systems for 1-MCP have been developed, but growers and storage operators lack experience and perspective on the best way to deploy them. We will evaluate the rate of 1-MCP emanations from available 1-MCP release technologies using gas chromatography. Release rate will be described as a function of temperature, humidity, solution agitation, and adsorption by non-target materials.

Obj. 2. Expand and translate fundamental plant biology to develop new storage technologies and plant materials that will enhance human nutrition and energy-efficient postharvest systems.

2.1. Mechanism and control of physiological disorders (NS, ONT, MI and NY)

Identify mechanisms involved in disorders by applying 'omic' studies (volatiles, metabolomics, quantitative proteomics including peptide dimethyl labeling and multiple reaction monitoring with LC/MS) for profiling features regulating disorders. 'Honeycrisp' apples will be harvested from multiple commercial orchards and stored under CA conditions (2.0 kPa O_2, 1.0 kPa CO_2) at 1°C and 3.5 °C. After 0, 3, and 7 months of storage plus 1 week at 20 °C, firmness, soluble solids, titratable acidity and soft scald will be evaluated. At each evaluation, peels and flesh from 12 apples will be sectioned, pooled, frozen in liquid N_2 and stored at -86 °C for further analysis. Volatile compounds in whole or homogenized fruit tissue will be collected from headspace by extraction with polymer traps or by using solid phase micro extraction (SPME) and analyzed by 2D-GC-MS. Untargeted (global) metabolomics will be applied for the comprehensive comparative analysis of metabolites. Protein extraction and quantification will be performed using a modified phenol extraction followed by ammonium acetate-methanol precipitation with detailed procedure for protein digestion and peptide desalting. The resulting peptides of total tryptic digestion from different biological samples will be labeled with isotopomeric dimethyl labels. Peptides analysis will be conducted on a mass spectrometer (Waters, q-TOF, Synapt, Milford, MA) equipped with a nano-electrospray source and nano-lock spray interface. Chromatographic separations will be conducted using a nano-Acquity UPLC system (Waters, Milford, MA).

2.2 Establish genetic maps of apple aroma, flavor volatile expression and bioactive compounds for improvement of fruit attributes and human nutrition (NS)

Fruit samples from an association mapping population (i.e., ABC, the biodiversity collection) will be harvested over two years at commercial harvest. Fruit samples will be stored at 3-4 °C. Texture, aroma volatiles, sensory attributes and bioactive compounds will be evaluated after 1 month. Metabolites involved with flavor volatiles will be measured using 2D-GC-MS. Volatile compounds in whole or homogenized fruit tissue will be collected from headspace by extraction with polymer traps or by using solid phase micro extraction (SPME) and analyzed by 2D-GC-MS. Untargeted (global) metabolomics that uses a Synapt qTOF (Waters Corp) liquid chromatograph-high resolution LC-MS will be applied for the comprehensive comparative analysis of metabolites. Genome-wide genotype data are available for the association mapping populations as part of ongoing research with Dr. Myles' lab. Data collected from storage experiments to phenotype fruit attributes at Kentville will be analyzed with genome-wide genotype data. All phenotyping data collected will be linked with genotyping data and possible genetic control mechanisms will be identified through linkage mapping or GWAS. Identifications and quantitation from proteomic, genomic and metabolic analysis will be combined to reveal further possible biochemical pathways and validate key regulatory mechanisms of flavor, texture and bioactive compound metabolism.

*2.3. Control of potato bruise symptoms after unloading from bulk storage conditions (ID).*Due to the inevitable contact with oxygen after potatoes are unloaded from bulk storage, treatment with 1-MCP and nitric oxide (NO) may minimize the blackening of pressure-flattened tissues. 'Russet Burbank' potatoes will be harvested and submitted to two conditions:1) without pile pressure – 0 lb ft^2 (control) and 2) constant force of ~375 lb ft^2. Tubers will be stored at 7°C and 95% RH for up to 8 months. Five days before unloading, potatoes will be treated with 1-MCP for 48 hours with 1 mMol/L NO for 5 hours. Unloaded potatoes will be air-dried and transferred to ambient conditions to allow pressure bruise browning to develop. Samples will be taken 0, 2, 4, and 6 days after unloading when bruises will be evaluated using a visual scale. Electrolyte leakage will be assessed, and samples will be taken for PPO and POD activity determination.

*2.4. Tracking senescence/freshness by monitoring changes in gene expression in broccoli and lettuce (FL and KS).*We will focus on (1) using RNA-seq to identify senescence-associated genes (SAGs) for broccoli and lettuce that are associated with physiological age and (2) determining which of those SAGs are most highly conserved while also being expressed only at discrete stages of senescence. Similarities and differences in gene expression between natural (on-plant) and postharvest senescence will be compared to develop a better understanding of both processes, particularly the possible role of abiotic stress in postharvest senescence. A goal is to develop a quick test device utilizing colorimetric quantitative loop-mediated isothermal amplification (qLAMP) to measure product physiological age based on the presence of diagnostic freshness indicator genes.

2.5. Predictors of chilling injury development during peach cold storage (MD). We will characterize differences in fruit ripening patterns, physicochemical properties, chilling injury incidence, and aroma volatile composition between sound fruit and fruit exposed to CI-inducing conditions. 'Red Haven' peaches stored at 0, 5 and 20°C, will be analyzed for the traits listed above after 1 to 30 days of storage and after 3 days at 20°C. Overall, our results contribute to the identification of potential key aroma volatile compounds that could be used as early predictors of chilling injury. Also, we plan to identify similar volatile markers in other susceptible fruit crops.

*2.6. Changes climacteric and non-climacteric plums and other fruits during postharvest storage (MD).*We will study the impact of changes in ethylene and sugars in flavonoid metabolism-related pathways of the climacteric and non-climacteric plums and other fruits. Fruits subjected to ethylene treatments will be examined for transcript profiles of structural and regulatory genes of flavonoid-related pathways and their associated metabolites in skin and flesh, integrated with multivariate analyses of ethylene and sugar metabolism.

*2.7 Characterization of aroma forming pathways in fruit (MI).*The contribution of the citramalate pathway in the formation of esters in apple will be explored using highly specific inhibitors of pathways related to branched-chain amino acid metabolism. Whole fruit will be treated with sulfonyl urea and the volatile profile determined using GC/MS. Tissue disks from treated and untreated fruit will be fed with ^{13}C-acetate and the incorporation of label into pathway constituents (alpha keto acids, amino acids, alcohols, aldehydes, and esters) determined. The impact of the suppression of the branched-chain esters on sensory perception will be assessed using a duo-trio test. The same pathway will be evaluated in banana and melon using molecular and biochemical approaches to characterize pathway enzymes. Initial work suggests a transcriptional modification leads to the synthesis of proteins lacking feedback regulation in a manner reminiscent of the citramalate pathway. We will explore the possibility that the ripening-dependent creation of a 'non-regulated' pathway is a common mechanism to enable precursor synthesis in ripening fruits and vegetables.

Obj. 3. Advance our fundamental knowledge of host-pathogen-microbe interactions to maintain high-quality fruits and vegetables while reducing food loss and waste.

3.1. Systems-based discovery of genetic resistance mechanisms in fruit and vegetables and fungal virulence genes(USDA-MD)

Specific apple accessions with resistance to bitter rot will be sequenced, assembled, annotated, deposited and made publicly available in the NCBI database. Our sequence data can be placed online for public access, and the publications, pipelines, and gene annotation programs used to develop these resources will also be available with open access.

We propose to use high-throughput Illumina platform sequencing to obtain whole genome sequence data for each of the 452 accessions that comprise 4 unique accessions with resistance. This will help identify causal SNPs and INDELs that coincide with a given phenotype.

High-quality wild apple (PI 369855 and GMAL 3689.h) and 4*Colletotricum fioriniae* genomes will be obtained, using a combination of short- (Illumina) and long-read (Nanopore or PacBio HiFi) sequencing technologies combined with high-throughput chromatin conformation capture sequencing (Hi-C), to achieve sufficient coverage and genomic organization to assemble near gapless host and pathogen genomes. After sequence data is obtained, raw reads will be assessed for quality and downstream applications (e.g. assembly and annotation) using a combination of online, command line, and bioinformatics tools (e.g. Geneious). We will use established pipelines to determine transposon number, type and gene location, AntiSMash to determine SM gene cluster identity and type CAZyme, TF, etc. and SNP calling between fungal isolates.

Once high-quality *C. fiorinae* and wild apple genomes are sequenced, assembled and annotated, the next step will be to obtain fruit from susceptible commercial cultivars and wild apples with immunity to bitter rot. Five individual fruits will be inoculated. This experiment will help elucidate the genetics modulating wound responses and will also help ascertain signaling networks in the fruit controlling induced antimicrobial compounds, resistance genes in the host and virulence factor genes in the fungus. Total RNA will be isolated using standard procedures to assess total RNA quality and quantity of the samples. Total RNA will be sent to a company for library construction, sequencing and QC analysis of output data. Transcriptome sequence data will be assembled using a genome-guided mapping approach coupled with open-source R-based differential gene expression analysis pipelines. We will derive the list of differentially expressed genes (DEGs) for groups 3 and 4 which represent the transcriptomic changes associated with defense against the target pathogens at 24 and 96 hpi, respectively.

3.2. Develop and optimize chemical and biotechnological tools to abate decay and maintain fruit and vegetable quality (MD, USDA-MD)

Based on previous work from Sun et al., (2016), we will test the following specific purified compounds (epi-catechin, chlorogenic acid, procyanidin B1, B2, C1, querticin-3-O-glucoside, quinic acid, phloridizin, and Phloretin-xylosyl-glucoside) at physiological concentrations found in PI 369855 wild apple fruit *in vitro* Petri plate assays. Additionally, genes encoding proteins involved in the production of specific biochemical compounds and intermediates, identified from our wild apple fruit inoculated with the bitter rot fungus from our dual RNAseq analysis, will also be examined. These purified compounds will be tested *in vitro* using conidial germination and mycelial growth assays to assess their impact on *C. fiorinae* growth. Concentrations to inhibit 50% (EC50) growth will be determined.

Purified compounds with demonstrated *in vitro* efficacy will be evaluated using assays with susceptible varieties (Honeycrisp, Gala, Fuji) to test their ability to control decay caused by bitter rot. Finally, any compound or combination that shows >90% inhibition of decay, will be added to Shellac based wax or other industry utilized coating (e.g. Shieldbright 40), and the above tests will be conducted to see if the compound can be applied with wax to reduce decay. Tests with each compound as dip treatments will also be conducted to mimic drench treatments. To ensure that the decay control findings are practical and potentially impactful, inoculated fruit will also be stored at 1 °C in regular atmosphere over a 6-month period and monitored monthly for decay with balanced number of untreated fruit as a control group. Since we do not have CA or DCA storage at Beltsville MD, we will replicate this study using DCA and or CA (as available) with other collaborators in the project to increase the rigor and variety of commercial environments.

In another experiment, we will screen for potential bacterial isolates and natural compounds with high antagonistic activity against a set of postharvest pathogens. A commercial biofungicide Cease, *Bacillus subtilis*, will be the positive control against diseases (such as *Colletotrichum acutatum and Alternaria alternata*). About 70 bacterial isolates and several natural compounds will be screened in a dual culture system *in vitro* (Lahlali et al 2020).

We will study physical and chemical treatments that trigger plant defense responses through signal molecules. We propose to generate a marker-free CRISPR/Cas9 system to create resistant *Colletotrichum* spp. mutants with the point mutation E198A in ß-tubulin gene and other targets that mediate fungal virulence as determined in dual RNAseq studies proposed in the previous sub-objective of this proposal.

Fitness tests and assessment of fungal virulence: At least five resistant transformants will be randomly selected and tested for their resistance stability in vitro as described previously (Hu et al. 2014). Fitness components including mycelial growth at different temperatures in vitro, osmotic sensitivity in vitro, oxidative sensitivity in vitro, and aggressiveness/spore production in vivo will also be determined.

3.3. Evaluate newly developed tools for their impacts on postharvest shelf life, sensory aspects, and customer acceptability. VA

Newly discovered compounds, coatings, essential oils, microbial consortia and/or treatments that can thwart postharvest rot will be tested for their impact on sensory and consumer acceptability using trained panels and consumer panels. Treatment effects on apple fruit color, starch, sugar level, titratable acidity, pH, firmness will be conducted with standard fruit varieties (Honeycrisp, Gala, Fuji).

We will test plant volatiles (essential oils) for their efficacy in *ex vitro*, and *in vitro* conditions for decay control in strawberry. Fruit quality parameters (weight loss, total soluble solids, titratable acidity, and fruit decay) will be assessed. We are also looking for ways to reduce unwanted side-effects (off-flavor) and increase the efficacy of antifungal properties of natural compounds.

For both experiments, commercially used antifungal materials will be used as a control.

Measurement of Progress and Results

Outputs

- Basic understanding of the action of 1-MCP and NO on the potato pressure bruise development after unloading from bulk storage.
- Storage recommendations for new fruit cultivars that maintain high quality through storage and marketing.
- Recommendations for optimizing environmental conditions that favor storage of fresh vegetables and herbs
- Nonchemical methods for long-term storage and quality preservation of organic and conventional apples.
- Predictive tools for identifying fruit and vegetable storage problems at harvest.
- Twenty published research papers

Outcomes or Projected Impacts

- Extended shelf life of fresh fruits and vegetables and reduced waste, improved postharvest practices and increased profitability along the supply chain.
- Greater availability of high quality fruits and vegetables for consumers.

Milestones

Outreach Plan

Results of this research will be made available through publications in refereed journals, grower and trade magazines, conference papers and proceedings, project reports, on-line sources (web), and presentations to industry. Several participants have partial extension appointments and develop outreach materials through fact sheets, web-based resources (e.g. https://www.uidaho.edu/extension/publications) and other extension publications. Extension publications include the NY Fruit Quarterly, the Good Fruit Grower, Spudman and the Potato Grower. In addition, states such as FL, MI, NY and WA have extensive formal industry venues for presentation of results to growers. These include storage workshops in MI and NY, which are held every two years, the New England Vegetable and Fruit Growers Conference, The NY Fruit and Vegetable Expo, Carolinas Fruit and Vegetable Expos, the NC Winter vegetable meeting, the SE Fruit and Vegetable Expo, Florida AgExpo, Annual Hood River Winter Horticultural Meeting, Pear Packers Pre-harvest meeting, Great Lakes Horticulture meeting, the Idaho Potato Conference (bilingual) and other fruit schools. Several project participants attend and are invited to present their results at grower meetings in other states and Canadian provinces. Overall, the project members have established excellent performance in ensuring that research results to colleagues in North America and to fruit growers and storage operators are made available in a timely manner.

Organization/Governance

One person at each participating agency is designated, with approval of the agency director, as a voting member of the Technical Committee. Other persons at agencies are encouraged to participate as non-voting members. The Chair, Secretary, and Administrative Advisor will conduct the activities of the multistate project between annual meetings. Officers can be any member, including the official voting representatives. The officers are elected every second year by voting members and serve a two-year term.

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Land Grant Participating States/Institutions

ME,NJ

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Moran, Renae	Yes	Maine - University of Maine	1	205 205	1110 1114	1020 1020	1.00	0.00	0.00	0	0

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
205-1110-1020	0.5	0	0
205-1114-1020	0.5	0	0
203-1122-1020	0.13	0	0
203-1123-1020	0.13	0	0
205-1122-1020	0.13	0	0
205-1123-1020	0.13	0	0
Grand Total:	**1.50**	**0.00**	**0.00**

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

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2336: Improving Quality and Reducing Losses in Specialty Fruit and Vegetable Crops through Storage Technologies

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
A key strength to this programme is the geographical spread of the participants. This diversity is essential for accelerating knowledge on the responses of crops grown in different environments. This knowledge will grow in importance as future food supply chains adapt to a volatile climate. Many of the researchers in this programme are world-leaders in the field, ensuring knowledge from this programme will also provide international as well as national impact. It is also pleasing to read that the programme will genuinely have a multi-crop focus, this is critical for increasing the likelihood of a scientific breakthrough that benefits all crops, rather than one. I would consider the proposed programme to be world-leading in this field.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2336: Improving Quality and Reducing Losses in Specialty Fruit and Vegetable Crops through Storage Technologies

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:
Good
5. Overall technical merit:
Excellent
Comments
This is an ambitious plan but the experience and capacity of the Project team are world-class and will achieve the stated objectives. The inherent value of this proposal is the multi-state (multi-growing region) approach which is important and should be applied in most trials where possible. In addition, it would be recommended to also compare season to season variations with the multi-state work. This is a lot of work, but it is essential to capture the variability required to make valid commercial recommendations for growers across seasons in different regions.
The diversion from the major apple focus is welcome. While apples are an important for many regional commodities, other fruit and vegetables are also very important for diverse regional communities and American consumer health.
It will be important to have strong communication and planning meetings between the state partners to ensure consistent methods and comparisons of results.
Some specific comments –
Methods 1.1 How will postharvest UV treatment be applied on apple fruit (ie round-shape) in a commercial bin?
Methods 1.2 There will be gradients of CO2 within the Janny bin. Who will these be monitored and accounted for? Are there any other sensors available to test maturity and quality?
Method 1.3 Comparison of the different seasons will be important to test the lower oxygen limits for the different varieties.
Method 1.4 Consider comparing the commercial standard wax with the new coating technology. It is well known that coating and waxes work. It will be important to compare the new technology with existing commercial treatments, and untreated controls.

Overall this is a great program of exciting work with a high degree of academic and commercial success. I thoroughly endorse this proposal.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2336: Improving Quality and Reducing Losses in Specialty Fruit and Vegetable Crops through Storage Technologies

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
The proposal is outstanding and well put together covering fundamental to applied science. The assembled team is globally recognised for its postharvest research and impact. The focus on the preharvest postharvest continuum is welcomed since there is a paucity of research in this area. Indeed the team rightly sets out to build on previous work but takes a new approach to implementing ultra cold storage and DCA, for example. Consideration should be given to the recent increased energy costs - this impacting feasibility of long term cold storage. However, the work seeks to centre research on adapting postharevst storage regimes to new cultivars - again this is timely and very much needed and has been overlooked in the recent past. Creating cultivar specific postharvest treatments may open up new higher value markets for the NE region. The proposed pathology work plan is welcomed. Outreach and governance are standard and well thought through.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP2336: Improving Quality and Reducing Losses in Specialty Fruit and Vegetable Crops through Storage Technologies

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
Hello,
The research topics are well described in proposal- NE1836: Improving Quality and Reducing Losses in Specialty Fruit Crops through Storage Technologies is directed to a global concern regard food losses and food security. This research program suggested to reduce and to prevent quality deteriorations of many different fruit types that are important to the different growing regions. The questions are good and targets the challenges using appropriate and innovative technological approaches. The research plan copes well with each of the challenges presented in the proposal. This proposal offers a good combination of applied and basic research that will improve the understanding of basic biological processes and may provide future application of the findings to additional agricultural products.

I would like to suggest a few recommendations you might consider:
• In the objective that points to adapt or develop harvest, handling and storage technologies to improve fruit quality, increase consumption and reduce food waste, there are experiments that aim to develop dynamic controlled atmosphere storage (DCA) technology for apples. This method determines the minimal oxygen levels for storage based on chlorophyll florescence. However, I would like to suggest to determine also carbon dioxide levels for this new technology and to avoid the damage of high carbon dioxide concentration during storage.
• In the objective to improve our understanding of the biology of fruit quality to further our development of harvest and storage technology and development of new plant materials, it is important to analyze the 'omics' of fruit treated with 1-MCP. This common commercial practice affects fruit physiology and postharvest disorders and losses (I am quite sure that the researchers included that in this research plan).
• In the experiments to prevent decay, it is recommended to include the common commercial practice as an additional control treatment (in addition to the untreated control fruit).

To conclude, proposal NE1836 has all the key elements to be successful. There is high possibility of success for this project as the research teams have extensive scientific and professional experience and in the past have succeeded with similar challenges.
Good Luck!
Your Recommendation:
Approve/continue project

Response to reviewers of the NE1836 proposal

We have read through the suggestions and have amended the proposal according to their suggestions. Below are specific responses to each suggestion. These changes to the methods are indicated in the revised proposal which was sent as an attached document. The revisions have also been uploaded to NIMSS.

Methods 1.1 How will postharvest UV treatment be applied on apple fruit (ie round-shape) in a commercial bin?

This will be addressed during the proposed phase of this project. The Univ. of MD plans to work with engineers to scale up UV-treatments. This is described in Objective 1.1.

Methods 1.2 There will be gradients of CO2 within the Janny bin. Who will these be monitored and accounted for? Are there any other sensors available to test maturity and quality?

Details on the monitoring of CO2 in the Janny bins has been included.

There are other more traditional sensors to measure maturity and quality, but were not described due to size limitations of the proposal.

Method 1.3 Comparison of the different seasons will be important to test the lower oxygen limits for the different varieties.

Year to year variation is expected, and will be accounted for as time allows.

Method 1.4 Consider comparing the commercial standard wax with the new coating technology. It is well known that coating and waxes work. It will be important to compare the new technology with existing commercial treatments, and untreated controls.

This was stated in the proposal, but the specifics of the controls were not mentioned due to size limitations.

Indeed the team rightly sets out to build on previous work but takes a new approach to implementing ultra cold storage and DCA, for example. Consideration should be given to the recent increased energy costs - this impacting feasibility of long term cold storage.

This is a good suggestion, but ultra cold storage does not appear in the proposal. Perhaps, they meant ultra low oxygen in Objective 1.3. DCA will be tested, and economic aspects of its implementation can be included. However, the team does not include an economist. Including an objective that addresses economics is something to consider in the future.

• In the objective that points to adapt or develop harvest, handling and storage technologies to improve fruit quality, increase consumption and reduce food waste, there are experiments that aim to develop dynamic controlled atmosphere storage (DCA) technology for apples. This method determines the minimal oxygen levels for storage based on chlorophyll florescence. However, I would like to suggest to determine also carbon dioxide levels for this new technology and to avoid the damage of high carbon dioxide concentration during storage.

This aspect of fruit stress is critical to successful storage of 'Honeycrisp' and will be included where the instrumentation is available. See amendments in the proposal under Objective 1.3.

• *In the objective to improve our understanding of the biology of fruit quality to further our development of harvest and storage technology and development of new plant materials, it is important to analyze the 'omics' of fruit treated with 1-MCP. This common commercial practice affects fruit physiology and postharvest disorders and losses (I am quite sure that the researchers included that in this research plan).*

'omics' analysis of apples has been performed previously by the USDA-WA lab. The plan for this may have been omitted due to the size constraints of the proposal, but the measurement of metabolites by this same lab is part of the plan under Objective 1.1.

• *In the experiments to prevent decay, it is recommended to include the common commercial practice as an additional control treatment (in addition to the untreated control fruit).*

This was part of the plan and is now mentioned in the proposal under Objective 3.3.

Angelos Deltsidis, Univ. of Georgia
Carolina Torres, Washington State Univ.
Chris Watkins, Cornell Univ.
Eleni Pliakoni, Kansas State Univ.
Elizabeth Mitcham, Univ. of California Davis
Gustavo de Almeida Teixeira, Univ. of Idaho
Jeff Brecht, Univ. of Florida
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NE_TEMP9: Conservation and Utilization of Plant Genetic Resources

Status: Submitted As Final

Duration	10/01/2023 to 09/30/2028
Admin Advisors:	[Olga I. Padilla-Zakour]
NIFA Reps:	

Statement of Issues and Justification

The Need: Agriculture in the United States contributes to both national and global food security, and supports many diverse industries (e.g., food, ornamental, textile, medicine). Sustainability and diversification of agricultural industries depend on the development of genetically enhanced cultivars to combat emerging pests and diseases, climate and environmental changes, and shifting consumer demands. Germplasm, or genetic resources (sources of genetic diversity), provides the foundation for crop improvement. However, diverse genetic resources are at risk due to reduced diversity in large-scale cultivation, changes in environmental conditions, degradation of native habitats, and international inaccessibility. These resources are difficult or can even be impossible to reconstitute if lost. This is especially true for fruit genetic resources, where preservation clonal identity is paramount.

The mission of the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Plant Germplasm System (NPGS) is to acquire, safeguard, document, and distribute plant germplasm, which is accomplished through a cooperative effort with State, Federal, and non-profit partners. NPGS serves research, breeding, and higher education as a public source of plant genetic diversity. The composition of NPGS collections includes landraces, older commercial cultivars, pre-breeding, elite breeding material, and crop wild relatives. As of 2022, more than 16,000 plant species in the form of more than 605,000 accessions were actively held by NPGS. An average of 296,488 samples per year were distributed during the five-year period 2018 – 2022; typically, 70% of these were domestic and 30% were foreign distributions.

The NPGS Plant Genetic Resources Unit (PGRU) located on the campus of Cornell AgriTech at the New York State Agricultural Experiment Station (NYSAES) in Geneva, NY is comprised of the Northeast Regional PI Station for seed crop collections, the National Clonal Germplasm Repository-Geneva, as well as the Apple Rootstock Breeding Program. In 2021, PGRU initiated the new NPGS Hemp (*Cannabis sativa*) collection. Major collections conserved are tomato, onion, celery, winter squash, brassica vegetable crops, radish, hemp, apple, cold-hardy grape, and tart cherry, including crop wild relatives. The Seed, Hemp, and Clonal repositories hold 12,723, 301, and 6,504 active NPGS accessions, respectively.

Safeguarding these genetic resources is critical to meeting future stakeholder demands, including states in the Northeast US, where many of these crops are principal sources of economic activity and potential commercial growth. The Northeast Regional Multistate Research Project, NE9, brings together representatives from 12 states (CT, DE, ME, MD, MA, NH, NJ, NY, PA, RI, VT, WV) and Washington DC to address mutual interests in plant breeding, research, and extension/education. Members of the Regional Technical Advisory Committee (RTAC) represent state universities, State Agricultural Experiment Stations (SAESs), and the USDA-ARS; the duty of Project Administrative Advisor is assigned to the director of Cornell AgriTech at the New York SAES. Breeding, research, and extension within the NE9 region are supported and strengthened by services and activities performed by PGRU and NPGS. Funding from the NE9 Project has been critical for the realization and sustainability of PGRU germplasm activities. PGRU Seeds and Clonal germplasm projects rely heavily on collaborations for evaluation trials and cultivar development, which are largely beyond the scope of NPGS.

Proposed Objectives: Objectives of this project are directed towards providing the required germplasm to assure stable and sustainable production of vegetable, hemp, and fruit crops in the Northeast USA and worldwide:

1. Efficiently and effectively acquire and maintain the safety, genetic integrity, health, and viability of priority genetic resources, and distribute them and their associated information worldwide.
2. Develop more effective germplasm maintenance, evaluation, and characterization methods and apply them to priority genetic resources. Record and disseminate evaluation and characterization data via the Germplasm Resources Information Network (GRIN-Global) and other data sources.
3. With other NPGS gene banks and Crop Germplasm Committees (CGCs) develop, update, document, and implement best management practices and Crop Vulnerability Statements (CVSs) for priority vegetable, hemp, and fruit genetic resources and information management.

4. Actively engage in and support the development of novel priority vegetable, hemp, and fruit germplasm that integrates diverse, useful genes from various resources and breed, release, maintain, and evaluate improved and regulatory compliant germplasm and cultivars. Devise and apply research tools, knowledge of genetics, and of the genetic control of priority traits to broaden the diversity available for agricultural production systems. The role of the PGRU staff in the development of novel priority germplasm will vary across different crops and projects, and can range from providing germplasm resources to projects, advising project planning and implementation, to direct action on data collection and analysis.

Note: Objectives 3 and 4 require collaboration. Developing strong collaborative relationships among PGRU staff and reliable and productive cooperators are viewed as part of these objectives.

Importance of the Work: The vegetable genetic resources, which includes tomato, onion, brassica, winter squash, celery, and asparagus, managed by this project represent approximately 36% of the combined dollar value of fresh and processing vegetables in the USA. The average production annual value from 2016-2021 in the US for PGRUs major vegetable collections was $5.145 billion (Appendix A, Table 1). During the past five years, PGRU maintained more than 12,600 accessions of tomato, onion, radish, winter squash, cabbage, cauliflower, broccoli, other brassica crops, celery, tomatillo, asparagus, other vegetables, and buckwheat, representing 29 genera, 151 species and 1,999 taxa. Approximately 150 – 200 seed crop accessions were regenerated per year to replenish stocks. In addition, 285 distinct inventories were acquired since 2017.

In 2021, the hemp germplasm repository achieved all federal and state compliance to initiate germplasm acquisition, characterization, evaluation, and distribution of regulatory compliant genetic stocks. This collection has grown rapidly to include over 300 accessions and is now the largest international public hemp germplasm collection. The collection contains feral, fiber, grain/oilseed, secondary metabolite, landrace, breeding lines, and other classes of diverse germplasm. Distribution of regulatory compliant materials began in 2022. Work is planned for 2023 to genotype the entire collection and to conduct population structure analysis. These genotyping efforts will guide collection and conservation priorities, development of mapping populations, and provide higher stakeholder utility as inputs into breeding programs.

PGRU maintains 4,940 accessions of *Malus*, 1,415 accessions of *Vitis*, and 149 accessions of tart cherries. Through introductions, explorations, and exchanges, PGRU acquired 104 *Malus*, 1 *Vitis*, and 13 *Prunus* accessions since 2017. The fruit crops maintained by PGRU account for about 49% of the value of US fruit and vine crop production (Appendix A, Table 1). Apples, grapes, and tart cherries contribute significantly to the US economy, with average annual values of $3.129, $5.908, and $0.062 billion from 2016-2021, respectively (Appendix A, Table 1).

Historically, the NE9 Project has made substantial contributions to the vegetable and fruit industries through distribution of germplasm and associated information for developing improved varieties with higher and more stable yield, disease and insect resistance, and improved quality. From 2017 – 2022, PGRU distributed 52,612 seed lots (47% domestic and 53% foreign) comprising 11,155 unique seed accessions. PGRU distributed 24,399 clonal crop samples (97% domestic and 3% foreign) comprising 3,790 unique accessions. In NE9 states, there were 4,930 seed samples from 3,788 accessions distributed and 8,778 clonal samples from 2,8236 unique accessions distributed (Appendix A, Tables 2-7). Close to 40% of distribution of PGRU fruit germplasm is directed to NE9 states. The collections have been extensively used worldwide to develop new cultivars and for other research purposes, such as genetic analysis of disease resistance, fruit quality, genetic diversity, and population structure. PGRU scientists characterize germplasm for priority traits to make the material more readily accessible. Much of this characterization and evaluation is performed in collaboration with scientists from the NE9 region and other regions in the USA and abroad. Research into quality and health-beneficial traits was initiated at the request of partners in various CGCs and has become increasingly emphasized.

Technical Feasibility and Value of a Multi-state Project:

: Acquisition, conservation, and characterization of germplasm collections are more efficient at a central location than through individual state organizations, which would result in unnecessary duplication of efforts. A cooperative approach among state partners and the PGRU allows for an efficient conservation of vegetable, hemp, and fruit germplasm while plant breeders and other researchers can take the lead in characterization and evaluation, especially for quantitative traits that require replicated field trials. Utilization of germplasm for crop improvement by geneticists and breeders at individual SAESs capitalizes on the genetic resources and the characterization/evaluation information maintained by the NPGS.

PGRU is primarily supported by appropriated funds authorized by Congress, which provides long term stability for performing basic genebank activities. It is located within a vibrant agricultural region at Cornell AgriTech on the NYSAES campus and is well suited to take maximum advantage of additional multi-state funds from the NE9 project for conservation and characterization/evaluation of vegetable, hemp, and fruit germplasm of important crops to the Northeast region.

Funding from NE9 provides critical resources for better management of the collections and quality service of germplasm distribution. It also supports major efforts in supplying germplasm to screen for high-priority traits, such as important disease and pest resistances and traits important to human health, much of which is done in collaboration with scientists from SAESs. The budget allocated to NE9 over the last 5 years was kept constant, even though salaries and expenses kept increasing. To retain skilled staff and maintain our current operational levels with the addition of a high-value crop, a 7% increase in the budget is requested, matching the 7% increase the Northeast region received during the last 5 years for Hatch and Multistate federal allocations. The budget will stay constant for the 5-year project. This increase will allow NE9 to maintain resources for a growing, valuable genetic resource.

Impact: Genetic resources in the PGRU repository will continue to prove useful in developing improved cultivars of vegetable, hemp, and clonal crops while supporting and stabilizing agricultural production. Expansion of global trade and complex food systems, increases the risk of introduction of exotic pests and diseases to the United States. Agricultural research in the United States has intensified efforts to improve the sustainability of national food production while reducing its deleterious impacts. For example, crops with genetic resistance to pests and diseases reduces dependency on pesticides and preventative chemical sprays and reduces risks for agricultural workers and impact on the environment. In response to changing environmental conditions, PGRU collections will be used as sources of resistance to environmental stresses to increase the range of adaptation of crops. The Northeastern United States is particularly well suited to embrace emerging agricultural opportunities, to which PGRU can provide well documented germplasm for our growing conditions.

Finally, maximizing the use of available germplasm at PGRU will help domestic producers thrive in a competitive global marketplace. For example, within the current NE9 project, PGRU has provided accessions of tomatillo and cabbage as sources of natural pigments for breeding programs aimed at emerging markets; screened the tomato collection for biotic and abiotic resistances; identified a wild tomato accession used for late blight resistance in cultivated tomatoes. In 2021-2022 PGRU has assembled the world's largest public hemp germplasm collection, collected approximately 100,000 data points for priority horticultural and agronomic traits, and these materials have been used to develop five long-read whole genome sequences to be deposited in the National Center for Biotechnology Information. The hemp germplasm collection has been used to develop fiber quality standards that will underpin an emerging domestic fiber industry. Apple germplasm has been utilized to develop new rootstocks with multiple disease resistances and improved plant architecture. Both apple and grape accessions have been screened for biotic and abiotic resistances, leading to new scion cultivars being developed in large, multi-state projects.

Germplasm from PGRU has proven useful in developing improved cultivars of vegetable and fruit in the Northeast region, the USA, and the world:

- Genes from wild tomatoes have been exploited to increase ease of harvesting, disease resistance, and for stress and drought tolerance.
- More than 20 genes from the PGRU tomato collection for bacterial speck, spotted wilt virus, tobacco mosaic virus, leaf mold, fusarium wilt, verticillium wilt, late blight, and nematode resistance have been bred into modern varieties.
- Phylloxera resistant grape rootstocks and hybrids derived from North American wild *Vitis* germplasm were instrumental in rescuing the European grape and wine industry.
- The recent spread of grape cultivation throughout the USA, especially in the northeast, has been made possible by use of the germplasm collection for breeding of new cultivars of *Vitis vinifera* and *Vitis vinifera – Vitis* species hybrids that are adapted to environments where *vinifera* could not previously be grown.
- Genetic resources for resistance to apple scab, fire blight, and wooly apple aphids maintained in the germplasm collection have been deployed in disease resistant apple rootstocks and cultivars. Insect and disease resistant apple cultivars can be traced back to the PGRU apple collection.
- PGRU apple germplasm, especially historic cultivars, has been used directly by growers to expand the US apple cider industry.

Germplasm maintained at PGRU is currently or will be used for crop improvement of fruits, hemp, and vegetables:

- Tomato accession are being tested for resistance to race 1 strains of Rs5 bacteria, ToBrFV virus, salt, and drought tolerance in growth chamber facilities. These phenotypic characterizations of the PGRU tomato germplasm collection will identify genomic regions controlling pathogen and stress tolerances and add significant value to our long-term breeding effort and cultivar development.
- Cucurbit accessions have been evaluated for resistance to the oomycete pathogen Phytophthora capsica, which result in outbreaks that are challenging to manage and can result in huge yield loss.
- Specialty cucurbit accessions from PGRU and other NPGS collections will be screened for powdery and downy mildew resistance, and critical agronomic traits as novel specialty crops to serve historically underserved communities in the Northeast.
- Hemp germplasm from PGRU has been used to create five of the highest-quality cannabis sativa reference genomes and will likely contribute to the development of a cannabis pan-genome.
- PGRU's hemp collection has been screened for plant architectural traits, fiber and grain agronomic characteristics, and secondary plant metabolite composition and profile. This data will enhance subsequent curation, distribution, and evaluation efforts.
- PGRU's vegetable, hemp, and fruit germplasm collections are being screened for medicinal and nutraceutical properties for development of cultivars that will improve the health benefits of consumption.
- Genomic resources developed for major apple progenitors from Central Asia and other wild*Malus* are key to accelerating breeding programs to introduce resistance and adaptability traits in modern apple cultivars.
- Grape germplasm will continue to be used in developing new grape cultivars for better resistance to disease and climate change.

Related, Current and Previous Work

PGRU is responsible for acquiring, conserving, distributing, and characterizing genetic diversity of an array of vegetable, hemp, and fruit crop taxa adapted to temperate regions, including crop wild relatives (CWRs). This reservoir of genetic diversity contributes to food security and meeting human nutritional requirements. Seed or clonal cuttings, pollen, and DNA of accessions plus associated information are distributed worldwide for purposes of breeding, research, and higher education. Gaps in collections must be filled to preserve traits that have evolved naturally or through human selection over thousands of years. Germplasm is systematically regenerated, quality tested, and securely backed-up to ensure long-term availability. Regeneration and cultivation protocols must be assessed and refined to overcome dynamically changing pest, disease, and weather conditions. Descriptions of traits including growth, morphology, phenology, production, disease resistance, and health beneficial components are collected to provide for targeted requests and efficient utilization of accessions. Community-wide expertise in best management practices is leveraged to improve efficiency of operations and to document the skills and knowledge required to effectively execute this complex project. Crop vulnerability statements (CVSs) are consulted and updated in collaboration with respective CGCs, ensuring long-term protection of major components of the vegetable and fruit industries.

Some of the on-going research cooperation includes:

- M. Mazourek, Horticulture, Cornell University and Dr. Z. Fei, Boyce Thompson Institute: germplasm diversity in winter squash.
- A. Shi, University of Arkansas: screening and evaluation of tomato genetic resources for biotic and abiotic stressors.
- S. Kousik and Dr. K. Ling, USDA-ARS USVL: evaluation of vegetable genetic resources for pathogen and viral resistance.
- S. Branham, Clemson University: development of a leafy brassica genome-wide association panel for priority trait evaluation.
- C. Brummer, UC-Davis: development, evaluation, and contribution of germplasm to the hemp collection.
- S. Ellison, UW-Madison: feral hemp germplasm collection, evaluation, and contribution of germplasm to the hemp collection.
- L. Smart, Cornell University: phenotyping and genotyping of the hemp germplasm collection.
- C. Delhom, USDA-ARS SRRC: germplasm evaluation and development of hemp fiber quality standards.
- M. Berhow, USDA-ARS NCUAR: germplasm evaluation and protocol development of hemp secondary metabolites.
- E. Cebert, Alabama A&M University: germplasm evaluation and regeneration.
- C. Smart, Cornell University: pathogen screening of PGRU hemp germplasm.
- P. Bates, Washington State University: hemp seed fatty acid and protein method development and germplasm screening of PGRU collection.
- S. Brown, Horticulture, Cornell University: (1) apple scion breeding and (2) characterization of fruit quality traits of the *Malus* collection.
- K. Xu, Horticulture, Cornell University: genetic and genomics of fruit quality and tree architecture traits.
- L. Cheng, Horticulture, Cornell University: evaluation of bitter pit using *Malus* germplasm.
- G. Peck, Horticulture, Cornell University: (1) importation of new cider apple varieties from England, (2) evaluation of *Malus* collection for hard cider production potential, and (3) establishing new hard cider varietal trials in NY.
- O. Hurtado-Gonzales, USDA APHIS: import/quarantine of apples, genetic characterization of incoming *Malus* germplasm, and virus testing of the apple collection.
- A. Khan, Plant Pathology and Plant-Microbe Biology, Cornell University: (1) screening *Malus* collection for new fire blight and scab resistance, (2) importation of international apple varietal set for apple scab screening and monitoring, (3) genetic mapping of fire blight resistance genes and leaf spot resistance gene, (4) allele mining of disease resistance genes in *Malus* and *Vitis*.
- M. Fuchs, Plant Pathology and Plant-Microbe Biology, Cornell University, study of viruses in *Malus* and *Vitis*.
- M. Rivera, Entomology, Cornell University: trapping and monitoring of black stem borer of Malus.
- B. Reisch, Horticulture, Cornell University: (1) grape scion breeding and (2) *Vitis*Gen project.
- L. Cadle-Davidson, USDA-ARS GGRU, Geneva, NY: (1) resistance gene mining for powdery and downy mildews, (2) high-throughput phenotype resistance screening (including AI data mining), and (3) *Vitis*Gen project.
- G.Y. Zhong, USDA-ARS GGRU, Geneva, NY: aromatic compounds in Vitis germplasm.
- C. Heinitz, USDA-ARS: fruit quality traits of Vitis cultivars.
- K. Gold, Plant Pathology, Cornell University: Resistance to various fungal pathogens in Vitis germplasm.
- R. Hestrin, Plant Biology, University of Massachusetts Amherst: Soil microbiome composition in Vitis germplasm soils, by species and root structure.
- J. Londo, Horticulture, Cornell University: climate change adaptations of Malus, Vitis, and Prunus genetic resources.
- K. Gasic, Plant and Environmental Sciences: Clemson University, cold-hardy evaluation of Prunus genetic resources.
- C. Gottschalk, USDA-ARS, Kearneysville, WV: (1) apple breeding, (2) genomic characterization of apple germplasm, (3) exploration of wild apple germplasm.
- Gennaro Fazio, USDA-ARS PGRU, genetic characterization and rootstock evaluation of apple genetic resources.
- Apple and Grape Vulnerability Statements updates, with members from Apple and Grape CGCs, including many scientists from Cornell University.

Acquisition:

Collecting and conserving geographic and ecological diversity of CWRs *in situ* and *ex situ* is of high priority, as many wild populations face extinction due to various factors such as pests and diseases, competition with invasive species, and permanent alteration or loss of habitats. With respect to acquiring novel germplasm, obtaining precise estimates of gaps in cultivars, breeding material, and landrace or weedy material presents challenges because of the predominant influence of human activities. Germplasm acquired to fill gaps in PGRU collections is based on enhancing diversity of collections to reduce risks to agriculture.

Significant accomplishments in the period 2017-2022 include:

- 285 new accessions or mapping populations were acquired of onion, celery, brassica, winter squash, buckwheat, tomatillo, radish, tomato, and miscellaneous taxa.
- 301 new accessions of hemp were acquired.
- 104 *Malus*, 1 *Vitis*, and 26 *Prunus* accessions were added.

Maintenance and regeneration:

For the PGRU seed repository, accessions are routinely generated as seed stocks are depleted through distribution and viability is reduced during storage. Regeneration and maintenance procedures must minimize genetic changes within accessions. Loss of genetic diversity through genetic drift and through unintentional selection is avoided to the extent possible by managing the optimal health and size of regeneration populations. Long term safety of collections is ensured by back-ups of 2,000 seed with a minimum of 85% viability per accession at National Seed Storage Laboratory (NSSL) in Ft. Collins, CO. Additional backup is at the Svalbard Global Seed Vault. For clonal crops, cryopreservation at NSSL serves as backup storage for *Malus* and tart cherry accessions. Wild *Malus* and *Vitis* seed accessions are maintained in cold storage by the PGRU and NSSL. A research strategy for long term storage of *Vitis* is being investigated by NSSL with cooperation from the National Clonal Germplasm Repository in Davis, CA, and PGRU.

Significant accomplishments in the period 2017-2022 include:

- Regenerations and germplasm rescues were completed for over 1,000 vegetable crop accessions in Geneva, NY and 192 accessions were regenerated via collaborator increases.
- 1,651 backup samples for seed crops were added or replaced at the NLGRP.
- A Cooperative Agreement with University of California, Davis was used each year to support the CM Rick Tomato Genetics Resources Center (TGRC) of unique wild and genetic stock tomato accessions.
- Hemp collaborative regens were initiated at Oregon State University, University of California-Davis, and Alabama A&M University.
- 250 *Malus* and 150 *Prunus* accessions submitted for cryopreservation. Section and propagation of 250 wild apple seedlings for permanent maintenance. Seedling blocks of >2,000 trees removed following propagation.
- Seeds of wild and hybrid grapes were collected to backup allelic diversity.

Characterization:

Grant proposals funded by USDA upon recommendation from CGCs serve as a unique and valuable resource with which to study high priority traits in NPGS germplasm collections. NPGS germplasm and associated information are provided to the investigative team and resultant data are deposited into GRIN-Global and/or other public data bases. As ex-officio members of any CGC that includes PGRU crops conserved, the PGRU curators have firsthand knowledge of proposals submitted for screening NPGS germplasm and whether they are funded. Criteria for funding include a current Crop Vulnerability Statement, scientific merit, the national need for evaluation data, the likelihood of success, and the likelihood that the evaluation data will be entered into GRIN-Global.

Significant accomplishments in the period 2017-2022 include:

- PGRU scientists in collaboration with Dr. Shi at the University of Arkansas are evaluating and conducting genome-wide association study and genomic prediction for bacterial wilt and salt and drought tolerance in the PGRU tomato germplasm collection.
- In collaboration with Dr. Mazourek and PhD student, Marlie Luckach, PGRU is evaluating exotic cucurbits (luffa/ridge gourd, bitter gourd, pepinillo, and calabaza) for biotic pathogen resistances and suitability for emerging New York markets.
- In collaboration with PGRU researchers, two large hemp Supplemental and Alternative Crop germplasm acquisition and characterization grants have been awarded. These grants will add an estimated 500 hemp accessions to the PGRU collection and collect phenotypic data for approximate 75 priority traits.
- PGRU researchers drafted the UDSA Hemp Phenotyping and Descriptor Manual. This manual is being implemented by multiple groups to standardize phenotyping protocols across diverse hemp germplasm.
- PGRU researchers are collaboration with other USDA-ARS and Cornell University researchers to develop a hemp Breeding Insights phenotyping and breeding platform to characterize and breed enhanced hemp genetic resources.
- Following a fire blight outbreak, PGRU completed a disease resistance evaluation of the entire Apple Collection, one of the largest evaluations of its kind (Dougherty et al. 2021).
- PGRU with its collaborators explored the genomic impact of apple domestication and developed genome and pangenome resources for wild *Malus* species focusing on domesticated apple's primary progenitors (Sun et al. 2020; Migicovsky et al. 2021).
- Additional genetic markers (20K SNP array) ~300 apple accessions were generated and will be used to evaluate the pedigree and taxonomic gaps of the Apple Collection.
- Summarized an evaluation of the *Vitis* collection for phenological diversity. PGRU identified unique germplasm that may be suited for cold-climate breeding objectives (Gutierrez et al. 2021).
- PGRU completed a five-year fruit quality evaluation of 135 tart cherry accessions, identifying key cultivars which could improve the nutritional value of the US tart cherry industry.

Documentation:

CGCs maintain and update descriptor lists for crop traits that are highly heritable and therefore can be evaluated without costly, replicated experimental trials (http://www.ars-grin.gov/npgs/cgclist.html). There are typically dozens or more traits for a crop which are grouped into the major categories of disease resistance, growth habit, fruit morphology, phenology, and production. We continue to test and implement new methods and technologies for high-throughput data collection, such as FieldBook to facilitate data collection. In addition, digital images of plants and their parts (e.g., fruit, flowers, bulbs) are collected and deposited into GRIN-Global.

Significant accomplishments in the period 2017-2022 include:

- Phenotypic data, including digital images, were collected for routine regenerations of tomato, onion, brassica and winter squash accessions using CGC descriptors for highly heritable traits.
- Over 11,000 original seed lot passport data were digitized and associated with accessions in GRIN.
- Over 100,000 data points were collected for hemp genetic resources and will be associated with GRIN.
- Protocols for in-house seed viability tests were adopted and implemented in 2016. Several–hundred tests on regeneration samples were successfully completed.
- Documentation and routine evaluation of apple was limited due to fire blight outbreaks in the collection. However, fruit weights and images of 367 accessions of wild and hybrid *Malus* were documented and will be used for taxonomic evaluations in the Apple Collection.

Distribution:

New cultivars developed from germplasm contribute to diversity in crops, expand variety in diets, and provide benefits to human health and nutrition. Progress in crop genetics, genomics, genetic improvement, and horticultural production are accelerated by the information and genetic resources supported by NE9. Information associated with collections is distributed through GRIN-Global and other public databases such as National Center for Biotechnology Information (NCBI) or Sol Genomics Network (SGN).

Significant accomplishments in the period 2017-2022 include:

- The GRIN-Global public website interface for ordering accessions and accessing information has been substantially improved to make it more user-friendly and compatible with a wide range of electronic devices.
- PGRU distributed 33,466 samples (49% domestic and 51% foreign) of 29,092 seed crop accessions. On average, 5,818 unique accessions were distributed per year, i.e., approximately 46% of the active seed collections each year (Appendix A, Tables 2 and 3).
- International and domestic hemp seed distribution has begun in 2022.
- PGRU distributed 22,097 clonal samples (Appendix A, Tables 4 and 5) in the form of scions, seed, fruit, pollen, DNA, leaves, or use of trees for controlled This included an increase in request for “Botany of Desire” seeds (open pollinated seeds of *M. sieversii)* from the USA and particularly from European countries such as Austria, England, Finland, France, and Germany

Research:

NE9 is a cooperative research project among the State Agricultural Experiment Stations within the Northeast region of the USA. PGRU strives to provide vigorous, pathogen-inspected, and quarantine-acceptable germplasm samples to researchers and breeders. PGRU curators continue to serve as co-PIs or close collaborators on experimental studies, to ensure success of the research and follow-up on depositing data into GRIN-Global or other public databases. Genotypic research focusses on the analyses of diversity within and among accessions and taxa, as well as identifying functional alleles. Phenotypic research prioritizes traits for genetic improvement, such as tolerance of environmental stresses and extremes, nutritional content, flavor, color, horticultural traits, and host-plant resistance to diseases and pests.

Significant accomplishments in the period 2017-2022 include:

- Significant progress was made in digitizing early accession correspondence and passport information. For example, over 13,000 paper forms were digitally scanned, associated with their respective accession, and linked digitally within GRIN-Global.
- PGRU began a long-term regeneration of the asparagus collection using 56 unavailable or jeopardized seed stocks. In addition, we initiated a genotype-by-sequencing study evaluating 116 unique asparagus cultivars from 29 countries. This work evaluates overall genetic diversity and population structure within the broader pool of asparagus germplasm, as well as putative genetic bottlenecks that occurred during domestication processes. PGRU has identified several critical sub-clusters of unique genetic diversity within asparagus germplasm using approximately 41K high-quality DNA markers.
- PGRU completed a genetic diversity study on tomatoes (Labate, 2021) including partial genome sequencing on 190 tomato stocks from the PGRU collection and will provide gene discovery tools and other genetic information to increase the efficiency of selection and breeding.
- The PGRU radish collection was evaluated for genetic diversity (Arro and Labate, 2022) using 152 diverse accessions. These efforts will support ongoing radish conservation and improvement efforts.
- PGRU completed an evaluation of *Brassica oleracea* diversity using a diversity panel of 225 accessions from 19 different morphotypes and eight crop wild relative species and examined patterns of relationships among them (Mabry et al., 2019). These analyses point to the closest living relatives of *oleracea* as *Brassica incana* and *Brassica cretica*, indicating support for origin of cultivation in the Mediterranean region, and recover evidence for multiple origins of kales.
- The Hemp Germplasm Repository has met USDA-ARS, USDA-AMS, DEA, and NY State Hemp Program regulatory compliance for required germplasm collection, conservation, distribution, evaluation objectives. Hemp germplasm collection, regeneration, characterization, and evaluation efforts are already underway. At time of writing, the PGRU hemp germplasm holds over 300 unique accessions spanning all usage classes. To our knowledge, this is the largest publicly available hemp germplasm collection. Hemp has been added as a new crop within the New Crops Crop Germplasm Committee.
- PGRU has developed the first hemp phenotyping and descriptor handbook to assist breeders and researchers in identifying accessions with specific traits to facilitate germplasm selection within hemp (Cannabis sativa L.) improvement programs (Stansell and Osatuke, 2021). These efforts will help to identify gaps in the existing hemp collections and help formulate strategies for future collection and conservation efforts, to designate and maintain a core collection of critical materials, to increase NPGS user utility and accessibility to hemp germplasm and associated data, and to identify duplicate accessions and reduce costs of hemp genetic resource conservation.
- Major apple phenolics were evaluated and characterized genetically. In particular, research was done on apple dihydrochalcones, a group of apple-specific phenolics associated with human nutrition and *Malus* evolution and physiology. These compounds could be critical in elucidating the natural histories of apple species and a way to enrich the nutritional quality of modern apple cultivars (Gutierrez et al. 2018).
- PGRUs tart cherry collection was evaluated for viruses, from which collaborators identified a novel trichovirus which infects sweet cherries (Brewer et al. 2020).
- Phased diploid and pan-genomes were developed for cultivated apple and its wild progenitors. These resources help to elucidate the domestication history of apple and allow future research to leverage genomic resources to characterize traits in apple relatives (Sun et al. 2020).
- The apple collection was evaluated for fire blight resistance during a severe outbreak. The evaluation covered over 2,500 accessions and 48 taxa, representing one of the largest of its kind. Genome mapping of historic fire blight evaluation data identified novel loci related to resistance (Thapa et al. 2021; Dougherty et al. 2021).
- The apple collection was further characterized for kinship using GBS markers. Of the 1,900 cultivars evaluated, close to 900 of them had at least one first degree relationship, signaling substantial redundancy within the collection (Migicovsky et al. 2021).
- Foxy aromas in grapes are predominantly from methyl anthranilate, originating from *Vitis labrusca* and its hybrids. PGRU germplasm was evaluated for foxy aromas, and a major gene controlling for methyl anthranilate was described. Though foxiness may contribute positively to table grapes (Concord as an example), for wine these aromas are unfavorable, limiting the utilization of *labrusca* germplasm for breeding. The genetic control described will allow breeders to select against foxy aromas using marker assisted breeding. Over 1,300 accessions were evaluated for this allele (Yang et al. 2020).
- Over 5 years the *Vitis* collection was evaluated for phenological traits, including budbreak, bloom, and veraison. These three developmental traits set the limits for grape production. PGRU evaluated over 1,500 accession across 20 taxa. Increasing heat requirements for bloom was strongly associated with increased percentage of *Vitis vinifera* the European grape (Gutierrez et al. 2021).
- Profiling of epigallocatechins in grape species identified a phylogenetic linkage across diverse *Vitis* species (Brillouet et al. 2022).

Objectives

1. Efficiently and effectively acquire and maintain the safety, genetic integrity, health, and viability of priority genetic resources, and distribute them and their associated information worldwide.
2. Develop more effective germplasm maintenance, evaluation, and characterization methods and apply them to priority genetic resources. Record and disseminate evaluation and characterization data via the Germplasm Resources Information Network (GRIN-Global) and other data sources.
3. With other NPGS gene banks and Crop Germplasm Committees (CGCs) develop, update, document, and implement best management practices and Crop Vulnerability Statements (CVSs) for priority vegetable, hemp, and fruit genetic resources and information management.
4. Develop novel priority vegetable, hemp, and fruit germplasm that integrates diverse, useful genes from various resources and breed, release, maintain, and evaluate improved and regulatory compliant germplasm and cultivars. Devise and apply research tools, knowledge of genetics, and of the genetic control of priority traits to broaden the diversity available for agricultural production systems.

Methods

1. Efficiently and effectively acquire and maintain the safety, genetic integrity, health, and viability of priority genetic resources, and distribute them and their associated information worldwide.

NE9 will continue to serve as a conduit for movement and exploration of valuable plant genetic resources from worldwide origins to the northeastern states and beyond (Appendix A, Tables 3-7). The PGRU is equipped with facilities and equipment for conducting its service, research, education, and outreach activities (Appendix D).

Acquisition

PGRU will identify collection gaps by reviewing taxonomic representation, geographic distribution, or missing allelic or phenotypic variation. These gaps will be filled through germplasm exchanges, cooperator donations, expired Plant Variety Protection materials, and explorations. Genetic diversity of tomato, *B. oleracea*, onion, winter squash, radish, and celery collections will be restored and enhanced by identifying gaps and sources of germplasm to fill the gaps. Genesys free online portal (https://www.genesys-pgr.org/welcome) will be used to explore sources of seed. Genesys is a plant genetic resources accession database that contains 3.6 million accession records from 481 institutes: the three largest being US-NPGS, Consortium of International Agricultural Research Centers (CGIAR), and European Cooperative Programme for Crop Genetic Resources Networks-European Internet Search Catalog (ECPGR-EURISCO). The USA became a Party to the Food and Agricultural Organization of the United Nations (FAO) International Treaty on Plant Genetic Resources for Food and Agriculture in March, 2017.

Taxonomic gaps in apple are especially important, as recent genetic studies identified incorrect classification due to genetic admixture in hundreds of PGRU accessions (Volk et al. 2022). We will pursue plant exchanges with international collections for specific cultivars and wild germplasm inaccessible within the United States. For apple, cider cultivars are still of particular interest to stakeholders and a target for acquisition. Duke cherry cultivars (sweet cherry × tart cherry hybrids) are underrepresented in the NGPS and are targets for acquisition to expand the *Prunus* collection. Explorations for wild germplasm will target key species underrepresented in the NPGS, particularly domestic explorations for North American apple, grape, and *Prunus* species.

Cannabis germplasm exploration and collection efforts are challenging due to a suite of complex international relationships. However, there are two hemp germplasm collection efforts in the early stages of planning, in Northwestern Vietnam and Uzbekistan. These regions have been identified by stakeholders as sources of locally adapted germplasm to abiotic stress tolerances and photoperiod insensitivity.

A recently funded USDA Postdoc collection and evaluation fellowship was awarded titled "*Investigating the utility of feral populations for Brassica crop breeding*" which describes a highly valuable and unique approach to address many important questions within the broader domain of crop genetic resource conservation and plant breeding. Specifically, this proposed work will investigate feralization within *Brassica* crops as a mechanism to both enhance conservation efforts while providing new insight into genetic control of priority traits (e.g., plant architecture, phenological development, yield, and domestication). This work would provide great value to the National Plant Germplasm System by identifying, collecting, and safeguarding vital genetic resources and support of breeding and other improvement efforts. NPGS curators will receive, accession, conserve, and help with characterization of genetic materials gathered during this work and ensure that phenotypic or other associated information generated from this research is associated with relevant accessions within GRIN-Global.

Preservation

We will use best management practices (BMP) to preserve and safeguard the vegetable, hemp, and fruit collections. We will ensure the long-term safety of collection by systematically completing backups of accessions at NLGRP, Fort Collins, CO. To meet distribution needs of NPGS customers and stakeholders, any accession that drops below minimum seed quantity (e.g., 1,000) or viability (80%) is routinely regenerated. Typically, 150 to 200 accessions are regenerated annually in-house or via collaborative increases. Regeneration planning and execution are being streamlined via prepopulated forms held in shared cloud storage. This approach mitigates numerous hand-off issues experienced between teams. Dr. Stansell has developed an automated, reproducible computational algorithm for identifying and assigning priorities for the approximately 60,000 unique inventories in the seed crop collection by evaluating inventories in greatest need of regeneration, rescue, and backup. Inventories are computationally processed within the R statistical environment, scored for seed age, quantity, viability, backup status, and stakeholder usage, and then assigned an aggregated regeneration/rescue/backup score. These reports are checked by hand against existing inventories and are then slated for future regenerations.

The PGRU Clonal fruit collections are primarily maintained as field collections and vulnerable to abiotic and biotic stress. To better safeguard field collections, PGRU will enhance orchard and vineyard maintenance practices. The largest threat to the apple collection is fire blight, a bacterial disease which spreads quickly through an orchard and can kill susceptible trees within a season (Dougherty et al. 2021). Apple rootstocks can mediate scion qualities (Marini and Fazio 2018; Singh et al. 2019), which could improve PGRU maintenance practices through enhanced resistance to fire blight and other biotic and abiotic stresses, improve grafting success rate and nursery viability, reduced root suckering, and reduced tree size to accommodate higher density plantings. To evaluate new rootstocks, we will propagate a diverse panel of 100 PGRU *Malus* accessions onto four Geneva series rootstocks: G.890, G.210, G.30, and CG.6006, with M7 as a control. Each is a semi-dwarf rootstock that produces a tree 10-20% smaller than M.7 and has improved disease resistance, with reduced suckering relative to M.7 and a low tendency for biennial bearing compared to M.7. *M. domestica* and wild *Malus* accessions will be selected for evaluation based on genetic diversity using genetic data, ploidy variation, and phenotypic diversity.

The grape collection is currently grown on an Umbrella Kniffin (UK) system, which requires yearly regrowth of canes and does not facilitate mechanical pruning. Vines will be shifted to a high wire cordon (HWC) to allow better canopy control through mechanical pruning, pesticide penetration, budwood growth, and air circulation around fruit. Highly vigorous accessions (primarily wild *Vitis*) will be repropagated to T-trellis systems over the next 5 years, and trained to a Munson system or Geneva Double Curtain (GDC) to better control shoot growth by inducing shorter internodes and fewer lateral shoots (Atucha and Wimmer 2018).

Long-term storage of biological material in liquid nitrogen through cryopreservation provides a way to preserve clonal material free from environmental pressures and serves as a backup to replace field accessions lost to disease or catastrophic events. Currently, 70% of the permanent apple accessions are successfully cryopreserved as budwood. We coordinate closely with NLGRP (G. Volk) for cryopreservation of apple and tart cherry genetic resources. As of 2021, 2,088 apple accessions were considered backed up in NLGRP. The goal is to ensure that >95% of permanent *Malus* and tart cherry accessions are cryogenically backed up. Currently, 43 tart cherries and 460 *M. domestica* accessions remain to back up. Cherries will be completed in 2 years, and we will target 75-100 apples each year over the five-year period. *Vitis* cryopreservation methods are too laborious to apply to the PGRU collection. As such, seeds of select wild and hybrid *Vitis* accessions will be collected to preserve allelic diversity in the collection. Other potential backup methods are also being investigated and considered for viability within larger germplasm collections.

Distribution

The distribution of genetic resources is our most direct and meaningful contribution to customers. Delivery of germplasm to stakeholders is managed through GRIN-Global, including customer information, germplasm use, and types of materials requested. Over the past 10 years, requests have significantly increased, particularly from individuals apparently not conducting research, breeding, or educational activities. Excessive requests hinder timely delivery and requires substantial input from PGRU staff. In response to increased public demand for germplasm, all requests are filtered through GRIN-Global administrators to restrict requests for personal use and target genuine research, conservation, commercial development, and genetic enhancement programs. PGRU provides germplasm openly to legitimate requestors and does not require Material Transfer Agreements for distribution. PGRU distributes germplasm in multiple forms including seeds, winter dormant cuttings, leaves, pollen, summer green cuttings, and DNA. Record keeping for order processing is maintained through GRIN-Global. Distribution of vegetable crops is directed towards research and crop improvement needs. The normal number of seed distributed is 50 seed per accession. Whenever seed is requested for an accession with low seed supply, it is given priority for regeneration. PGRU also extensively provides germplasm to researchers and extension personnel in fruit crop related projects including many cooperations within the NE9 region.

2. Develop more effective germplasm maintenance, evaluation, and characterization methods and apply them to priority genetic resources. Record and disseminate evaluation and characterization data via the Germplasm Resources Information Network (GRIN-Global) and other data sources.

We will cooperate with scientists from ARS and other public and private sectors to characterize priority traits in vegetable, hemp, and fruit collections. PGRU will carry out the characterization and evaluation of key morphological, horticultural, genetic, and biochemical attributes of accessions. We will optimize the protocols and develop/adapt new methods for data collection for collections. Characterization and evaluation data are distributed via GRIN-Global and other databases.

Local regenerations will continue to be the primary method of maintaining the genetic resource collections PGRU. The viable lifetime of regenerated seed varies between different crop species, ranging from a few years to ~50 years, and is a major factor in deciding which accessions get regenerated in a given year. Total regenerations will be increased substantially by adopting more efficient approaches to field regeneration via method development and application to optimize seed production. For example, running greater numbers of regenerations of fewer taxa per production cycle will enable efficient scaling and throughput. Regeneration planning and execution are being streamlined via prepopulated forms held in shared cloud storage. This approach mitigates numerous hand-off issues experienced between teams with phenotyping integration in FieldBook. Typically, 150 to 200 accessions are regenerated annually in-house or via collaborative increases.

High-throughput hemp germplasm regenerations will be a critical early stage of supporting NE9 efforts. Pollination must be extremely carefully controlled in hemp. Hemp, unlike other annual crops maintained at PGRU, is anemophilous (wind pollinated) and the anthers typically produce many very small (~ 25 µm) pollen grains. Prevention of genetic drift and contamination requires more resources to be invested in regeneration on a per accession basis compared to self-pollinated crops such as tomato. Of note, outdoor pollination is not possible due to pollen drift. Currently, hemp pollen drift is not well understood, but there is an expectation that pollen can travel up to five miles by wind. It is almost certain that contaminating pollen sources exist within this radius of PGRU's outdoor vegetable seed production facilities. We will work closely with Cornell researchers to coordinate pollen control within any cannabinoid trials. Several methods have been developed for PGRU's hemp production systems: isolating a single population within a controlled environment growth chamber, isolating a single population (> 25 plants) within a greenhouse, isolating a single population in hemp pollination cages, isolating individual inflorescences in pollination bags with the addition of pollen, and collaborator increases using the above methods. PGRU has three greenhouses with the capacity for regeneration of one population at a time, four shared 10 x 10' controlled environment growth chambers, two 6 x 12' customized pollination cages, and customized hemp pollination bags and is developing relationships with collaborators capable of preforming routine regenerations. Based on these current resources, we estimate that between 15 to 50 populations can be regenerated per year. Cultural practices will follow PGRU's in-house controlled environment protocols to produce healthy, virus and pathogen-tested plants and seed. Fertility and pest control will be controlled in accordance with Cornell Cooperative Extension recommendations for indoor hemp production. PGRU is exploring distributing hemp pollen as an alternate or complementary approach to seed distribution. Specific questions regarding storage longevity, scaling, implementation, and other technical elements are currently being investigated. PGRU is developing large-scale capture and storage solutions for hemp pollen, including developing microfiltration vacuum systems, running pollen longevity experiments in 20, 4, -20, and -80 °C conditions, and evaluating viability using tetrazolium testing.

In apple, fire blight can be present in asymptomatic tissues (Tancos et al. 2017) and carried over during propagation which later proliferates in regenerated tissues, impacting PGRU propagation, distribution, and potentially cryopreserved germplasm. We hypothesize that cryopreservation techniques will significantly reduce fire blight inoculum in apple propagules. Currently, it is uncertain whether *E. amylovora* can survive cryopreservation, although other microbes have been reported to survive cryotemperatures (Bajerski et al. 2020). To determine the impact of cryotreatments on fire blight-infected apple scions, dormant buds will be inoculated with fire blight (Bell and Van Der Zwet 1987) and processed following NLGRP protocols (Volk et al. 2020). Cryotreatments will occur early to mid-January in Geneva, NY, following at least three successive days at or below 0 . Scions will be cut into 2 cm nodal sections and:

1. Desiccated to 25-30% moisture content at -5 .
2. Frozen to -30 at the rate of 1 /hour.
3. Exposed to liquid nitrogen vapor for at least 1 hour.

Sample subsets will be removed during each progressive step and stored at 5 °C prior to *E. amylovora* culturing, including lab inoculated and non-inoculated controls. Each treatment will include five biological replicate samples, each with 10 to 20 buds. Five buds from each treatment replication will be grafted to determine bud viability. Depending on results, second-year samples will expand to cover more genetic diversity, including apple cultivars and wild progenitors amenable to cryopreservation. ANOVA will be used to determine statistical variation between treatments. Linear mixed models will be used to determine the effects of species and genotype diversity assay in the second year. If successful, methods for dormant bud cryopreservation could develop into a SOP for regeneration of priority and diseased accessions or international germplasm distribution. In a pilot study, we observed up to a 96% reduction of fire blight colony forming units (CFUs) in 'Gala' samples exposed to all three cryopreservation stages, compared to untreated, inoculated samples, but additional sampling is needed to determine significance.

Evaluation of fruit quality traits are of high importance to stakeholders to identify accessions with desirable or undesirable fruit qualities within the apple, grape, and tart cherry collections. All data collected, including genotypic and phenotypic data will be deposited in GRIN-Global under the respective crop descriptors page. For grapes, metabolite profiles will be developed for aroma, color, and flavor compounds in accessions from approximately 130 grape hybrids from PGRU and approximately 340 *Vitis vinifera* cultivar accessions from the NPGS NCGR-Davis germplasm collection of wine grapes and warm season adapted grapes. PGRU germplasm will be selected based on GRIN-Global descriptors, with an emphasis on *V. labrusca* hybrids with foxy aromas (Yang et al. 2020). NCGR-Davis accessions were selected through analyses of genetic marker data to represent the diversity of *V. vinifera*, and excludes clones and first-degree relatives (Myles et al. 2011) and include accessions previously evaluated for phenolic diversity (Liang et al. 2011). Surveying broad genetic variation is challenging because each accession matures at a different time; both sites contend with personnel constraints for repeated BRIX measurements to determine maturity. Our strategy will be to collect samples of approximately 200 grams of berries from each of the selected accessions, from at least four clusters across each vine, at the end of the season, during which most of the selected accessions have reached maturity. Sampling will repeated be over three years.

In apple, the following traits related to fruit quality will be evaluated: fruit size, fruit weight, soluble solids concentrations, tannin content, fruit texture, titratable acidity and pH, and juice volume (Kumar et al. 2021). Additionally, digital images of fruit at maturity will be collected. Fruit maturity will be determined using the cortex starch pattern index (Blanpied and Silsby 1992). Major fruit sweetness and acidity genes will be genotyped (Zhen et al. 2018; Kumar et al. 2021). Sets of 300 accessions will be evaluated for a two-year period for fruit quality traits, evaluating up to 900 accessions over the five-year period, representing about 63% (900/1,432) of the *M. domestica* cultivars in the collection. Sampling strategies will include biological replicates where possible, including repeated sets of commercial standards in each subset to make statistical inferences of the differences among the cultivars.

Phenological characterization of perennial germplasm is critical for determining suitability of cultivars for an environment and identifying targets to breed for climate adaptations. Phenology studies the relationship between seasonal climate changes and the timing of biological events, including loss of dormancy leading to budbreak, onset of flowering, and fruit ripening. Phenological events are driven by environmental conditions, genetic factors, and the interaction of the two. Certain events, such as bloom in grapevine, may have a significant genetic component making it a target for genetic enhancement (Gutierrez et al. 2021). Timing of budbreak and flowering is particularly important due to the threat of frost damage resulting in crop loss. Acclimation and de-acclimation of cold hardiness are related to dormancy and budbreak, a key trait that limits crop production in certain areas where climate-adapted cultivars are not available.

Cold-hardiness and bloom phenology are intrinsically connected to productivity as late-season frosts are among the leading reasons for cherry crop failure in the US. Acclimation and de-acclimation of cold-hardiness will be measured in the cherry collection. Weekly fine-scale monitoring of bud sensitivity to seasonal temperature changes will be completed using four species *Prunus cerasus* (‘Montmorency’), *P. avium* (‘Black Gold’) and *P. fruticosa* (‘Dwarfrich’), and the early-blooming Japanese species *P. nipponica*. A larger set (64) representing the diversity of the collection will be evaluated each month. Initial cold-hardiness will be determined at each timepoint, and the remaining samples will be maintained at 20°C and measured in 3 to 7-day intervals for up to four weeks to determine cold deacclimation; the interval of deacclimation testing decreases as accessions lose dormancy towards spring. Sampling will begin following the first frost (typically October) and repeated each week until budbreak (late March or early April), across three consecutive seasons. Budbreak and bloom phenology will be scored weekly in the field using the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale for cherries (Fadón et al. 2015). Cumulative growing degree days (GDD) will be determined for *Prunus* and analyzed following approaches described in grape germplasm to determine heritability and species effects (Gutierrez et al. 2021).

For the cold-hardy *Vitis* collection, a test of bud hardiness is a critical missing descriptor from the collection. Previous research studies have evaluated 61 of the accessions for low temperature exotherm (LTE) (Londo and Kovaleski 2017; Londo and Kovaleski 2019), 43 of which were one species, *Vitis riparia*. Current evaluations of the *Vitis* core collection were successful, which includes 90 accessions (15 species). Testing will increase to 330 accessions per year to complete bud testing of the full germplasm collection within five years. The sampling frequency will be once a month from December through February. Each year, the evaluations will include cultivars ‘Concord’, ‘Riesling’, and ‘Merlot’, key industry standards in cold climate regions, as hardiness controls, for between-freezer and between-year controls. Nine buds per accession will be collected and processed at time of analysis.

Differential thermal analysis (DTA) will be used to determine the LTE or lethality point for *Prunus* and *Vitis* germplasm. LTE is measured as the release of heat during ice formation when temperatures exceed the supercooling point, indicating the bud lethality point. Bud LTE will be assayed on thermoelectric modules in a programmable freezer from 4 to -40 with a rate of 3.4 /hour. Methods in grapevine are described by Londo and Kovaleski (2017) and will be used here for grape and cherry testing. Detailed weather data within 5 km of the evaluation site is available at Network for Environment and Weather Applications (www.newa.cornell.edu). Data analysis includes analysis of covariance to determine significant differences between accessions LTE curves. Significant explanatory variables were determined previously in grapevine germplasm and will be reevaluated in these studies (Londo and Kovaleski 2017). These variables include species and a linear and quadratic function of the time covariate, and a statistically determined temperature index to correct for temperature shifts before sample collection.

3. With other NPGS gene banks and Crop Germplasm Committees (CGCs) develop, update, document, and implement best management practices and Crop Vulnerability Statements (CVSs) for priority fruit and vegetable genetic resources and information management.

The knowledge, expertise, and experience of CGC members, and staff at other NPGS and international germplasm repositories will be leveraged to strengthen and improve germplasm conservation through best management practices (Rao, Dulloo et al. 2016). Other NPGS sites with shared taxa include NC7 (*Brassica*, *Cucurbita*), S9 (*Cucurbita*), and W6 (*Allium*, Grape, *Prunus*). Curators and other scientists meet on a regular basis at scientific conferences, CGC meetings, Regional Technical Advisory Committee meetings, and Plant Germplasm Operations Committee meetings. This provides many opportunities for mutually beneficial consultation, exchange of information, formulation of new ideas, and soliciting recommendations. All components of PGRU operations will be reviewed and documented as SOPs with sufficient detail to reduce risk of any lapse in operations. Thereafter, the finalized PGRU Operations Manual will be reviewed and updated annually.

4. Develop novel germplasm that integrates diverse, useful genes from various resources and breed, release, maintain, and evaluate improved germplasm and cultivars.

This objective is primarily met through collaborations among members of the SAESs of the Northeast (Appendix E) and the PGRU and other ARS scientists (Appendix F).

We are working with breeders in the vegetables through collaborative characterizations/evaluations to provide the germplasm necessary for improvement of disease and insect resistance. Additionally, we provide germplasm to cooperators to improve traits such pathogen, drought, and salt cold tolerance resistance in tomato, locally adapted germplasm to southern Africa, and novel cucurbit germplasm to support historically underserved communities in the Northeastern US.

Hemp germplasm is being evaluated at multiple testing locations including Geneva, NY and in collaborator nurseries in Normal, AL, Madison, WI, Corvallis, OR, Pullman, WA and Davis, CA. The same group of accessions will be tested at these locations for a least two years. Multiple environment testing will support development of unique hemp germplasm through routine total floral THC and CBD testing, dissemination of pertinent phenotypic data, and through characterization and elucidation of priority traits. Planting design will be consistent across locations with accessions replicated across three blocks in a RCBD. High quality photos of the plants growing in the field, stems, and seeds will be deposited in GRIN-Global. Genotype-by-environment effects and interactions will be calculated for each measured trait. Traits with high broad-sense heritability values will be selected as breeding targets. Accessions will be selected for hybridations based on trait performance within and across locations.

Through discussions with researchers, breeders, and CGCs, we continue to update phenotyping and genotyping efforts of the PGRU fruit collections to provide information useful for crop improvement and cultivar development. In collaboration with the GGRU, Cornell University, Appalachian Fruit Research Station (ARS) in West Virginia, Washington State University, University of Minnesota, and other institutions in the USA and worldwide, we will continue to identify new sources of disease resistance and other useful traits. Where possible, pre-breeding populations will be developed to advance stakeholder objectives.

Measurement of Progress and Results

Outputs

- Identified priority germplasm to enhance collections and addition of new accessions with targeted characteristics.
- Improved field conditions for better maintenance and improved regeneration protocols
- Germplasm security, viability and availability secured through germination testing and back-ups.
- Collected data for growth, morphology, phenology, and production of priority accessions.
- An expanding GRIN-Global database with passport, characterization, and evaluation data including digital images.
- Organized germplasm and increased efficiency of filling orders allowing timely distribution of germplasm and associated information.
- Improved usability of germplasm for genetic research and cultivar development.

Outcomes or Projected Impacts

- Gaps in PGRU collections filled.
- Optimized regeneration protocols and cultivation conditions.
- Healthy germplasm with genetic integrity.
- Optimized characterization and documentation methods.
- Publicly available data and images for accessions.
- Updated PGRU Operations Manual and updated Crop Vulnerability Statements.
- Improved germplasm and cultivars.

Milestones

(**2023**):• Regenerate seed for 150 vegetable accessions. • Backup up 100 vegetable and hemp accessions and fully backup tomato core collection in NLGRP. • Improve barcoding and tracking of regeneration to manage vegetable crop regeneration efforts more effectively in real-time. • Rescue 50 jeopardized vegetable accessions in greenhouse regeneration. • Consult with plant pathologists to identify chronic diseases during seed regenerations, identify winter squash accessions with no seed set or production of low viability seed. • Apply FieldBook application to increase throughput of vegetable and hemp phenotyping efforts. • Develop effective tissue-culture protocols for hemp genetic resource conservation. • Regenerate 25 hemp accessions in controlled-environment conditions. • Upload 10,000 phenotypic data points to describe hemp germplasm collection. • Acquire over 20 novel hemp genetic resources. • Backup clonal accessions and test viability of cryo treated buds. • Collect descriptor data and digital images of clonal accessions. • Determine protocols and treatments for fire blight cryotherapy of apple. • Evaluate genetic data currently available for apple and grape to determine genetic gaps. • Evaluate fruit quality traits of apple and grape. • Improve maintenance and grapevine quality in the vineyard via trellis system changes. • Evaluate abiotic and biotic resistances in Vitis accessions. • Consult with CGC members to prioritize traits for evaluation of collections. • Draft outline for PGRU Operations Manual. Participate in CGC meetings and consult with members on status and plans for CVS.

(**2024**):• Regenerate seed for 150 vegetable accessions. • Backup up 100 vegetable and hemp accessions at NLGRP. • Rescue 50 jeopardized vegetable accessions in greenhouse regeneration. • Apply cucurbit phenotyping protocols developed with Cornell collaborators to critical genetic resources. • Apply treatments to reduce chronic diseases during seed regenerations, consult with experts for recommendations on regenerating winter squash accessions with no seed set or production of low viability seed. • Develop pre-breeding hemp populations for priority traits. • Regenerate 25 hemp accessions in controlled-environment conditions. • Upload 10,000 phenotypic data points to describe hemp germplasm collection. • Acquire over 20 novel hemp genetic resources. • Backup clonal accessions and test viability of cryopreserved buds if resources are available. • Evaluate 300 of 900 (set 1) apple cultivars for fruit quality. Year 1 evaluation of 470 accessions for grape juice metabolites. • Cryotherapy technique applied to diverse set of apple germplasm. • Identify apple germplasm and suitable rootstocks for the orchard system trial. Establish an apple nursery for rootstock evaluation. Retrain vines from the UK to TWC system. Identify accessions that could grow better on a T-trellis system. • Evaluate abiotic and biotic resistances in Vitis accessions. • Identify sources to fill genetic gaps through exchanges and explorations. • Compile and edit content of Operations Manual and contribute to updated CVS.

(**2025**):• Regenerate seed for 150 vegetable accessions. • Backup up 100 vegetable and hemp accessions at NLGRP. • Rescue 50 jeopardized accession in greenhouse regeneration. • Continue to refine regeneration protocols of seed crops. • Improve soil and assess onion bulb production. • Develop parents for hemp MAGIC populations with collaborators. • Regenerate 25 hemp accessions in controlled-environment conditions. • Upload 10,000 phenotypic data points to describe hemp germplasm collection. • Acquire over 20 novel hemp genetic resources. • Backup clonal accessions and test viability of cryo treated buds. • Evaluate apple nursery for rootstock compatibility. • Re-evaluate set 1 apple cultivars for fruit quality. Re-evaluate 470 accessions for grape juice metabolites (year 2). • Evaluate abiotic and biotic resistances in Vitis accessions. • Incorporate cryotherapy treatments, if successful, into standard practices. • Fill genetic gaps through exchanges and explorations. • Compile and edit content of Operations Manual, check for deficiencies in information and finalize content.

(**2026**):• Regenerate seed for 150 vegetable accessions. • Backup up 100 vegetable and hemp accessions at NLGRP. • Rescue 50 jeopardized accession in greenhouse regeneration. • Continue to refine regeneration protocols of seed crops. • Map critical genes underpinning priority traits in hemp. • Regenerate 25 hemp accessions in controlled-environment conditions.

• Upload 10,000 phenotypic data points to describe hemp germplasm collection. • Acquire over 20 novel hemp genetic resources. • Backup clonal accessions and test viability of cryo treated buds. • Plant apple nursery trees in high-density blocks. • Evaluate new set (set 2) of 300 apple cultivars for fruit quality. Re-evaluate 470 accessions for grape juice metabolites (year 3). • Evaluate abiotic and biotic resistances in Vitis accessions. • Fill genetic gaps through exchanges and explorations.

(**2027**):• Regenerate seed for 150 vegetable accessions. • Backup up 100 vegetable and hemp accessions at NLGRP. • Rescue 50 jeopardized accession in greenhouse regeneration. • Begin development of hemp MAGIC populations with collaborators. • Regenerate 25 hemp accessions in controlled-environment conditions. • Upload 10,000 phenotypic data points to describe hemp germplasm collection. • Acquire over 20 novel hemp genetic resources. • Continue to refine regeneration protocols of seed crops. • Backup seed and clonal accessions and test viability of cryo treated buds. • Re-evaluate set 2 apple cultivars for fruit quality. Summarize grape evaluation. • Update the CVSs.

Outreach Plan

Improved documentation and utilization of PGRU germplasm will better support breeding and research objectives, and subsequently benefit consumers in the US and abroad with stable production, nutritionally enhanced crops with reduced risk of susceptibility to pests, diseases, and changing environments. Customers looking for sources of rare or exotic fruit or vegetable varieties, for example, small growers serving local niche markets, are also frequent requestors and recipients of our germplasm. This project provides a secured supply of germplasm, ensuring the availability of genetic diversity required by US fruit, hemp, and vegetable producers to remain successful and competitive in global markets.

Primary stakeholder groups include international horticultural communities, including farmers; government and academic scientists including plant pathologists, physiologists, food scientists, entomologists; and scientists at private companies are major users of the germplasm and data from this project. Outreach objectives include publication of germplasm evaluation data in peer-reviewed journals; presentations at academic and industry meetings; and deposition of data into GRIN. To better engage stakeholders, we host several tours of our collections, including an annual open house to inform the general public about our program. Additionally, we engage with Cornell AgriTech and contribute to their outreach events aimed at serving underrepresented populations. Educational tours serve various groups including: primary, secondary, college and graduate students, growers, commodity groups, researchers, and botanical gardens, arboreta, and garden clubs. Additionally, PGRU hosts several undergraduate interns each year in an effort to train the next generation of germplasm researchers https://www.ars.usda.gov/northeast-area/geneva-ny/plant-genetic-resources-unit-pgru/docs/intern-corner/).

Organization/Governance

Regional Research Project NE9 can be effective only through federal, state, and private cooperation. The federal agency ARS, through acquisition, maintenance, characterization, documentation, and distribution activities, will make plant genetic resources available for evaluation and utilization research. ARS will provide support, staff, facilities, equipment, and specialized technical assistance at both the regional and national levels. The SAESs provide facilities, additional support staff, equipment, utilities, and local assistance. The NE9 Regional Technical Advisory Committee (RTAC) will provide technical guidance in this effort. This committee is composed of an Administrative Advisor, Regional Coordinator, plus technical representatives invited to participate from each of the Northeastern SAESs plus the District of Columbia. ARS representatives from the National Program Staff, the National Germplasm Resources Laboratory, and the PAGRP are also included on the committee as ex officio members. The names, affiliations, and areas of specialization of these individuals are presented in Appendices E and F. This committee has annual meetings with the PGRU staff at locations throughout the NE9 region which provides yearly review of genetic resources research in the region and provides technical advice to PGRU scientists.

Other committees contribute to the planning and management and are active participants in the NPGS. These include:

1. The ARS Plant Germplasm Operations Committee (PGOC) evaluates and recommends foreign/domestic exploration proposals, and assists the NPGS, ARS National Program Staff and other officials with plans needed to manage the NPGS.
2. CGCs have been established for 42 crops (or crop groups) to help advise the NPGS with regard to genetic vulnerability, gaps in current collections, operational procedures, evaluation needs, and current enhancement and utilization research associated with their specific commodity.

Project scientists have monthly meetings to discuss progress in meeting milestones and to modify activities in order to obtain goals. Scientists have regular meetings with support staff to ensure all activities are coordinated and directed towards project milestones. Scientists within the USDA-ARS Geneva, NY Location meet on a regular basis to discuss new technologies and methodologies and their potential application to project research. An annual Plant Germplasm Operations Committee (PGOC) meeting provides a forum for NPGS scientists to discuss germplasm issues with each other and National Program leaders. Shared network folders are used for storage and use of common files, both documents and data. Our major source of stakeholder feedback is from the Crop Germplasm Committee (CGCs) members who meet annually to discuss germplasm user needs and concerns pertaining to the various crop collections. We also regularly discuss with our collaborators the research and regeneration activities through emails, conference calls, discussions at scientific meetings, and site visits. Progress towards meeting project milestones is reported annually in the project annual report (AD 421). Any changes in activities necessitated by unforeseen circumstances that will affect progress towards meeting project milestones are also documented in the project annual report.

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Land Grant Participating States/Institutions

NJ

Non Land Grant Participating States/Institutions

Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Goffreda, Joseph		New Jersey - Rutgers University	1,2,4	202 202 202 202 203 212	1110 1111 1113 1114 1119 1119	1081 1081 1081 1081 1081 1081	0.60	0.00	0.00	0.1	201

Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
202-1110-1081	0.1	0	0
202-1111-1081	0.1	0	0
202-1113-1081	0.1	0	0
202-1114-1081	0.1	0	0
203-1119-1081	0.1	0	0
212-1119-1081	0.1	0	0
Grand Total:	**0.60**	**0.00**	**0.00**

Program/KA	Total FTE
201	0.03
Grand FTE Total:	**0.1**

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP9: Conservation and Utilization of Plant Genetic Resources

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
Overall, this is a well written project plan for Conservation and Utilization of Plant Genetic Resources. I only had very minor comments and thoughts as I reviewed this project. These are listed below.

NE_TEMP9: Conservation and Utilization of Plant Genetic Resources.
The amount (296,488 / year) of plant genetic resources of various plant species that NPGS provides to customers and stakeholders is truly commendable. I do know many researchers at Universities and ARS who place a very high value on the ability of GRIN to provide seeds, clones etc. of high value crops. I too have requested seeds from GRIN-Global over the years for many of my studies and have not been disappointed.
Para 1: However, genetic resources are at risk due to reduced cultivation?? The other part of the statement is conceivable. However, why should reduced cultivation reduce genetic resources? The resources with USDA GRIN should still be there. Please modify this statement if possible.
The four proposed objectives are sound and can be accomplished by the group.
Objective 4: Support development of novel…
I assume that researchers in Universities and USDA ARS will be the ones developing the novel priority vegetable, hemp and fruit germplasm?? The authors do state that these will be done in collaboration with other entities and PGRU will help provide the starting germplasm? Or will PGRU scientists directly work on some of these projects?

Importance of Work: There is no doubt that this work is extremely important for a sustainable future.

In 2021, the hemp germplasm…
How diverse is the hemp germplasm. Have detailed genotyping (GBS, WGRS) studies of the entire hemp collection been done??

Related, current and Previous work: Germplasm maintained at PGRU is currently .....
Several researchers are also working on resistance in tomatoes and other solanaceous crops to ToBrFV which has become a major problem across the world.
Cucurbit Accessions have also been evaluated for resistance to Phytophthora capsici by various groups.
If there is space these can be mentioned as well.

Some of the ongoing research cooperation include:

Kousik and Ling work on various vegetable crops but not Asparagus according to the USDA ARS website.

Is it 2017-2022 or 2018-2022?? In some instances, it is 2017 to 2022 and others it is 2018 to 2022. Please check and modify as needed.
Methods:

Objective 1: Can the scientists elaborate a little bit more on the exploration part? Specifically, exploration will be carried out for which crops (apart from the one described for apple and Prunus species)? Whether such explorations will be national or international and will it be a team travelling through various continents etc.?

Milestones:
A general question: Based on distribution record and accession viability, how long (approx..) does one generation of regenerated seed for 150 vegetable accessions last. The researchers may want to point this out in the text.
150 vegetable accession that will be regenerated? Or this will depend upon the progress that is being made with specific individual vegetable accessions?
2027 Milestone: Will PGRU develop the MAGIC population mor will this be done in collaboration with University or other ARS researchers? Developing large MAGIC populations is a significant endeavor.
Table 2.
56% of the seed samples were distributed to foreign entities (26,530 / total: 47,140). The Global impact of USDA GRIN cannot be understated.

Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete
Project ID/Title: NE_TEMP9: Conservation and Utilization of Plant Genetic Resources

Rate the technical merit of the project:

1. Sound Scientific approach:
Approve/continue project
2. Achievable goals/objectives:
Excellent
3. Appropriate scope of activity to accomplish objectives:
Excellent
4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent
5. Overall technical merit:
Excellent
Comments
This proposal leverages decades of germplasm conservation work in a wide variety crops that provide a broad, durable and high priority impact. NE9's support for the project will enhance this vital long-term work in an area where climate impacts (pathogenic as well as environmental) create uncertainties for the crops involve on a scale rarely seen in recent memory and demonstrates the wisdom of a robust and diverse conservation effort to ensure that germplasm material for such radically and unpredictably changing conditions. The project proposes a comprehensive and strategic approach to high priority areas that will support ongoing collection development, dissemination of material, and information management while also applying genetic analysis to understand the ways in material in the conservation programs can provide better performance based on highly-heritable traits.
With my expertise is in malus conservation with specific reference to historical cultivars for both fresh eating and cider, the proposal makes clear both the work that needs to be done with fruit like apples while also serving an incredibly broad set of public constituencies and research communities. It is in an elegant and well-conceived approach to pressing problems in each crop area. Knowing in some significant detail the impact the work of the germplasm collections to support research on fruit conservation, plant breeding, and other research with impacts locally, regionally, nationally and internationally, it is easy to see how this project will deepen and expand on previous work in such a vital area. The team’s proposal addresses critical needs and provides ways to answer important questions while doing so efficiently and collaboratively. It is clear the team has strong conceptual approaches but even more important is that their proposal reflect very careful coordination and prioritization. I was impressed by that element of the proposal.
Your Recommendation:
Approve/continue project

Appendix G: Peer Review (Submitted)
Status: Complete
Project ID/Title: NE_TEMP9: Conservation and Utilization of Plant Genetic Resources
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:
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4. Potential for significant outputs(products) and outcomes and/or impacts:
Excellent

5. Overall technical merit:
Excellent

Comments:
Overall, this is a well written project plan for Conservation and Utilization of Plant Genetic Resources. I only had very minor comments and thoughts as I reviewed this project. These are listed below.

NE_TEMP9: Conservation and Utilization of Plant Genetic Resources.

The amount (296,488 / year) of plant genetic resources of various plant species that NPGS provides to customers and stakeholders is truly commendable. I do know many researchers at Universities and ARS who place a very high value on the ability of GRIN to provide seeds, clones etc. of high value crops. I too have requested seeds from GRIN-Global over the years for many of my studies and have not been disappointed.

Para 1: However, genetic resources are at risk due to reduced cultivation?? The other part of the statement is conceivable. However, why should reduced cultivation reduce genetic resources? The resources with USDA GRIN should still be there. Please modify this statement if possible.

Thank you for this helpful comment.
We have clarified and removed confusing text, changed the following sentence:

However, diverse genetic resources are at risk due to reduced ~~diversity in large-scale~~ cultivation, changes in environmental conditions, degradation of native habitats, and international inaccessibility.

The four proposed objectives are sound and can be accomplished by the group.

Objective 4: Support development of novel…

I assume that researchers in Universities and USDA ARS will be the ones developing the novel priority vegetable, hemp and fruit germplasm?? The authors do state that these will be done in collaboration with other entities and PGRU will help provide the starting germplasm? Or will PGRU scientists directly work on some of these projects? Importance of Work: There is no doubt that this work is extremely important for a sustainable future.

This is an important point to clarify. We have changed the Proposed Objectives 4 text to specific the role of the PGRU curatorial staff in ongoing and planned breeding efforts:

Actively engage in and support the development of novel priority vegetable, hemp, and fruit germplasm that integrates diverse, useful genes from various resources and breed, release, maintain, and evaluate improved and regulatory compliant germplasm and cultivars. Devise and apply research tools, knowledge of genetics, and of the genetic control of priority traits to broaden the diversity available for agricultural production systems. The role of the PGRU staff in the development of novel priority germplasm will vary across different crops and projects, and can range from providing germplasm resources to projects, advising project planning and implementation, to direct action on data collection and analysis.

In 2021, the hemp germplasm…

How diverse is the hemp germplasm. Have detailed genotyping (GBS, WGRS) studies of the entire hemp collection been done??

Wonderful question! Thank you for asking.
We have appended the paragraph with text outlining out genotyping plans:

"Work is planned for 2023 to genotype the entire collection and to conduct population structure analysis. These genotyping efforts will guide collection and conservation priorities, development of mapping populations, and provide higher stakeholder utility as inputs into breeding programs."

Related, current and Previous work: Germplasm maintained at PGRU is currently .....

Several researchers are also working on resistance in tomatoes and other solanaceous crops to ToBrFV which has become a major problem across the world.

Changed text to include Rs5 bacteria and ToBrFV virus testing.

Cucurbit Accessions have also been evaluated for resistance to Phytophthora capsici by various groups. If there is space these can be mentioned as well.

Added text: "*Cucurbit accessions have been evaluated for resistance to the oomycete pathogen Phytophthora capsica, which result in outbreaks that are challenging to manage and can result in huge yield loss.*"

Some of the ongoing research cooperation include: Kousik and Ling work on various vegetable crops but not Asparagus according to the USDA ARS website.

We have been coordinating with Dr. Kousik and Ling at the USDA-USVL in Charleston, SC to plan these efforts, but they have not yet been formally established.

Is it 2017-2022 or 2018-2022?? In some instances, it is 2017 to 2022 and others it is 2018 to 2022. Please check and modify as needed.

Thank you for identifying this error. Corrections have been made throughout.

Methods:

Objective 1: Can the scientists elaborate a little bit more on the exploration part? Specifically, exploration will be carried out for which crops (apart from the one described for apple and Prunus species)? Whether such explorations will be national or international and will it be a team travelling through various continents etc.?

Thank you for this suggestion. We have information regarding upcoming hemp and vegetable exploration efforts:
"Cannabis germplasm exploration and collection efforts are challenging due to a suite of complex international relationships. However, there are two hemp germplasm collection efforts in the early stages of planning, in Northwestern Vietnam and Uzbekistan. These regions have been identified by stakeholders as sources of locally adapted germplasm to abiotic stress tolerances and photoperiod insensitivity.

A recently funded USDA Postdoc collection and evaluation fellowship was awarded titled "Investigating the utility of feral populations for Brassica crop breeding" which describes a highly valuable and unique approach to address many important questions within the broader domain of crop genetic resource conservation and plant breeding. Specifically, this proposed work will investigate feralization within Brassica crops as a mechanism to both enhance conservation efforts while providing new insight into genetic control of priority traits (e.g., plant architecture, phenological development, yield, and domestication). This work would provide great value to the National Plant Germplasm System by identifying, collecting, and safeguarding vital genetic resources and support of

breeding and other improvement efforts. NPGS curators will receive, accession, conserve, and help with characterization of genetic materials gathered during this work and ensure that phenotypic or other associated information generated from this research is associated with relevant accessions within GRIN-Global."

Milestones:
A general question: Based on distribution record and accession viability, how long (approx..) does one generation of regenerated seed for 150 vegetable accessions last. The researchers may want to point this out in the text. 150 vegetable accession that will be regenerated? Or this will depend upon the progress that is being made with specific individual vegetable accessions?

Added additional clarifying text:

"The viable lifetime of regenerated seed varies between different crop species, ranging from a few years to ~50 years, and is a major factor in deciding which accessions get regenerated in a given year."

2027 Milestone: Will PGRU develop the MAGIC population mor will this be done in collaboration with University or other ARS researchers? Developing large MAGIC populations is a significant endeavor.

Yes, this work will occur with numerous collaborators. We have changed the milestone text to acknowledge the role of collaborations in this challenging process.

Develop parents for hemp MAGIC populations with collaborators.
Begin development of hemp MAGIC populations with collaborators.

Table 2.
56% of the seed samples were distributed to foreign entities (26,530 / total: 47,140). The Global impact of USDA GRIN cannot be understated.

Your Recommendation:Approve/continue project

Appendix G: Peer Review (Submitted)

Status: Complete

Project ID/Title: NE_TEMP9: Conservation and Utilization of Plant Genetic Resources
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:
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5. Overall technical merit:
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Comments
This proposal leverages decades of germplasm conservation work in a wide variety crops that provide a broad, durable and high priority impact. NE9's support for the project will enhance this vital long-term work in an area where climate impacts (pathogenic as well as environmental) create uncertainties for the crops involve on a scale rarely seen in recent memory and demonstrates the wisdom of a robust and diverse conservation effort to ensure that germplasm material for such radically and unpredictably changing conditions. The project proposes a comprehensive and strategic approach to high priority areas that will support ongoing collection development, dissemination of material, and information management while also applying genetic analysis to understand the ways in material in the conservation programs can provide better performance based on highly-heritable traits. With my expertise is in malus conservation with specific reference to historical cultivars for both fresh eating and cider, the proposal makes clear both the work that needs to be done with fruit like apples while also serving an incredibly broad set of public constituencies and research communities. It is in an elegant and well-conceived approach to pressing problems in each crop area. Knowing in some significant detail the impact the work of the germplasm collections to support research on fruit conservation, plant breeding, and other research with impacts locally, regionally, nationally and internationally, it is easy to see how this project will deepen and expand on previous work in such a vital area. The team’s proposal addresses critical needs and provides ways to answer important questions while doing so efficiently and collaboratively. It is clear the team has strong conceptual approaches but even more important is that their proposal reflect very careful coordination and prioritization. I was impressed by that element of the proposal.

Your Recommendation:

Approve/continue project

Date: 2023 05 24

ACCESSION NO. SUBFILE: CRIS
PROJ NO: NYG- **AGENCY**:
PR0JTYPE: HATCH **PROJ STATUS**: REVISED **MULTISTATE PROJ NO**: NE9
START: 01 OCT 2023 **TERM** 30 SEP 2028

INVESTIGATORS: Zhong, G-Y.; Gutierrez B.; Stansell, Z.; Galarneau, E.
PERFORMING INSTITUTION:
HORTICULTURAL SCIENCE, NY AGRICULTURAL EXPT. STATION
GENEVA, NEW YORK 14456

NE-9 Budget Proposal for Fiscal Year 2023 through 2028

Period - October - September
In collaboration with USDA, ARS, Plant Genetic Resources Unit, Geneva, NY 14456
- ARS Project No. 8060-21000-024-00D "Conservation and utilization of priority vegetable crop genetic resources and associated information"
- ARS Project No. 8060-21000-025-00D "Management and Development of Apple, Cold-Hardy Grape, and Tart Cherry Genetic Resources and Associated Information"

Proposed budget includes 5% salary increase from FY23 to FY24 and then 3% inceases for each of the following years. Operational cost increases of 2% each year are proposed.

	FY22/23		FY23/24		FY24/25		FY26/27		FY27/28	
Salary costs:	**Dollars**	**FTE**	**Dollars**	**FTE**	**Dollars**	**FTE**	**Dollars**	**FTE**	**Dollars**	**FTE**
Farm Manager - Fruit Germplasm	$63,499	1	$66,674	1	$68,675	1	$70,736	1	$72,859	1
Field Assistant III	$49,848	1	$52,341	1	$53,912	1	$55,530	1	$57,196	1
Field Assistant III	$48,388	1	$50,808	1	$52,333	1	$53,903	1	$55,521	1
Field Assistant III	$45,488	1	$47,763	1	$49,196	1	$50,672	1	$52,193	1
Temp Agricultural worker (1 - 6 Month @18/hr)	$18,270	0.5	$19,184	0.5	$19,760	0.5	$20,353	0.5	$20,964	0.5
Temp Agricultural worker (1 - 6 Month @18/hr)	$18,270	0.5	$19,184	0.5	$19,760	0.5	$20,353	0.5	$20,964	0.5
Total Salaries:	$243,763	4.00	$255,954	4	$263,636	4	$271,547	4	$279,697	4
Operational costs:										
Supplies	$6,426		$4,890		$3,922		$2,925		$1,897	
Field research - land maintenance, pesticides, etc.	$17,976		$13,678		$10,970		$8,181		$5,307	
Field equipment repairs	$7,002		$5,328		$4,273		$3,187		$2,067	
Seed storage, vernalization, etc.	$9,770		$7,434		$5,962		$4,446		$2,884	
Seed testing	$9,813		$7,467		$5,988		$4,466		$2,897	
Total operational costs:	$50,988		$38,797		$31,115		$23,204		$15,054	
TOTAL NE9 BUDGET ESTIMATE FOR 5 YEARS:	**$294,751**		**$294,751**		**$294,751**		**$294,751**		**$294,751**	

Other Sources of Funding

		FY2023		FY2024		FY2025		FY2026		FY2027	
		Dollars	**FTE**	**Dollars**	**FTE**	**Dollars**	**FTE**	**Dollars**	**FTE**	**Dollars**	**FTE**
Salaries:	8060-21000-028-00D - Fruit Germplasm	$ 577,380.00	6.90	$ 588,927.60	6.90	$ 600,706.15	6.90	$ 612,720.28	6.90	$ 624,974.68	6.90
	8060-21000-027-00D - Vegetable Germplasm	$ 718,047.00	8.02	$ 732,407.94	8.02	$ 747,056.10	8.02	$ 761,997.22	8.02	$ 777,237.17	8.02
	8060-21000-030-00D - Hemp Germplasm	$ 614,567.00	7.08	$ 626,858.34	7.08	$ 639,395.51	7.08	$ 652,183.42	7.08	$ 665,227.09	7.08
	Total Salaries:	$ 1,909,994.00		$ 1,948,193.88		$1,987,157.76		$2,026,900.91		$2,067,438.93	
Note: Fringe benefit rate is 42%											
Travel		$ 48,000.00		$ 48,960.00		$ 49,939.20		$ 50,937.98		$ 51,956.74	
Transportation		$ 20,000.00		$ 20,400.00		$ 20,808.00		$ 21,224.16		$ 21,648.64	
Rent, Communications, Utilities		$ 3,500.00		$ 3,570.00		$ 3,641.40		$ 3,714.23		$ 3,788.51	

Printing and Reproduction		$ 13,000.00		$ 13,260.00		$ 13,525.20		$ 13,795.70		$ 14,071.62			
Contracts and shipping		$ 140,098.00		$ 142,899.96		$ 145,757.96		$ 148,673.12		$ 151,646.58			
RSA support-Hemp		$ 10,000.00		$ 10,200.00		$ 10,404.00		$ 10,612.08		$ 10,824.32			
RSA support - Clonal		$ 25,300.00		$ 25,806.00		$ 26,322.12		$ 26,848.56		$ 27,385.53			
RSA support - Seeds		$ 11,100.00		$ 11,322.00		$ 11,548.44		$ 11,779.41		$ 12,015.00			
Extramural (Short Day Onion)		$ 15,548.00		$ 15,858.96		$ 16,176.14		$ 16,499.66		$ 16,829.66			
Extramural - Hemp		$ 125,000.00		$ 127,500.00		$ 130,050.00		$ 132,651.00		$ 135,304.02			
Facility and admin support		$ 515,334.00	3.39	$ 525,640.68	3.39	$ 536,153.49	3.39	$ 546,876.56	3.39	$ 557,814.09	3.39		
Supplies		$ 497,850.00		$ 507,807.00		$ 517,963.14		$ 528,322.40		$ 538,888.85			
	Total Operational Costs:	$ 1,424,730.00	25.39	$ 1,453,224.60	25.39	$1,482,289.09	25.39	$1,511,934.87	25.39	$1,542,173.57	25.39		
	Total:	$ 3,334,724.00		$ 3,401,418.48		$3,469,446.85		$3,538,835.79		###########			

<u>APPENDIX A.</u> Supporting tables on NE9 crop importance and the PGRU conservation and distribution activities.

Table 1. Importance of the PGRU's vegetables and fruits to the U.S. Based on US Production Data from National Agricultural Statistics Service (2022).

Crops	**Average U.S. Production Value from 2016-2021 in millions of dollars**
Artichokes	$67.01
Asparagus	$87.66
Broccoli	$825.92
Cabbage	$451.25
Cauliflower	$403.35
Celery	$381.01
Onions	$997.69
Squash	$218.12
Tomatoes	$1,713.47
Total vegetables	**$5,145.48**
Apples	$3,129.97
Grapes	$5,908.48
Tart Cherries	$62.20
Total fruits	**$9,100.66**

Table 2. Number of **samples** distributed from PGRU **seed** collections from 2018-2022 by cooperator type.

Cooperator Type	**2018**	**2019**	**2020**	**2021**	**2022**	**Total**
Foreign, Commercial Companies	5872	3908	366	6851	592	**17589**
Foreign genebank/genetic resource units	6	30	-	64	123	**223**
Foreign Individuals	771	47	170	6	27	**1021**
Foreign Public Organizations (gov)	2190	1259	965	1227	2056	**7697**
US State Agencies & All Universities	1713	2131	2000	2756	1069	**9669**
USDA, ARS	32	439	185	91	17	**764**
USA Commercial Companies	1988	1193	813	2219	790	**7003**
Other USA Federal Agencies	169	480	7	3	37	**696**
USA Individuals	263	693	380	167	164	**1667**
US Non-profit Organizations	614	61	102	7	27	**811**
Total	**13618**	**10241**	**4988**	**13391**	**4902**	**47140**

Table 3. Number of **unique accessions** distributed from PGRU **seed** collections from 2018-2022 by cooperator type.

Cooperator Type	**2018**	**2019**	**2020**	**2021**	**2022**
Foreign, Commercial Companies	4890	3493	343	6546	584
Foreign genebank/genetic resource units	6	29	-	64	123
Foreign Individuals	770	46	170	6	27
Foreign Public Organizations (gov)	1661	1185	790	1047	1524
US State Agencies & All Universities	1297	1510	1561	1902	942
USDA, ARS	32	398	125	76	17
USA Commercial Companies	1485	1059	686	1977	668
Other USA Federal Agencies	169	463	7	3	36
USA Individuals	255	647	359	163	149
US Non-profit Organizations	611	60	102	6	27

Table 4. Number of **samples** distributed from PGRU **clonal** collections from 2018-2022 by cooperator type.

Cooperator Type	**2018**	**2019**	**2020**	**2021**	**2022**	**Total**
Foreign, Commercial Companies	7	-	-	4	-	**11**
Foreign genebank/genetic resource units	1	123	-	-	-	**124**
Foreign Individuals	132	68	102	54	80	**436**
Foreign Public Organizations (gov)	16	34	1	-	8	**59**
US State Agencies & All Universities	1911	1314	395	161	274	**4055**
USDA, ARS	331	1265	664	9	446	**2715**
USA Commercial Companies	684	412	240	80	136	**1552**
Other USA Federal Agencies	9	3	8	14	10	**44**
USA Individuals	4737	4558	2557	274	354	**12480**
US Non-profit Organizations	255	204	147	1	14	**621**
Total	**8083**	**7981**	**4114**	**597**	**1322**	**22097**

Table 5. Number of **unique accessions** distributed from PGRU **clonal** collections from 2018-2022 by cooperator type.

Cooperator Type	**2018**	**2019**	**2020**	**2021**	**2022**
Foreign, Commercial Companies	7	-	-	4	-
Foreign genebank/genetic resource units	1	117	-	-	-
Foreign Individuals	71	28	36	22	28
Foreign Public Organizations (gov)	15	29	1		8
US State Agencies & All Universities	1028	426	371	154	264
USDA, ARS	315	1184	664	9	437
USA Commercial Companies	457	335	211	72	128
Other USA Federal Agencies	6	3	8	14	10
USA Individuals	1586	1671	1209	184	304
US Non-profit Organizations	167	170	133	1	14

Table 6. Number of samples and unique accessions distributed to NE9 states from 2018-2022.

State	Clonal		Seeds	
	Samples	**Accessions**	**Samples**	**Accessions**
Connecticut	77	70	18	18
Delaware	35	35	2	2
Maine	639	484	62	60
Maryland	748	667	99	90
Massachusetts	263	201	102	98
New Hampshire	95	88	106	106
New Jersey	85	83	187	177
New York	5065	2074	3992	3253
Pennsylvania	895	583	318	296
Rhode Island	27	27	1	1
Vermont	268	230	15	9
West Virginia	581	450	28	27
Total to NE9	**8778**	**2823**	**4930**	**3788**

Appendix B: PGRU References from 2018 to 2022

2018

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Appendix C: Facilities and Equipment

Facilities

1. PGRU is divided between three buildings located on the campus of the New York State Agriculture Experiment Station, Cornell University, Geneva, New York.

    a. USDA Building (592 m² or 6,372 sq ft) houses the laboratory, administration, components as well as facilities for clonal crops

        i. Five offices (114 m² or 1,227 sq ft), including Research Leader, Computer Specialist, Molecular Biologist, Computer support staff and Administrative Support Staff.
        ii. Laboratory Space (157 m^2 or 1,690 sq ft)
        iii. Three Clonal Greenhouses (160 m^2 or 1,722 sq ft)
        iv. Headhouse (92 m² or 990 sq ft)
        v. Characterization room (30 m² or 323 sq ft)
        vi. Cold Storage (26 m² or 280 sq ft)

    b. Clonal Office Building was finished in July 2001 with 4 scientist offices at 100 sq ft each. Technician room with 10 cubicle workstations for the clonal program technicians and breeding program technicians at 400 sq ft. One unisex bathroom and open storage area.

    c. The Seed Processing Building houses the NERPIS office, seed processing and storage facilities

        i. Office space (858 sq ft), contains three enclosed offices for Vegetable Curator/Horticulturist, Statistician and the Operations Manager. Desk space for Greenhouse Manager, three Agricultural Science Technicians and a Biological Science Aid.
        ii. Vernalization chamber (291 sq ft) held at 20 °C and ambient humidity.
        iii. Seed cold storage room (47 m^2 or 529 sq ft) held at 0° C and 20% relative humidity.

iv. Cold storage anteroom (27 m^2 or 330 sq ft) held at 4° C and 30% relative humidity.
v. Restrooms (47 m^2 or 506 sq ft) handicap equipped, separate for male and female.

2. Crop and seed production facilities include approximately 24.1 ha of land and 0.10 ha of greenhouses.

a. Wellington farm (14 ha or 34.58 acres), is located 1.2 km (about 1 mile) north of the Cornell Agritech campus. The PGRU has a lease-to-own contract with Cornell University. The following site improvements have been made:

i. Comprehensive field drainage system was installed in 1988.
ii. 1105 m^2 (11,895 sq ft) field laboratory which includes a 277 m^2 (2,982 sq ft) rodent proof storage area for pollination cages and bee keeping equipment, an 483 m^2 (5,200 sq ft) farm equipment storage and workshop area and a 350 m^2 (3,768 sq ft) heated field lab for planting, harvesting and seed cleaning operations was built in 1989.
iii. Twenty-five hive apiary on a gravel pad was established in 1992.
iv. Trickle irrigation was installed in 1992. The farm was divided into 8 irrigation zones which can be individually scheduled using electronic timers. The system includes a 18,920 liter (4,000 gallon) water storage tank and an injection fertigation system.
v. Electrified deer fence was installed in 1992. A deer fence now encloses both the Wellington Farm and the adjacent McCarthy Farm which is used by the NCGR.
vi. 3-sided equipment shed

b. McCarthy Farm (Approximately 20 ha or 50 acres) is located 1.3 km (about 1 mile) North of the NYSAES Campus. The PGRU maintains a long-term lease with Cornell University for this property.

i. Comprehensive field drainage system was installed in 1984-85.
ii. Trickle irrigation was installed in 1984-85. There are 9 risers from the system which are normally controlled.
iii. Electrified deer fenced was installed in 1984-85. The fence was modified/extended in 1992 to encompass the Wellington Farm.
iv. 4.05 ha (10 acres) are leased from Cornell University on the Station Nursery Farm which is located 0.4 km (0.2 mile) north of the Wellington farm. Site improvements include trickle irrigation and field tile drainage.

c. Greenhouse Facilities

i. Construction was completed on two permanent USDA, ARS greenhouses (450 m^2 or 5,000 sq ft) in 1992. One house (PGH-1) is equipped with aluminum-framed rolling benches, the other (PGH-2), has sand bed floors to accommodate pollination cages. Both greenhouses contain computerized environmental controls, automated drip irrigation systems, ratio:feeder fertilizer injector, hot water bottom heat for benches and ground beds and 1,000 watt sodium lights and are heated with steam. Approximate capacity is 5,000 1-gallon pots.
ii. Construction was complete in 2002 on a permanent USDA, ARS greenhouse (2,000 sq ft). The house (PGH-3) is equipped with sand bed floors and computerized environmental controls.
iii. Adjoining headhouse (148 m^2 or 1,600 sq ft) contains 12.43 m^2 (134 sq ft) potting bench space, 11 soil bins (4.5 hl), 2 walk-in vernalization coolers (92 m^2 or 990 sq ft),

steel shelving (30 m^2 or 323 sq ft) for storage, and vented steel chemical storage cabinet for pesticide storage and was completed in 2004.

Information Management

Computer resources include a Dell server [PowerEdge R510 (4TB and 8TB HD, 64GB RAM, 1GB Network)] and Red Barn server [Supermicro SYS-8027R-TRF+ (20TB HD, 512GB RAM, 10GB Network)] housed by the Cornell University Computational Biology Service Unit for bioinformatics. Cloud storage solutions are available, allowing for the maximization of research and information security. An onsite USDA IT specialist is at the location, which helps to bridge the gap between university and USDA IT systems. Cornell University supplies high-speed Internet2 rated traffic. Our collaboration tools use voice over internet protocol (VoIP), Microsoft Teams, and Zoom.

Field Equipment

Equipment used for field maintenance and distribution of seed and clonal collections are listed below:

- 3 pt. hitch Spinner Spreader
- 4 Bottom Plow
- Air blast sprayer
- Air Column (5)
- ATV (4)
- Auger
- Boom Sprayer 110 gallon
- Brush Chopper, 5'
- Brush Machine
- Cargo van
- Cleaner & Tester Mill Seed
- Clipper (2)
- Crop care Mulch lifter
- Cultivator
- Cultivator - Vineyard
- Cutter Mower – Sickle-bar
- Debarder
- Disk
- Drill
- Flatbed trucks (5)
- Forklift
- Generator
- Gravity Separator
- Herbicide Sprayer (2)
- Mower (2)
- Mower Walk Behind
- Mulch Layer
- Mulch Transplantor
- Mulcher Pulvi/Teeth Notched
- Multi-Crop Shredder
- Plow Coulter-Chisels SL
- Power Pruners
- Rotary mower
- Rotovator
- Seed Counter
- Snowmobile
- Sprayer
- Straw Mulcher
- Thresher (3)
- Tiller, 42" Rotovator
- Tiller, 68" Rotovator
- Tractor (14)
- Tractor Wagon
- Trailer (2)
- Vegetable Seed Separator (2)
- Ventilation bin controller

Laboratory and Characterization Equipment

Equipment used for characterization and research of seed and clonal collections are listed below:

- Autoclave
- Balance (3)
- Calculator, DNA/RNA
- Centrifuge (7)
- Centrifuge (refrigerated)
- Cold Chamber (2)
- Digital imager/Analysis
- Dishwasher
- DJI Phantom Drone
- Fotosystem 1000
- Freezer, -20°C (2)
- Freezer, -80°C (9)
- Genetic Analyzer
- GenoGrinder (2)
- HPLC Systems (3)
- Hybridization oven (2)
- Ice machine
- Incubator (2)
- Laminar Flow Hood (2)
- Liquid nitrogen tank
- Lyophilizer system
- Microplate Reader
- Oven (2)
- PCR Machine (8)
- Penetrometer, digital
- pH meter (2)
- Plate reader
- Printer
- Refractometer (2)
- Repeater Thin/Thin
- Rotor (8)
- Shaker, Environmental (2)
- Shaker, Junior Orbital (2)
- Speedvac (2)
- Still
- Titrator, automated
- Transfer lamp
- Tristimulus Colorimeter (3)
- Uninterrupted Power Supply (2)
- Vacuum Centrifuge
- Vacuum Manifold
- Water Filtration System

APPENDIX D: Project participants for the NE-9 Regional Research Project

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APPENDIX E: Projected participation, allocation of resources of state and federal participants for Regional Research Project NE9: Plant Genetic Resources Conservation and Utilization.

Participant Name, Email Address and Phone Number	Institution and Department	Research						Objectives			
		CRIS Codes			Personnel						
		RPA	SOI	FOS	SY	PY	TY	1	2	3	4
Gan-Yuan Zhong GanYuan.zhong@usda.gov 315-787-2482	PGRU, USDA, ARS	202-1110-1080; 202-1112-1080 202-1130-1080; 202-1131-1080 202-1132-1080; 202-1139-1080			0.25	-	1.25		X	X	X
Benjamin Gutierrez ben.gutierrez@usda.gov 315-787-2439	PGRU, USDA, ARS	202-1110-1080; 202-1112-1080 202-1130-1080; 202-1131-1080 202-1132-1080; 202-1139-1080			1.00	-	2.00	X	X	X	X
Zachary Stansell zachary.stansell@usda.gov 315-787-2454	PGRU, USDA, ARS	202-1110-1080; 202-1112-1080 202-1130-1080; 202-1131-1080 202-1132-1080; 202-1139-1080			1.00	-	2.60	X	X	X	X
Erin Galarneau erin.galarneau@usda.gov 315-787-2438	PGRU, USDA, ARS	202-1429-1080; 202-1430-1080 202-1451-1080; 202-1460-1080 202-1469-1080			1.00	-	6.60		X	X	X
Peter Bretting peter.bretting@usda.gov 301-504-5541	NPS, USDA, ARS, National Program Leader NP301	202-1110-1080; 202-1112-1080 202-1130-1080; 202-1131-1080 202-1132-1080; 202-1139-1080 202-1429-1080; 202-1430-1080 202-1451-1080; 202-1460-1080 202-1469-1080			0.10	-	-	X	X	X	X
Gary Kinard gary.kinard@usda.gov 301-504-5951	National Germplasm Resources Laboratory USDA, ARS	202-1110-1080; 202-1112-1080 202-1130-1080; 202-1131-1080 202-1132-1080; 202-1139-1080 202-1429-1080; 202-1430-1080 202-1451-1080; 202-1460-1080 202-1469-1080			0.15	1.00	-	X		X	

Participant Name, Email Address and Phone Number	Institution and Department	Research						Objectives			
		CRIS Codes			Personnel						
		RPA	SOI	FOS	SY	PY	TY	1	2	3	4
Karen Williams karen.williams@ usda.gov 301-504-5421	National Germplasm Resources Laboratory USDA, ARS	202-1110-1080; 202-1112-1080 202-1130-1080; 202-1131-1080 202-1132-1080; 202-1139-1080 202-1429-1080; 202-1430-1080 202-1451-1080; 202-1460-1080 202-1469-1080			0.15	-	0.15	X			
Christina Walters christina.walters@ usda.gov 970-495-3202	National Center for Genetic Resources Preservation; USDA, ARS	202-1110-1080; 202-1112-1080 202-1130-1080; 202-1131-1080 202-1132-1080; 202-1139-1080 202-1429-1080; 202-1430-1080 202-1451-1080; 202-1460-1080 202-1469-1080			0.10	0.10	0.05	X			
Gayle Volk gayle.volk@usda.gov 970-492-7607	National Center for Genetic Resources Preservation; USDA, ARS	202-1110-1080; 202-1112-1080 202-1130-1080; 202-1131-1080 202-1132-1080; 202-1139-1080 202-1429-1080; 202-1430-1080 202-1451-1080; 202-1460-1080 202-1469-1080			0.10	0.10	0.05	X			
Christopher Richards christopher.richards@ usda.gov 970-495-3201	National Center for Genetic Resources Preservation; USDA, ARS	202-1110-1080; 202-1112-1080 202-1130-1080; 202-1131-1080 202-1132-1080; 202-1139-1080 202-1429-1080; 202-1430-1080 202-1451-1080; 202-1460-1080 202-1469-1080			0.10	0.05	X	X			X
Total SY, PY, TY and FTE	X	X						X	X	X	X

[1] Research Problem Area(s) (RPA), Subject(s) of Investigation (SOI), and Field(s) of Science (FOS) [2] SY = scientist years, PY = professional years, TY = technician years

Project: NE-2140 Sustainable Management of Nematodes in Plant and Soil Health Systems
Nominating Region: Northeastern
Nominator: G.W. Bird, L. Schumacher, K.-H. Wang, A. Westphal, J. Kotcon, N. Mitkowski, E.C. Bernard, W.T. Crow
Administrative Advisor: Anton Bekkerman

ISSUE, PROBLEM, or SITUATION ADDRESSED: The U.S. food industry—valued at $2.3 trillion in 2021—represents approximately 10% of the national GDP, with an additional $140 billion generated by the ornamental and landscaping product sectors. But the security and trust in the U.S. food and agricultural systems depends critically on meeting consumers' expectations of high-quality, readily available, and affordable food and ornamental products. A long-standing challenge to this has been the damaging impacts of nematodes—an undoubtedly national concern but one that has been acutely concentrated and impactful on agricultural systems in the Northeast. Nematode infestations can cause crop losses of 10-15%, which can have seriously damaging impacts on the high-value cropping operations in the northeast, reduce regional food sustainability, and raise environmental hazards from increased food imports. Moreover, these risks have increased because intensive crop production in the region has gradually deteriorated soil quality, which itself reduces productivity and exacerbates the high population densities of plant-parasitic nematodes that compound the risks of lower yields, poorer quality, and lower profitability.

The ubiquitous presence of nematodes and their hazards to Northeast agricultural system and the challenges of managing these pests in ways that minimize adverse impacts on beneficial species—bacterial-feeding, fungal-feeding, omnivores and predatory nematodes that are key components of soil food webs, essential for soil nutrient cycling, and may contribute to biological control—make sustainable nematode management an important contributor to at least two *Grand Challenges*: Food Security and Environmental Stewardship. And the urgency is only growing, as rapid consequences of climate change in the Northeast are increasing the pest's occurrence and spread, enabling endemic species from warmer climates (such as FL, CA, and HI) to spread to more temperate regions, and worsening the impacts of yield, quality, and disease spread, such as the beech leaf disease. Since the 1950s, the research group associated with NE-2140—the group's current iteration—has conducted collaborative research and educational programs that has significantly benefited economic viability and soil health of Northeast farms and forests through sustainable and reliable nematode management methods such as host plant resistance, crop rotation with nematode-antagonistic cover crops, soil amendments, trap cropping, biological control agents, and reduced-risk nematicides. The group's long-term impacts have increased agroecosystem sustainability, resilience of natural resources, and recreational values of the region.

OBJECTIVES:

1. Develop and integrate management tactics to control plant-parasitic nematodes including biological, cultural (e.g.,rotation, cover crops, plant resistance), and chemical controls.
2. Determine the ecological interactions between nematode populations, nematode communities, ecosystems, soil health and climate resilience.
3. Detect and evaluate the distribution/movement of invasive and emerging nematode pests.
4. Compile, present, and publish guidance on nematode management and management effects on soil health for different crops under different conditions.

ACCOMPLISHMENTS:

Short-term outcomes: Between 2016-2022, members of NE-2140 (previously NE-1640) generated 214 peer-reviewed publications or abstracts related to plant-parasitic nematodes and soil health management. Multiple grower education seminars and workshops were conducted in participating states every year.

Medium-term outcomes: Although nematodes have been used as comprehensive biological models in studying basic science and have recently been utilized as important biological indicators of soil health, comparatively little is known about the relationships between soil health and management of plant-parasitic nematodes regarding crop yield. Within the last 5 years, NE-2140 had contributed frontline research on the use of biological control organisms and breeding for nematode resistance to protect agronomic crops. Other members of NE-2140 documented the relationship between soil health parameters to soil nutrient cycling, soil greenhouse gas emission, plant-parasitic nematode suppression, natural weed suppression, water conservation, above ground insect pest mitigation and wcontinues to integrate nematode management recommendations into soil health management programs designed for agronomic and horticultural production systems.

Long-term outcomes: Since 1954 as NE-34 with 17 scientists from ten northeastern states, the overarching mission of NE-2140 remains a robust multistate infrastructure for nematologists in academia and USDA-ARS to collaborate on research and educational initiatives that lead to impactful regional and increasingly national solutions to ever-emerging nematode challenges. This has been especially critical because private sector enterprises do not have the nematology resources and institutional structures necessary to fulfill this critical need.

These include progress in identifying alternative nematode management options for: fruits such as apples, grape, peaches, strawberries; almonds and walnuts; vegetables including beans, carrots, garlics, peppers, potatoes, tomatoes; specialty crops like hemp; and row crops including peanuts, cotton, and soybeans, based on using nematode suppressive cover and rotational crops, biofumigants, resistant cultivars, and effective biological control agents, such as *Pasteuria penetrans* and *P. thornei*. Suppressive rotation crops have reduced severity of strawberry black root rot without the need for nematicides or soil fumigation. Free-living nematodes were assessed as indicators of soil health in several long-term crop rotation trials, with significant findings on the impact of reduced tillage mitigate environmental stresses on the soil food web. The efficacy of new and reduced risk nematicidal products was evaluated, with the findings that different nematodes varied in their response and their potential impact on soil health.

Impacts: NE-2140 and its predecessors have: 1) enhanced farm economic viability and reduced environment-human health risks associated with nematicide usage; 2) changed farmers' behaviors to increase IPM of nematodes through Extension and outreach, thereby increasing sustainable soil health of small, medium, and large farms; and 3) increased farmer, consultant, industry and government understanding about the nature of nematodes, the damage they can cause, and the importance of maintaining soil health. One recent example showed that after the research and Extension activities of the Soybean Cyst Menatode Coalition—which is in part led by members of NE-2140—soybean growers rotating with non-host crops increased from 71% to 77%, growers planting SCN resistant varieties increased from 59% to 66%, growers rotating genetic sources of

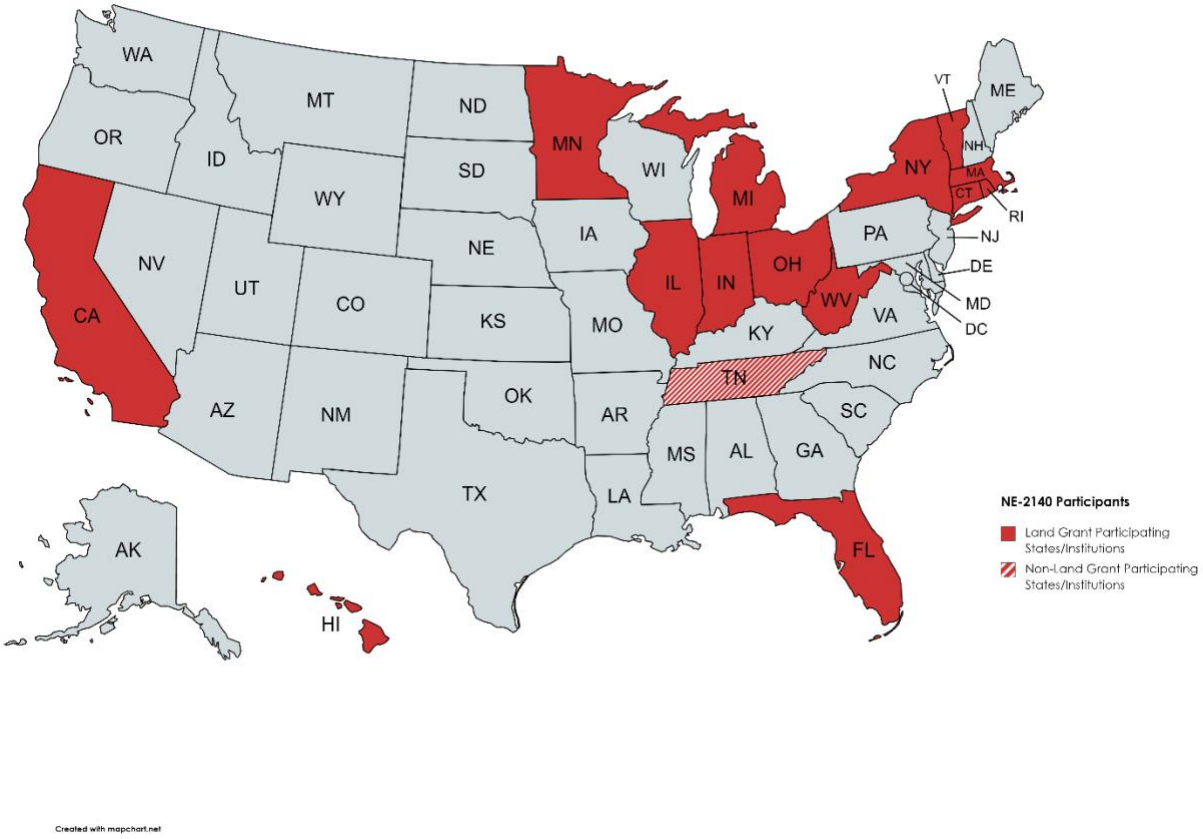
resistance increased from 39% to 49%, and growers using nematode protectant seed treatments increased from 22% to 40%. Additionally, the impact's from the group's research and Extension have expanded to include other important communities such as urban gardening and forested ecosystems challenged by new invasive nematode of beech trees.

Importantly, NE-2140's impacts adapt to the evolving issues across U.S. regions. For example, the group is at the forefront of the young domain of soil health biology and has produced actionable, practical solutions in the face of recent projections that approximately 85% of Earth's animals are nematodes. To ensure continued impacts, the project is committed to training future generations of nematology researchers and Extension professionals—a practice dating back to NE-34, when project participants acquired NSF funding to train 30 young nematologists under the guidance of the 30 world's best nematologists. This created an impactful foundation for nematology research and Extension in the decades that followed. The commitment to training the next generation of scientists remains foundational to the group, with NE-2140 members developing 9 Master's, 9 PhD, 2 post-doctoral scholars, 1 research associate and 2 research assistants in FY21–23.

ADDED-VALUE AND SYNERGISTIC ACTIVITIES: NE-2140 is a large and diverse group of world-class leaders in nematological research whose work not only affects the Northeast, but stretches across the country, the continent, and the world. NE-2140 includes 15 official members located at 12 institutions and 1 USDA-ARS station who have backgrounds in nematology, plant pathology, organic farming, sustainable pest management, crop science, soil health, turf and landscape pest management, animal production, and Extension. NE-2140 embraces soil health policy-relevant research that is national in scope but applicable to regional and local issues. The project maintains a sustained multi-generational, multi-institutional, and inter-disciplinary membership of scholars. Many mid-career and early career scholars benefitted from participating in this project's predecessors (NE-1040, NE-1640), leading to an expansion of membership and, geographic scope of its research beyond the Northeastern. Today, NE-2140 objectives are informed by in-depth knowledge from New England to the Northern Great Plains, Great Lakes, Mississippi Delta, Southeast, West, and the Pacific Islands. Many committee members have formal Extension responsibilities, and their expertise provides the group with stakeholder-informed knowledge of issues across diverse farming communities. Discussion and input about ongoing and new research efforts occur during well-attended, vibrant annual meetings. The education, training, and networking opportunities offered through NE-2140 collaborators has helped applied scholars to build capacity to better serve both large-scale crop producers and smaller-scale farmers and hobbyists, and inform policy makers about nematode and soil health management.

EVIDENCE OF MULTI-INSTITUTIONAL AND LEVERAGED FUNDING: NE-2140 projects have been critical to garnering multiple external research funding. In FY21–23, project participants secured over $7.6 million in funding (attributed to NE-2140 participants) for 26 proposals from NIFA, SARE, NRCS, and multiple state agencies and foundations. Seven of these projects include multiple NE-2140 participants, with one—the Soybean Cyst Nematode Coalition project—including four NE-2140 members and 24 states not represented in the group. These leverage and extend key NE-2140 research and outreach efforts to broaden the impacts of mitigating yield loss from plant-parasitic nematodes, converting agriculture from carbon emission to carbon storage operation, protecting farm soil from erosion and conserve soil moisture to be better prepared for unexpected drought.

PARTICIPATING INSTITUTIONS and UNITS:



The fifteen NE-2140 official participants represent 12 land-grant universities and 1 USDA-ARS stations, represent all four regions of Agricultural Experiment Stations and Cooperative Extension Services.

Appendix A. FY 21-23 NE-2140 Projects, Funds Leveraged, Students/Post-Docs, Multi-Institutional Projects, Impacts and Impact Metrics.

Projects (Lead Institution)

1. Implementing a nematode management system for almonds using chemical and biological treatments (CA)
2. Pre- and post-plant remedie for nematode infestations in walnut orchards (CA)
3. Is there risk ofmplant parasitic nematodes in pistachio on current and future rootstocks? (CA)
4. Use of endophytic microorganisms as nematicidal biocontrol agents (FL)
5. Preservation of SDHI chemistry on golf turf (FL)
6. Regenerating soil health in shelterbelts, sheet mulching and tree mulch plant-available N calculator (HI)
7. Organic sweet potato IPM and soil health management for small- and mid-size farms (HI)
8. Rooting for ecosystem services (IL)
9. Turfgrass nematode management (MA, RI)
10. Applied nematology research (MI)
11. Michigan potato soil health dynamics (MI)
12. The SCN Coalition (MI)
13. Putting the heat on seed-borne pathogens of garlic (NY)
14. Bacterial-plant interactions (OH)
15. Beech leaf disease nematode biology and management (RI)
16. Multi-trophic effects of agricultural microplastics:implications for soil biological health (TN)
17. Solanaceous pest, virus and nematode survey (TN)
18. Grape commodity survey (TN)
19. Susceptibility of *Cannabis sativa* to the southern root-knot nematode, *Meloidogyne incognita* (TN)
20. Investigating the severity of nematode and fungal pathogens in Tennessee soybean production (USDA-ARS, TN)
21. Long-term impacts of crop rotations and cover crops on soybean yield and disease (USDA-ARS, TN)
22. Organic amendments to suppress disease in organic vegetable and dairy systems (VT)
23. Food systems sustainability starts with soil. (VT)

24. Evaluation of nematode population dynamics in long-term organic farming systems (WV)
25. Role of nematode biological control agents in suppressive soils (WV)
26. Management of dagger nematodes in peach and apple production (WV)

Funds Leveraged ($7,578,462)[1]

1. $350,000 (Implementing a nematode management for almonds using chemical biological treatments)
2. $107,040 (Pre- and post-plant remedie for nematode infestations in walnut orchards)
3. $126,070 (Is there risk ofmplant parasitic nematodes in pistachio on current and future rootstocks?)
4. $116,665 (Use of Endophytic Microorganisms as Nematicidal Biocontrol Agents)
5. $107,604 (Preservation of SDHI Chemistry on Golf Turf)
6. $771,442 (Regenerating soil health through shelterbelt planting and PAN calculator)
7. $740,876 (Organic sweet potato IPM and soil health management for small- and mid-size farms)
8. $1,499,090 (Rooting for Ecosystem Services)
9. $1,199,527 (Applied Nematology Research)
10. $24,000 (Michigan Potato Soil Health Dynamics)
11. $124,692 (Putting the heat on seed-borne pathogens of garlic)
12. $675,000 (The SCN Coalition)
13. $750,000 (Multi-Trophic Effects of Agricultural Microplastics: Implications for Soil Biological Health)
14. $60,000 (Solanaceous pest, virus and nematode survey)
15. $50,000 (Grape Commodity Survey)
16. $15,000 (Susceptibility of *Cannabis sativa* to the southern root-knot nematode, *Meloidogyne incognita*)
17. $75,000 (Investigating the severity of nematode and fungal pathogens in Tennessee soybean production)
18. $25,000 (Long-term impacts of crop rotations and cover crops on soybean yield and disease)
19. $123,528 (National USDA Uniform Soybean Tests/Yield, Disease Resistance and Quality Traits
Evaluation of Public Breeding Lines)

20. $89,998 (Organic amendments to suppress disease in organic vegetable and dairy systems)
21. $599,983 (Food Systems Sustainability Starts with Soil)
22. $38,247 (Link between soil and human health)
23. $592,911 (Diversifying Appapachia's pastures tio improve soil health)
24. $749,999 (Unrvling the effects of manure treatments on pests in organic field crops)
25. $499,817 (Overcoming barriers to transitioning small ruminants to organic production)

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[1]$7,578,462 is a 2021-2023 mixture of total grant awards and portions of awards allocated to NE-2140 scientists.

Students and Post Docs

M.S.	9
Ph.D.	9
Post Docs	2

Multi-Institutional Projects

1. Organic sweet potato IPM and soil health management for small- and mid-size farms (HI, AL, NC)
2. Turfgrass nematode management (MA, RI)
3. Applied nematology research (MI, ND, ID)
4. Michigan Potato Soil Health Dynamics (MI and NY)
5. The SCN Coalition (MI, NY, OH, MN and twenty-four other states not formally part of NE-2140)
6. Support development of the Western Cover Crops Council and enhance cover crop educational resources and adoption in the Western U.S. (OR, CA, HI, UT)

7. National USDA Uniform Soybean Tests/Yield, Disease Resistance and Quality Traits Evaluation of Public Breeding Lines (MS, TN, MO, NC, LA, AL, GA, AR, SC, KS, VA)

Impact Statements

1. First documentation of nematicide resistance to plant-parasitic nematode resistance.
2. Higher sweet potato yield and health index was positively related to the joint impact of higher cover crop biomass in combination with biopesticides, encouraging organic sweet potato farmers to practice cover cropping and biopesticides or other organic fertilizers.
3. Between 2012 and 2022, there were significant increases in soil organic matter, active carbon, and available soil water potential associated with Michigan potato production.
4. Soybean growers improved their knowledge about active management of the soybean cyst active and their SCN practices.
5. Several OMRI-listed pesticides were found to be ineffective against bloat nematode.
6. Poultry based compost enhanced survival of enteric pathogens in soil more than dairy-based compost. This may shift the choice away from poultry products that are currently used by vegetable farmers for economic reasons and may affect their acceptance by organic certification programs.
7. To engage the potential of soil health to enhance beneficial outcomes for farms and society, farmers and practitioners need guidance on what indicators to measure, how and when to measure them, how to interpret them, and guidance to translate them into recommendations.
8. *Meloidogyne incognita*, identified by bioassays and molecular analysis, was found in one-third of the 137 samples analyzed. *M. hapla* was collected in 5% of the samples. *M. enterolobii* was not detected.
9. *Xiphinema americanum* was identified morphologically and molecularly from 12 of 51 vineyards, while *X. rivesi* was detected in three vineyards. Virus symptoms were not observed on the vines.
10. Distribution of nematodes in general and densities of plant-parasitic nematodes were dependent upon species of pasture grass and soil texture rather than fertilizer or overseeding treatments.
11. Hemp susceptibility to *M. incognita* is dependent on cultivar, with a few cultivars being nearly immune (reproduction almost non-existent) to others that are excellent hosts (reproduction greater than 20× initial inoculum).

12. Western Region Cover Crop Council was formed to promote cover crop adoption in CA, OR, HI, and UT from regular outreach activities to facilitating local cover crop seeds production.
13. Soybean maturity groups IV and higher are more susceptible to soybean cyst nematode compared to earlier maturity groups (000-III).

Impact Metrics

1. Following guest lectures through the GoFarm Hawaii new farmers' training program for food crop production, farmers identified improvement of soil health (54.4%), and weed suppression benefits-soil moisture conservation (9.1%) as the key factors for adoption of cover cropping. In addition, all participants indicated managing soil health as their top priority in farm management plus the high cost of fertilizer and improving water retention for their cropping systems.
2. Between 2012 and 2022, soil organic matter, active carbon and available water potential increased in 68 of 68 geopositioned sampling sites associated with six Michigan commercial potato farms.
3. A pre-SCN Coalition survey of 950 soybean growers from multiple states and a follow-up survey three years later demonstrated that growers rotating with non-host crops increased from 71% to 77%, growers planting SCN resistant varieties increased from 59% to 66%, growers rotating genetic sources of resistance increased from 39% to 49%, growers recognizing Peking as a source of SCN resistance increased from 15% to 25%, and growers using nematode protectant seed treatments increased from 22% to 40%. The five changes were all statistically significant (P = 0.05).
4. Based on the Tennessee statewide nematode survey for newly emerging plant-parasitic nematodes of concern: *Meloidogyne incognita* was present in one-third of the 137 Solanaceous samples analyzed. *M. hapla* in 5% of the samples, and *M. enterolobii* not detected. *Xiphinema americanum* was identified morphologically and molecularly from 12 of 51 vineyards, while *X. rivesi* was detected in three vineyards.
5. Eighty-three garlic growers participated in onion bloat nematode educational sessions.

NE-2140 Technical Committee Members

CA	Andreas Westphal
FL	Billy Crow
HI	Koon-Hui Wang
IL	Carmen Ugarte
MA	Robert Wick
MI	Marisol Quintanilla
	Haddish Melakeberhan
	George Bird
NY	Frank Hay
OH	Christopher Taylor
RI	Nathaniel Mitkowski
TN	Ernest Bernard
	Lesley Schumacher
VT	Deborah Neher
WV	James Kotcon

NE-1640 Technical Committee Members Not Actively Participating in NE-2140

CT	James Lamondia	Retired
MN	Senyu Chen	

NE-2140 Brief Historical Synopsis

Although nematodes have been recorded throughout history as pathogens of humans, animals, and plants, the discipline of plant nematology was not formalized until the 20th Century. NE-2140 began in 1956 as NE-34 and has since played a major role in the development of the discipline of nematology. NE-34 began with 17 scientists representing ten northeastern states. Today, NE-2140 has 15 nematologists representing 12 states including Florida, California, and Hawaii. Throughout the years, the initiative has played a highly significant role in increasing the overall understanding of nematode biology and management options. The project provided resources for training multiple generations of nematologists and outreach interactions with the Cooperative Extension Services throughout the region and beyond. In an early example, NE-34 facilitated acquisition of NSF funds for an intensive short course for training of 30 young nematologists under the guidance of the 30 world's best nematologists. Throughout the years, under the leadership of numerus excellent Administrative Advisors, the project has been able to remain on the cutting edge of many highly significant economic issues. As the northeast region changed, the project adapted to include urban, suburban, and environmental quality matters. Recently, NE-1640 and NE-2140 have been at the forefront of the young domain of soil health biology. Now that advances in molecular biology have projected that about 85% of the animals on our planet are nematodes, NE-2140 remains key to a vast number of quality-of-life issues.