ORG Project Details

Award Year 2018

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Decision Support Tool to Optimize Establishment and Management of Alfalfa as Trap Crop in Organic Strawberry Productions

Accession No.	1017173
Project No.	CA-D-ENM-2491-CG
Agency	NIFA CALB\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2018-51106-28771
Proposal No.	2018-03574
Start Date	01 SEP 2018
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Grant Amount	$499,992
Grant Year	2018
Investigator(s)	Nansen, C.; Mauck, KE, E.; Lin, JO, .
Performing Institution	UNIVERSITY OF CALIFORNIA, DAVIS, 410 MRAK HALL, DAVIS, CALIFORNIA 95616-8671

NON-TECHNICAL SUMMARY

Lygus is considered the most serious insect pest on strawberry in California. Currently, organic strawberry growers do not have effective control strategies for Lygus management. However, it is well-known for decades that strawberry is not a preferred host plant for Lygus - alfalfa is much more attractive! Some efforts have therefore been made the last couple of deacdes to introduce alfalfa as a possible perimeter trap crop around strawberry fields. However, a major limitation to these efforts has been that no real efforts were made to control the Lygus populations in the trap crop\... Thus, there is a major risk of Lygus being drawn to the alfalfa and then afterwards gradually spread into strawberry fields. The goals of this project are to demonstrate that establishment of an alfalfa-based trap crop at the perimeter of organic strawberry fields combined with an optimized combination of vacuuming, strip cutting, and releases of natural enemies - can be used to effectively suppress Lygus pest populations in strawberry production systems. The project focus is to simultaneously optimize: 1) alfalfa-based trap crop establishment (not establish more alfalfa trap crop than is needed, and 2) required management efforts (identify when management is needed and also identify the most feasible and cost-effective combination of vacuuming, strip cutting, and releases of natural enemies). This project aligns with all four program research priorities, and the overall research hypothesis is that establishment and careful management of an alfalfa-based trap crop along the perimeter can be used to successfully manage Lygus infestations. As project outcome to strawberry growers, we will, through extensive grower involvement, develop and disseminate a user-friendly web-based decision support tool. This project has a significant educational component, as project outcomes will be included into undergraduate curricula (lectures and lab/field sessions) by the PDs at UC Davis and UC Riverside.

OBJECTIVES

OPTIMIZED ESTABLISHMENT AND MANAGEMENT OF ALFALFA TRAP CROP TO CONTROL LYGUS IN ORGANIC STRAWBERRY PRODUCTIONSThe overall project hypothesis is that establishment and careful management of an alfalfa-based (Medicago sativa) trap crop along the perimeter of organically grown strawberry

(Fragaria ananasa) can be used to successfully manage Lygus (Lygus spp. and Lygus hesperus in particular) infestations. The main project outcome to strawberry growers is a user-friendly web-based decision support tool to successfully manage Lygus pests based on alfalfa trap cropping. The innovative component of this project is that establishment of an alfalfa-based trap crop at the strawberry field perimeter is combined with an optimized combination of vacuuming, strip cutting, and releases of natural enemies to suppress Lygus populations in the alfalfa trap. Simply put - planting a highly attractive host plant immediately adjacent to a high-value crop is likely exacerbating the risk of pest infestations in the crop, unless innovative and timely targeted management tactics are deployed to suppress pest populations developing in the trap crop. Consequently, growers transitioning to trap crop based management of Lygus must be willing to invest both time and resources into management of the pest population in the trap crop. Such commitment by growers will only happen if: 1) there is clear financial evidence of this strategy being viable, and 2) it is clearly demonstrated how this strategy can be practically feasible. It is therefore critically important to determine the minimum amount of strawberry production area to be assigned to the trap crop in order to achieve effective and sustainable pest management. And it is equally critical to optimize the trap crop management to minimize the investments and to maximize the level of practical feasibility. In order to accomplish such optimization, the only viable approach is to conduct experimental research and to thoroughly analyze the generated data through a comprehensive agro-ecological modeling framework. Such modeling can be used to identify optimized recommendations to strawberry growers. However, for such model-based Lygus management recommendation to be meaningful and adoptable by strawberry growers, a user-friendly, and reliable web-based decision support tool is necessary. Furthermore, it is critically important that a user-friendly decision support tool is both developed and explained in close collaboration with strawberry growers, extension agents/educators, and agricultural consultants. This project is composed of five complementary objectives:1) Optimize production area assigned to alfalfa-based perimeter trap cropRationale: Growers must assign productive land area to plant trap crops. If alfalfa provides sufficient pest control services, this trade-off is profitable. However, at present, there is no knowledge of how much established alfalfa area is necessary to reliably ensure Lygus attraction, retention, and suppression in the trap crop.2) Maximize Lygus attraction and retention in alfalfa-based perimeter trap cropRationale: Lygus feeding enhances attractiveness of alfalfa plants to conspecifics. Our preliminary research suggests that this occurs via effects of salivary proteins on activation of anti-pathogen defense pathways. Thus, cultivars with strong anti-pathogen defenses may be more attractive to, and retentive of, Lygus bugs. Consequently, alfalfa cultivar selection will be critical to ensuring trap crop effectiveness.3) Development of cost-effective control strategiesRationale: Trap crop establishment along strawberry field perimeters as a stand-alone practice does not ensure effective Lygus management in the strawberry crop. Alfalfa must be managed, and we propose an optimized integration of three suppressive tactics, namely: vacuuming, strip cutting, and releases of natural enemies. Currently, growers have limited information and knowledge on the relative effective performance of these suppressive tactics, and there is no available decision support tool to optimize their integration and adoption by strawberry growers.4) Development and dissemination of a web-based decision support toolRationale: There is growing acknowledgement of long-term sustainable pest management solutions requiring in-depth understanding of the biological and ecological variables driving agro-ecological food webs. Furthermore, such biological and ecological understanding needs to be parameterized and integrated so that reliable and user-friendly decision support tools can be developed and used to optimize their implementation. The focus of the decision support tool is to simultaneously optimize: 1) alfalfa-based trap crop establishment (not establish more alfalfa trap crop than is needed to successfully manage Lygus populations, and 2) required management efforts (identify when management is needed and also identify the most feasible and cost-effective combination of management practices - such as: vacuuming, strip cutting, and releases of natural enemies). Data collected in Objectives 1-3 will provide the necessary information to produce such a tool for organic strawberry production.5) Education and capacity buildingRationale: The USDA\'s 2008-2010 Strategic Workforce Plan indicates a dearth of trained candidates for FANH occupations critical to the fulfillment of the USDA mission. And in relation to US demographics, proportionally few underrepresented minorities are being trained to fill these vacancies. The proposed project will provide numerous hands-on learning opportunities to recruit and educate students about FANH sciences and help prepare them for careers within the agricultural sector.

APPROACH

PDs already have access to greenhouse space, experimental vacuuming equipment, and equipment needed for volatile collection and phyto-hormone analyses. These assets represent major in-kind contributions to this project.Project objective 1 - optimize alfalfa establishment as perimeter trap cropActivity 1.1 (Extension): Stakeholder involvement. Throughout the project, we will maintain an \"open-door policy\" so that strawberry growers and other stakeholders can provide feedback to the project team.Activity 1.2 (Research): Establish experimental alfalfa trap crop designs in strawberry fields. Based on stakeholder involvement and feedback obtained as part of activity 1.1., we will establish designs for experimental field work in a minimum of four organic

strawberry fields.Project objective 2 - maximize Lygus attraction and retention in alfalfa stripsActivity 2.1 (Research): Preference bioassays. To determine whether alfalfa cultivars differ in capacity to attract and arrest Lygus, we will employ field and laboratory preference assays that relate Lygus behavior to the chemical characteristics and disease resistance traits of chosen cultivars. Volatiles will be trapped on filters containing 40mg of a broad-spectrum adsorbent (Hayesep Q), eluted with solvent, and quantified and identified using gas chromatography and mass spectrometry (as performed previously by the co-PD, see Mauck et al. 2010, Mauck et al. 2014a).Activity 2.2 (Research): Volatile analysis. Significant facilities and equipment at UC Riverside are being made available as in-kind contributions to this project activity Complementary laboratory-based volatile collection assays \83\, phyto-hormone profiling \84\, and targeted preference assays (Y-tube olfactometer choice tests) \85\ will be done using undamaged and Lygus-damaged plants to confirm field results under more controlled conditions and determine whether screening for volatile or defense-related biomarkers could function as a phenotyping tool to identify highly attractive/retentive alfalfa cultivars.Activity 2.3 (Research): Field validation. In project years 2 and 3, we will perform field validation of the two most attractive/highest volatile emitting cultivars and two least attractive/lowest volatile-emitting cultivars planted in experimental fields (see activity 1.2).Project objective 3 - development of cost-effective control strategiesActivity 3.1 (Research): Monitoring of Lygus and natural enemy population dynamics. In experimental field plots established under activity 1.2., we will conduct seasonal sampling as demonstrated in Fig. 2c. Using R software, we will examine spatial and temporal trends to natural enemy data in response to seasonal effects, distance from field edge, vacuuming, and strip cutting.Activity 3.2 (Research): Optimization of vacuuming performance. Significant facilities and equipment at Cap Poly are being made available as in-kind contributions to this project activity (for details, see proposal attachments entitled: \"facilities and other resources\" and \"equipment\"). Three of the most widely used Lygus vacuum designs within the California strawberry industry will be assessed and evaluated\87\. A tractor mounted encoder will measure vacuum application speed. Field population dynamics will be assessed via the protocol of activity 3.1. Pre- and post-vacuum measurements will follow the procedures developed by the California Strawberry Commission\93\. Confidence intervals, variance analysis, and comparison tests will be performed using MATLAB Statistics and Machine Learning toolbox\94\.Activity 3.3 (Research): Experimental testing of strip cutting of perimeter alfalfa trap crop. In experimental field plots established under activity 1.2., we will establish alfalfa strips that are cut in four-weekly intervals and we will have other plots without (control) cutting of alfalfa strips.Project objective 4 - development and dissemination of a web-based decision support tool.Activity 4.1 (Research): Temperature-dependent thresholds and requirements of Lygus. Developmental time of Lygus will be determined at seven constant temperatures (ranging from 13oC to 35oC).Activity 4.2 (Research): Field temperature data collection. At the experimental field sites developed under activity 1.2, we will place thermal and humidity sensors in strawberry and alfalfa canopies to monitor diurnal and seasonal variations in temperature conditions. Collection of thermal and humidity data from strawberry and alfalfa canopies is critically important for the temperature-dependent modeling of the Lygus population dynamics (see project activity 4.4.).Activity 4.3 (Project management): Data management. PD Nansen is responsible for quarterly updating of a cloud-based repository of project data.Activity 4.4 (Research): Decision support tool development. Experimental and field data sets collected under activities described above will be compiled and used to develop a simulation model of Lygus population dynamics in response to: 1) seasonal temperature and humidity in strawberry and alfalfa canopies, 2) landscape configuration, and 3) effects of imposed management practices. Economic data (fuel and labor costs associated with vacuuming and strip cutting, equipment maintenance/depreciation costs, costs of releases of natural enemies, strawberry yield data, and the market values of organic strawberry) will be obtained through statistics from the California Strawberry Commission.Activity 4.6 (Extension): Participatory training sessions. In addition to the grower meetings described under activity 1.1., we will contribute to Driscoll\'s seasonal kick-off meetings in Oxnard and Santa Maria and wrap-up meetings in Salinas (all major strawberry production areas in California). In addition, Driscoll\'s arranges grower tours each season. We will also work closely with the California Strawberry Commission and UC Cooperative Extension and contribute to their meetings and training sessions.Activity 4.7 (Extension): Extension dissemination. The main project outcome is a freely available web-based decision support tool to optimize Lygus management that will be hosted on a server managed by PD Nansen and will be linked directly to the UC IPM website for Lygus management in strawberry \2\. The UC IPM Director Dr Farrar has provided a strong endorsement of this proposal (see attached letter of support). In addition, we will create a social media account to share with growers and other stakeholders and provide regular project updates and links to YouTube videos and one-pagers describing the project progress. We will invite growers and other stakeholders to sign up and participate in the social media based discussion of the project.Activity 4.8 (Research). Research dissemination. We predict that a minimum of five peer-reviewed research articles will be produced on the basis of this project, and all PDs, postdocs and students contributing will be co-authors.Project objective 5 - education and capacity buildingActivity 5.1 (Education): Lectures and laboratory protocols at UC Davis. PD Nansen is in the process (his proposal to a revised curriculum is currently being reviewed by the university) of becoming the sole instructor of the course, ENT110 Arthropod Pest Management at UC Davis.Activity 5.2 (Education): Lectures and laboratory protocols at UC Riverside. Co-PD

Mauck serves a faculty mentor for UC Riverside\'s College of Agricultural and Natural Sciences \"RISE\" and \"PERSIST\" programs - both of which are targeted at providing paid internships for students to work full time in a mentor\'s laboratory.

PROGRESS

2018/09 TO 2021/08 Target Audience: Nothing Reported Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? Nothing Reported How have the results been disseminated to communities of interest? Nothing Reported What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported

IMPACT

2018/09 TO 2021/08 What was accomplished under these goals? As the project was never initiated and the funds returned in full, no products/outputs were generated. **PUBLICATIONS (not previously reported):** 2018/09 TO 2021/08 No publications reported this period.

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Ecology, Transmission and Control of Salmonella and Shigatoxin Producing E. Coli in Integrated Crop-livestock Farm

Accession No.	1017121
Project No.	MD-ANSC-03570
Agency	NIFA MD.\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2018-51106-28809
Proposal No.	2018-03570
Start Date	01 SEP 2018
Term Date	29 FEB 2020
Grant Amount	$268,079
Grant Year	2018
Investigator(s)	Biswas, D.; Parveen, SA, .; Buchanan, RO, L.; Millner, PA, .; Hashem, FA, .

NON-TECHNICAL SUMMARY

Increasing demand for organic food often is not met due to the limited number of producers engaging/adopting the management complexities fundamental to organic system and required to meet production parity with conventional systems. Complexities involve soil fertility/nutrients, diverse soil biological communities, soil health, and use of cover crops and animal manures often on integrated crop-livestock farms (ICLF). Microbial ecology on ICLF also can impact food safety through pathogen transfer between animals and crops. Recent reports of pathogen contaminated products sold in local organic retail, roadside and farmers markets highlight the need for assessment of the ecological patterns of bacterial pathogens, when they occur on ICLFs, and their survival/transfer to crops through use of raw or composted manure-based soil amendments, or by workers, equipment, and vectors. We propose to assess the ecological patterns of two major bacterial pathogens, Salmonella enterica (SE) and shigatoxin-producing E. coli (STEC), including their survival/transmission pathways and develop control strategies. The project objectives are to a) Determine the sources, survival ability in the farm environment and contamination pathways of SE and STEC; b) Develop practical means to control SE and STEC farm environment and eliminate from produce by using alternative natural antimicrobial components; and c) Disseminate outcomes of the project for controlling contamination by on-farm practices and the best options to eliminate these pathogens from the produce to diverse stakeholders. The study will generate extensive data on the dynamics of these bacterial pathogens in ICLF and its products, and offer effective control strategies.

OBJECTIVES

Major goals of this project is to evaluate the persistence of Salmonella andshigatoxin producing E. coliin the environment of intigrated crop-livestock farms (ICLFs), or in hosts/reservoirs, and the process by which produce becomes contaminated. This project will also develop effective practices to control contamination of ICLF products by these pathogens at the pre-harvest stage and assess the role of natural antimicrobial to eliminate these emerging zoonotic agents from ICLF products at the post-harvest level. The goals of this project include identification of ecological factor dynamics and paths of major produce-borne microbial pathogens in ICLFs and develop farm-friendly post-harvest processing practices to protect fresh produce from pathogen contamination sold directly or through farmer\'s markets to consumers. Development and use of science-based food production, harvesting, and handling practices will not only improve profitability for small to mid-sized organic fresh produce growers and on-farm processors, but also improve consumer/buyer confidence and reduce the risk of

gastrointestinal infections in human from consumption of contaminated produce. To achieve our research goals, we will pursue the following objectives: 1. Determine the sources, survival and persistence ability in the various components of the ICLF environment and contamination pathways of SE and STEC; 2. Develop practical means to control SE and STEC in ICLF environment and eliminate their survival on harvested fresh produce by developing and using novel farm-friendly manure composting methods and natural antimicrobial sanitizers and wash aids; 3. Disseminate outcomes of the project and measures for controlling fresh produce contamination through on-farm practices and post-harvest processing to diverse stakeholders.

APPROACH

Methods to achieve Objective 1: Determine the sources, survival ability in the farm environment and contamination pathways of major foodborne zoonotic bacterial pathogens, SE and STEC.Sources and number of samples and sampling procedure: Four major produce samples including lettuce, spinach, carrots, strawberry and tomato will be directly collected from the ground of ICLFs/backyard farms, organic crop only farm and conventional crop only farms and these samples will be treated as pre-harvest samples. In addition, as sources of pathogens, soil, compost, animal manure, animal feed, water, fecal sample from wild birds and animals, workers\' skin and instrument swabs will be collected for microbiological analysis. Similarly, four produce samples including lettuce, spinach, carrots, strawberry and tomato will be collected at post-harvest level from farmer\'s and retail markets including organic retailers such as Moms and Whole Foods and conventional retailers such as Giant and Safeway. A total of 600 samples including 200 pre-harvest produce samples, 200 environmental and sources of pathogen, and 200 post-harvest samples will be collected per visit and we will arrange at least 10 visits. A grand total of 6,000 samples will be collected by 10 visits and processed for detect of both of these bacterial pathogens, SE and STEC. In addition, manure, compost, compost-amended and unamended soil samples will be used to determine the manure and soil microbiome profiles.Sample processing and culture for bacterial pathogens: About 25 g of each sample will be cut into small pieces with a sterile scalpel and placed in a Whirl-Pak bag containing 225 ml of peptone (0.1 %) water, mechanically homogenized and centrifuged at low speed (500 × g) for 5 min to remove the debris. Then 0.1 mL of filtered suspension will be diluted and plated for direct quantitative count and the rest (0.9 mL) will be added to 9 mL of nutrient broth by overnight incubation at 37°C. After incubation, 1 mL of enriched culture will be transferred to 9 mL of selective liquid media and incubated overnight at 37°C. Bacterial pathogens will be grown on agar plates at 37°C. The whole culture methodology for isolation and identification will be performed following our previously developed methodology by Dr. Biswas. Colonies characteristics of the target bacteria will be isolated up for downstream confirmation by biochemical tests as well as by PCR assays.Phenotypic and biochemical characterization for SE and STEC isolates: The biochemical assays of SE and STEC presumptive positive isolates will be conducted according to methods described previously by Dr. Biswas. SE and STEC isolates will be confirmed using PCR assay and the pulsed-field gel electrophoresis (PFGE) method will be performed according to the PulseNet protocol published by CDC.Microbiome analysis: To investigate the composition of soil microflora and the relation to soil health, soil and compost samples collected from ICLFs, will be assayed for total bacterial and fungal communities using a metagenomic approach conducted at the Institute for Genome Sciences, University of Maryland School of Medicine under the direction of Collaborator, Dr. Ravel.Methods to achieve Objective 2: Develop practical means to control SE and STEC in ICLF environment and eliminate from produce by using alternative natural antimicrobial components.Inoculation of bacterial pathogens on lettuce, spinach, carrots, carrots, strawberry and tomatoes: The efficacy of BPE and CVO as alternative antimicrobials will be tested to remove bacterial pathogens from lettuce, spinach, carrots, strawberries and tomatoes. Two to three cultivars of each produce type, exhibiting phenotypic variation in the edible portion of each plant, e.g. semi-savoy, savoy and smooth-leaved spinach et al., will be purchased from ICLFs. Produce will be transported to labs at UMD and ARS-USDA for pathogen inoculation. Briefly, bacteria will be grown in appropriate culture media overnight, and then serially diluted in phosphate buffer saline (PBS) to final cell densities of 106 cfu/mL. Each replicate sample (leaves, root or fruit) will be sterilized in 2.5% bleach, rinsed 3 times, then spot inoculated with 100 µl aliquots, up to 1 mL per sample, and incubated at 4°C and 22°C at 65% RH for up to 3 days. Samples will then be treated with varying concentrations of BPE or CVO for different periods of time, 1 min, 20 min, 40 min, 60 min and pathogen recovery for quantification over a 48-72 h time series will be carried out on appropriate enumeration media. A series of variables will be investigated for each pathogen-commodity pair, cultivar, storage temperature, antimicrobial type and concentration.Efficacy of antimicrobials through dip treatment: Produce (lettuce, spinach, carrots, strawberry and tomatoes) will be inoculated with each bacterial strain. Inoculated produce will then be dipped in 5 L of individual bioactive extract treatment. As with the dip treatment, natural bioactive BPE, and CVO will be purchased. For BPE, the total bioactive compound concentrations used will be 0 (negative control), 1.0, 2.0 and 4.0 gm GAE per liter of deionized water. For CVO, the concentration will be 0 (control), 1.0, 5.0 and 10.0 gm/L in deionized water. Additionally, citrus oil and water pomace treatments will be combined and evaluated in a follow up study. The antimicrobial treatment combinations will be applied as single and/or multiple interventions utilizing dip applications. Inoculated produce

will be dipped in the antimicrobial for 30, 60, and 120 sec and allowed to drip for 5 min before packing. After the antimicrobial application phase, the produce will be wrapped and stored at room temperature. Cross-contamination in wash water will be evaluated using lettuce inoculated 48 hr prior to wash tests, and not inoculated, and stored at 4°C according to reported methods. A microfluidic device will be used to test pathogen inactivation at very rapid exposures at a range of dosages using procedures previously reported by other researchers.At the end,the farm level trial will be conducted at the participating farmer processing facilities.Methods to achieve Objective 3: Disseminate outcomes of the project controlling the contamination processes on farm practices and post-harvest processing to diverse stakeholders.Targeted training programs will be developed for ICLF owners, processors, workers, regulators and consumers to improve practices and their evaluation on adopting new processing technologies developed through the current project. Content suitable for delivery through Extension will be developed jointly by CFS3-UMD, ARS-USDA, and UMES.Research seminar/webinar: Design and implement a one-day applied research seminar/webinar to demonstrate the effectiveness of farming practices and the application of BPE and/or CVO in controlling contamination of SE and STEC. This initial program will be targeted to academic, government oversight personnel (USDA, FDA, etc.), third party auditors (consultants), and ICLF owners. The program will be held at CFS3-UMD, UMES and ARS-USDA. An extensive, spiral-bound notebook of the seminar materials will be provided to each participant, to include PowerPoint™ presentations, diagrams, charts, tables and other supporting information about the process. Instructors for this seminar would primarily be the researchers of this proposed grant. This seminar would also be held in Northeastern U.S.Fact sheets/bulletins development: Develop fact sheets, extension bulletins on the effectiveness and methods of use for technologies for industry and consumers. A video demonstrating the effectiveness of various technologies and their efficacy in reducing microbial contamination on produce and produce processing environment will be made available on You-Tube. The economic feasibility of each method of decontamination will also be presented. **Progress** 09/01/18 to 08/31/23 **Outputs** Target Audience:In this study, major audiences including integrated crop-livestock farmers, on farm workers at the pre-harvest level, workers/persons involved in processing, transportation, displaying and selling, people involved roadside/onfarm/farmers markets, academic staffs involved in extension activities, students, researchers, and policy makers were targeted to deliver data-based outcomes of the potential risks of cross-contamination possibility of the products produced in integrated crop-livestock farms (ICLFs). ICLFs, also known as polyface or mixed crop-livestock farms, is a common type of commercial agricultural practice associated with the production of both crops and animals on one farm which increase the possibility of cross-contamination of ICLF products. After decades of large commercial farming performance, ICLF practices are being returned and growing rapidly in the US, especially in the Mid-Atlantic and Northeast regions. A large number of ICLF farms are non-certified organic or in organic transition and contributing a significant amount of food products particularly fresh produce and meat products specifically lamb, goat meat to the US organic food supply chain. Products from ICLFs are sold in either farmers markets, roadside stands and/or local retail markets. The major efforts of this study were to evaluate the persistence of two major foodborne bacterial pathogens, Salmonella enterica (SE) and shiga toxin producing E. coli (STEC) in the environment of ICLFs, or in hosts/reservoirs, and the process by which produce becomes contaminated. This project also recommended the effective practice methods to control contamination of ICLF products by these pathogens at the pre-harvest stage and assess the role of natural antimicrobial to eliminate these emerging zoonotic agents from ICLF products at the post-harvest level. The goals of the project were to improve the safety and quality of organic fresh produce grown in ICLFs by developing and implementing a natural, easy to use and affordable antimicrobial at operational production system and/or handling practices. All stakeholders involved in producing and processing ICLF-based food products were provided data-base information with symposium, workshop, and presentation as well as one to one meeting. In addition, data collected from this study were presented in various scientific conferences as posters and oral presentations and published in reported journals for broader audiences. A website has also been developed to deliver the information to all stakeholders. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?In this study (Goal-1 and Goal-2) we visited selected farms (eight)and collected samples including environmental, animals and pre-harvest food products multiple times for microbiological analysis. We isolated Salmonella and shiga-toxin producing E. coli and determined the potential sources of these two pathogens and their route of contaminating plant products grown in the ICLFs at the pre-harvest levels. All potential risks of cross-contaminating pathways were pointed out and disseminated to farmers and workers of ICLFs. In addition, we have developed a washing method using natural antimicrobial (extract of Berry pomace/byproduct, known as BPEs) by which both pathogens were removed from the contaminated plant products (such as spinach and lettuce) within a short period of time and economically. BPEs was extracted berry (either blue or black berry). It is antioxidant and human consumable. Therefore, it can enhance the quality of leafy green product. We have also delivered this information to farmers and workers. Information was shared all participant farmers and workers in one-on-one meetings during farm visits and presented at workshops at both campuses, University of Maryland College Park and University of Maryland Eastern Shore. We also presented these outcomes at various conferences including the International Association of Food Production and American

Society for Microbiologist. In addition, twelve undergradute and sixgraduate students and twopostdoctoral fellows involved in this project learned microbiological techniques including culture, molecular analysis of isolation, identification and 16S sequencing, and data analysis. How have the results been disseminated to communities of interest?Yes, we havevisited all selected farmsand presented the outcomes of this study specifically all potential risks of cross-contaminating pathwaysto farmers and workers of these ICLFs. In addition, we have also shown thema washing method using natural antimicrobial (extract of Berry pomace/byproduct, known as BPEs) by which both pathogens were removed from the contaminated plant products (such as spinach and lettuce) within a short period of time and economically. We also explained to them why BPEs are important (It was extracted berry (either blue or black berry), and itis antioxidant and human consumable. Therefore, it can enhance the quality ofleafy green products). We have disseminated the information to the all participant farmers and workers in one-on-one meetings during farm visits.We have also organized workshops and symposiums at both the University of Maryland Eastern Shore and University of Maryland College Park andpresented using PowerPoint slides/photographs. Major workshops/symposiums/presentations for appropriate stakeholders were: 1) Contamination Risks Associated with Biological Soil Amendments on the Eastern Shore of Maryland. UMES Annual Small Farms Conference, UMES, Princess Anne, MD, November 2023. 2) Ecology, transmission and control of Salmonella and Shiga toxin producing E. coli in integrated crop-livestock farms. Accepted at International Association for Food Protection (IAFP), Toronto, Canada, July 2023. 3)Ecology, transmission and control of Salmonella and Shiga toxin producing E. coli in integrated crop-livestock farms. USDA-NIFA-OREI Meeting, Washington DC, April 2023. 4) Brian Goodwyn and Salina Parveen. 2022. Reducing Cross Contamination between Animals and Produce. UMES Annual Small Farms Conference, UMES, Princess Anne, MD, November 2022. 5) Impact of seasonal variation on the composition of soil microbiome in conventional dairy farms and associated risk of pathogen transmission. International Association of Food Protection, Pittsburgh, PA, August 2022. 6) Reducing Cross Contamination between Animals and Produce. UMES Annual Small Farms Conference, UMES, Princess Anne, MD, November 2021. 7) Prevalence of Salmonella and shiga toxin producing E. coli in organic/pasture foods in Maryland/Washington DC area. University of Maryland Extension Meeting/Cornerstone Program, UMD, 2020. 8)Safer food production in Integrated Crop-Livestock Farms, Center for Food Safety and Security Systems, UMD, October 2019. Finally, we have developed a website (https://biswas-mixedfarms.umd.edu/) and added all data from this study using PowerPoint slides and photographs for all types of audience including farmers and workers. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported **Impacts** What was accomplished under these goals? For achieving goal-1, we have collected more than 5,656 samples from eight ICLFs in Maryland specifically Eastern shore (Princess Anne County) and College Park (Prince George County) and Washington DC areas for determining the prevalence of Salmonella enterica (SE) and pathogenic E. coli, particularly shiga toxin producing E. coli (EHEC). The sample categories included water, feces, feed, soil, compost, bedding, grass from the grazing areas, and bedding. From these samples, we have identified 315 SE and 281 EHEC using microbiological culture. Then, we confirmed the isolates using molecular techniques including PCR and qRT-PCR. Analyzing various sources of these pathogens, environment specifically manure or animal waste particularly feces are the major sources of these bacterial pathogens. In addition, wild animals, birds, pest, and rodents also possess risks of cross-contamination of food products and spread zoonotic diseases. We have aimed to characterize the influence of aged or composted manure application on the diversity of soil bacterial community in ICLFs. Soil samples from six ICLFs in Maryland were collected before (pre-crop) and during the season (2020-2021) and use to analyze soil bacterial microbiome by 16S rDNA sequencing. Results showed that both phylum- and genus-level alterations of soil bacterial communities were associated with amendment of aged or composted manure. Particularly, Proteobacteria and Actinobacteria were enriched, while Acidobacteria, Bacteroidetes, Planctomycetes, Firmicutes, and Chloroflexi were reduced after manure product application. Meanwhile, the relative abundance of Bacillus was decreased, while two zoonotic pathogens, Salmonella and Listeria, were enriched by manure amendments. Overall, animal manure amendment of soil increased the phylogenetic diversity but reduced the richness and evenness of the soil bacterial communities. Although manure composting management in ICLFs benefits agricultural sustainable production, the amendments altered the soil bacterial communities and were associated with the finding of two major zoonotic bacterial pathogens, which raises the possibility of their potential transfer to fresh horticultural produce crops that may be produced on the manured soils and then subsequently consumed without cooking. We have also collected additional composite soil samples pre- and post-manure/compost amendment from ICLFs into sterile Whirl-Pak bags. These soils were sent to AgroLab in Harrington, DE per their instructions for the basic soil fertility (BSF) soil test analysis, which used the Mehlich 3 extraction method. The BSF test showed how manures/compost introduction affected the following soil properties: pH, buffer pH, Phosphorous, Potassium, Calcium, Magnesium, sodium, saturation ofH, KCa, Mg, Na, and Organic Matter. The results showedthe effectiveness of using biological soil amendments to replenish soil health. We have also collected forearm skin swabs from farm workers and the control group, and investigated the environmental influences introduced by livestock/poultry operations on forearm skin microflora of on-site farm workers. A total of 30 human skin swab samples were collected from 20 animal workers in dairy or

integrated farms and 10 healthy volunteer controls. The skin microbiome was 16S metagenomics sequenced with Illumina MiSeq system. For skin microbial community analysis, the abundance of major phyla and genera as well as alpha and beta diversities were compared across the groups. We identified distinctive microbial compositional patterns on the skin of workers in farms with different animal commodities. Workers in integrated farms containing various animals were associated with a higher abundance of epidermal Proteobacteria, especially Pseudomonas and Acinetobacter, but lower Actinobacteria, especially Corynebacterium and Propionibacterium. For those workers with frequent dairy cattle operations, their Firmicutes in the forearm skin microbiota were enriched. Furthermore, farm animal operations also reduced Staphylococcus and Streptococcus as well as modulated the microbial biodiversity in farm workers\' skin microbiome. The alterations of forearm skin microflora in farm workers, influenced by their frequent farm animal operations, may increase their risk in skin infections with unusual pathogens and epidermal diseases. This data is published in the journal of Microorganism. For achieving goal-2, we have developed a washing method for reducing pathogenic microbes in produce samples. In avoiding the use of antibiotics and chemicals, as well as the non-professional harvesting and packaging methods, there is an increased risk of cross-contamination with zoonotic pathogens. In this study, we have evaluated the efficiency of new post-harvest disinfection processes using natural berry pomace extracts (BPE) to reduce the bacterial load found in two common leafy-greens, spinach, and celery. Both spinach and celery were inoculated with a fixed bacterial load of Salmonella Typhimurium (ST) and later soaked in BPE supplemented water (wBPE) for increasing periods of time, and at two different temperatures (24°C and 4°C). The remaining live bacteria were quantified in Log of colony forming units (CFU)/leaf and compared to that of vegetables soaked in only water. The relative expression of virulence genes (hilA1/C1/D1, invA1/C1/E1/F1) of wBPE treated ST was determined. Results show that in the case of spinach, there was a significant (p\<0.05) reduction of ST from a range of 0.2 to1.2 log CFU/mL and 0.5 to 5 log CFU/mL at 24°C and 4°C, respectively. For celery there was also a significant (p\<0.05) reduction of ST at either 24°C or 4°C. The changes in relative expression of virulence genes of ST isolated from spinach and celery varied depending on the treatment conditions but showed a significant downregulation of inv genes when treated at 24°C for 1440 minutes (p\<0.05). After 7 uses of wBPE, the concentration of total polyphenolic compounds remains at the effective concentration. This research suggests that soaking these vegetables with BPE containing water at lower temperatures can still reduce load of ST enough to minimize risk of infection and alter the virulence properties. This data is published in the journal of Food Protection. For achieving goal-3, wehad a great opportunity to work with a group of farmers in two different regions of Maryland, six from Price Geroge County/Washing DC metro area and sic from Princess Anne County. All farmersare kind enough to work with us and allowed us to visit their farms and collect samples including preharvest products and skin swab of their workers. During these research activities, we had the opportunity to learn about their farming practices and discussed the potential ways to improve the safety practices. When we organized the workshop and symposium, we also had the opportunity to share our research outcomes with additional farmers, workers, researchers, students, and consumers. We have visited several farms repeatedly and collected samples. We have also developed a washing method of produce using natural antimicrobial components for controlling bacterial contamination. We expect this information will help the farmers to add natural antimicrobial products to water to clean the products before displaying for sale. In addition, we have provided information on the ways in which products could be cross contaminated through the consumer touches and handing/shipping. Finally, we have organized symposiums and workshops in both regions of Maryland anddeveloped a website, open to everyone, specifically farmers. On the website, we have added guidelines for the audience for better practice and improving the safety of food products produced in ICLFs. **Publications** - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Goodwyn, B, Punchihewage Don, P., Millner, P., Schwarz, G., Meredith, J., D. Biswas, D., Parveen, S. Presence of Indicator and Foodborne Pathogens from Pre- and Post-Harvest Integrated Crop-Livestock Farm Environments and Fresh Produce on the Eastern Shore of Maryland. Accepted, Annual Meeting of International Association for Food Protection, July 31 to August 3,2022, Pittsburg, PA - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Goodwyn, B, Punchihewage Don, P., Millner, P., Schwarz, G., Meredith, J., D. Biswas, D., Parveen, S. Examination of the Presence of Salmonella Typhimurium in Integrated Crop-Livestock Farm Environments on the Eastern Shore of Maryland. Accepted, Annual Meeting of International Association for Food Protection, July 31 to August 3,2022, Pittsburg, PA - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Goodwyn, B., Punchihewage A., Millner, P., Biswas, D., and Parveen, S. Examination of the Presence of Shiga Toxin-producing Escherichia coli in Integrated Crop-Livestock Farm Environments on the Eastern Shore of Maryland. American Society for Microbiologist (ASM) annual meeting, Washington, DC, June 9-13, 2022. - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Aditya, A., Peng, M., Suh, G and Biswas, D. Prevalence and Antibiotic Resistance of Pathogenic E. coli in Dairy Farm. International Association for Food Protection (IAFP), Jul 18-21, 2021, Phoenix, Arizona. - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Alvarado-Martinez, Z. and Biswas, D. Purified plant-derived phenolic acids on Salmonella Typhimurium and in ex vivo simulated gut microbiota. International Association for Food Protection (IAFP), Jul 18-21, 2021,

Phoenix, Arizona. - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Alvarado-Martinez, Z. and Biswas, D. Prevalence of Salmonella in Integrated Crop-Livestock and Dairy Farms in Maryland-Washington DC. International Association for Food Protection (IAFP), Jul 18-21, 2021, Phoenix, Arizona. - Type: Journal Articles Status: Published Year Published: 2023 Citation: Aditya, A., Tabashsum, Z., Alvarado-Martinez, Z., Tung, C. and Biswas, D. 2023. Diarrheagenic E. coli and Their Antibiotic Resistance Pattern in Dairy Farm and Its Microbial Ecosystems. Journal of Food Protection 86(3): 100051 - Type: Journal Articles Status: Published Year Published: 2021 Citation: Peng, M., Tabashsum, Z., Miller, P., Parveen, S., and Biswas, D. 2021. Influence of Manure Application on the Soil Bacterial Microbiome in Integrated Crop-Livestock Farms in Maryland. Microorganisms 9(12): 2586. - Type: Journal Articles Status: Accepted Year Published: 2023 Citation: Goodwyn, B., Punchihewage, A., Millner, P., Meredith, F. Hashem, F., Biswas, D. and Parveen, S. 2023. Survival and Persistence of Indicator Bacteria and Foodborne Pathogens in Spatially Separated Integrated Crop-Livestock Farm Environments. Frontiers in Sustainability, Volume 7, \| https://doi.org/10.3389/fsufs.2023.1287371 - Type: Journal Articles Status: Published Year Published: 2023 Citation: Alvarado-Martinez Z, Julianingsih D, Tabashsum Z, Aditya A, Tung C-W, Phung A, Suh G, Hshieh K, Wall M, Kapadia S, Canagarajah C, Maskey S, Sellers G, Scriba A and Biswas D. 2023 Assessment of the prevalence, serotype, and antibiotic resistance pattern of Salmonella enterica in integrated farming systems in the Maryland-DC area. Front. Microbiol. 14:1240458. doi: 10.3389/fmicb.2023.1240458 - Type: Journal Articles Status: Published Year Published: 2020 Citation: Alvarado-Martinez, Z., Bravo, P., Kennedy, N., Krishna, M., Hossain, S., Young, A., and Biswas, D.* 2020. Antimicrobial and ant virulence impacts of phenolics on Salmonella Enterica Serovar Typhimurium. Antibiotics, 9, 668; doi:10.3390/antibiotics9100668. - Type: Journal Articles Status: Published Year Published: 2020 Citation: Peng, M. and Biswas, D.* 2020. Environmental influences of high-density agricultural animal operation on human forearm skin microflora. Microorganisms, 8(10), 1481; https://doi.org/10.3390/microorganisms8101481 - Type: Journal Articles Status: Submitted Year Published: 2023 Citation: Aditya, A., Julianingsih, D., Tabashsum, Z., Alvarado-Martinez, Z., Tung, C., Wall, M. and Biswas, D. 2023. Dominance of diarrheagenic E. coli in integrated crop-livestock farms and their antibiotic resistance patterns. - Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Alvarado-Martinez, Z., Tabashsum, Z., Aditya, A. Tung, C. and Biswas, D. Analyzing predominant serotypes and antibiotic resistance profiles of Salmonella enterica isolated from integrated farms in the MD/DC area. International Association for Food Protection (IAFP), Jul 16-19, 2023, Toronto, Canada. - Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Goodwyn, B., Punchihewage, A., Millner, P., Meredith, F. Hashem, F., Biswas, D. and Parveen, S. Detection and Prevalence of Major Foodborne Pathogens in Integrated Crop-Livestock Farms and Post-Harvest Products on the Eastern Shore of Maryland. International Association for Food Protection (IAFP), Jul 16-19, 2023, Toronto, Canada. - Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Goodwyn, B., Punchihewage, A., Millner, P., Meredith, F. Hashem, F., Biswas, D. and Parveen, S. Persistence of Foodborne Pathogens in Biologically Amended Soils and Produce on Integrated Crop-Livestock Farms on the Eastern Shore of Maryland. International Association for Food Protection (IAFP), Jul 16-19, 2023, Toronto, Canada. - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Aditya, A and Biswas, D. Impact of Seasonal Variation in Soil Bacterial Microbiome of Dairy Farms and Risks Associated with Pathogen Transmission. International Association for Food Protection (IAFP), Jul 29- August 4, 2022, Pittsburg, Pennsylvania. - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Goodwyn, B, Punchihewage Don, P., Millner, P., Schwarz, G., Meredith, J., D. Biswas, D., Parveen, S. Examination of the Presence of Shiga Toxin-producing Escherichia coli in Pre- and Post-Harvest Farm Environments at the Eastern Shore of Maryland. Accepted, American Society for Microbiology Annual Meeting, May-2022, DC. **Progress** 09/01/21 to 08/31/22 **Outputs** Target Audience:Food safety researchers and professionals including academicians, scientists, researchers, students specifically graduate students, quality control and HACCP representatives, farmers, animal feed suppliers, veterinarians, animal food product processing plant staff, and state/federal (USDA-NIFA, FSIS, FDA) regulatory authorities. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?Under this project activities, several graduate students and a postdoctoral fellow were trained. They were extensivelyexperiencedin microbiological analysis specificallypathogen detection and confirmation and determine antibiotic resistant patterns. During our extension activities, we provided the information about the potential ways to improvethe mixed crop-livestock farming andmore than 20 farmers were able to join there. How have the results been disseminated to communities of interest?As I mentioned above, we have disseminated the outcomes of our research partly to the farmers, graduate students, and researchers. What do you plan to do during the next reporting period to accomplish the goals?A website is under preparation. On the website, we will provide details outcome of our study to guide the farmers and other stakeholder to improve their practice for producing safer foods which are particularly produced in mixed crop-livestock farms. In addition, we will publish rest of the data in respective journals. **Impacts** What was accomplished under these goals? So far, we have collected >5,000 specimens including soil, compost, animal feces, and produce from various farm

environments. We also performed microbiological analysis to detect the major foodborne bacterial pathogens specifically Salmonella and EHEC. Following the standard microbiological culture methods, we isolated >265 Salmonella and >250 EHEC (including previous isolates) and confirmed the pathogens using molecular techniques and determined the antibiotic resistance pattern. We also tested alternative antimicrobial components against the isolated pathogens to analyze their effects as inhibiting or controlling agents using produce sample. Now we are preparing manuscripts. **Publications** - Type: Journal Articles Status: Published Year Published: 2021 Citation: Influence of Manure Application on the Soil Bacterial Microbiome in Integrated Crop-Livestock Farms in Maryland. **Progress** 09/01/20 to 08/31/21 **Outputs** Target Audience:Graduate students, researchers, extention specialists, and farmers Changes/Problems:Due to the Covid-19 restrictions, we experienced some delays and are trying to catch up. What opportunities for training and professional development has the project provided? Nothing Reported How have the results been disseminated to communities of interest?Information is being disseminated to farmers, students, researchers, extension agents, and other stakeholders through scientific presentations. What do you plan to do during the next reporting period to accomplish the goals?We will continue to collect more farm samples and perform microbiological analysis. We will also determine the antimicrobial resistance pattern of the isolated pathogens and develop alternative inhibiting or controlling agents. We will prepare more manuscripts, present in conferences, and organize workshops. **Impacts** What was accomplished under these goals? We have collected more than 1,000 samples including soil, compost, animal feces, and produce from various farm environments that were cultured for microbiological analysis. We confirmed the pathogens using molecular techniques and determined the antibiotic resistance pattern. We also tested alternative antimicrobial components against the isolated pathogens to analyze their effects as inhibiting or controlling agents in the sample produce. We presented the research in conference and extension activities at the UMD. **Publications** - Type: Journal Articles Status: Published Year Published: 2020 Citation: Environmental Influences of High-Density Agricultural Animal Operation on Human Forearm Skin Microflora. Microorganisms 8(10). DOI: 10.3390/microorganisms8101481 **Progress** 09/01/19 to 08/31/20 **Outputs** Target Audience: Nothing Reported Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?Four graduate students and a postdoctoral fellow are involved in this project and they have learned microbiological techniques including culture, molecular analysis of isolation, identification and 16S sequencing, and data analysis. How have the results been disseminated to communities of interest? Nothing Reported What do you plan to do during the next reporting period to accomplish the goals?We are planning to revisit the farm and collect more produce samples and soil samples for microbiological and molecular analysis including 16S sequencing for comparing soil contamination during 'green' fertilizer application and while crops are growing. We are also planning to disseminateour research results to the appropriate stakeholders including farmers,food producers, processors, researchers and regulators. **Impacts** What was accomplished under these goals? To achieve goal-1, we have collected800 samples from 4 mixed farms in Maryland and Washington DC areas for determining the prevalence of Salmonella and pathogenic E. coli. The sample categories included water (n=101), feces (n=114), feed (n=95), soil (n=113), compost (n=45), bedding (n=31), grass (n=50), and bedding (n=251). For Salmonella, we are still performing PCR confirmation for the total of 114 (14.25%) presumptive isolates. For pathogenic E. coli, we identified 10.89% positive rate for water samples, 0.88% for fecal samples, 7.37% for feed samples, 1.77% for soil samples, 6.45% for bedding samples, 6.00% for grass samples, and 8.76% for produce samples, which provide a total positive rate of 6.00% among all the samples collected from the mixed farms.Among the total of 70 PCR-confirmed pathogenic E. coli isolates, 14.29% was identified as Enteropathogenic E. coli (EPEC), 24.29% was Shiga toxin-producing E. coli (STEC), 51.43% was Enteroinvasive E. coli (EIEC), 10.00% was Enteroaggregative E. coli (EAEC). We also collected forearm skin swabs from farm workers and the control group, and investigated the environmental influences introduced by livestock/poultry operations on forearm skin microflora of on-site farm workers. A total of 30 human skin swab samples were collected from 20 animal workers in dairy or integrated farms and 10 healthy volunteer controls. The skin microbiome was 16S metagenomics sequenced with Illumina MiSeq system. For skin microbial community analysis, the abundance of major phyla and genera as well as alpha and beta diversities were compared across the groups. We identified distinctive microbial compositional patterns on skin of workers in farm with different animal commodities. Workers in integrated farms containing various animals were associated with higher abundance of epidermal Proteobacteria, especially Pseudomonas and Acinetobacter, but lower Actinobacteria, especially Corynebacterium and Propionibacterium. For those workers with frequent dairy cattle operations, their Firmicutes in the forearm skin microbiota were enriched. Furthermore, farm animal operations also reduced Staphylococcus and Streptococcus as well as modulated the microbial biodiversity in farm workers' skin microbiome. The alterations of forearm skin microflora in farm workers, influenced by their frequent farm animal operations, may increase their risk in skin infections with unusual pathogens and epidermal diseases. To achieve goal-2, we have developed a washing method for reducing pathogenic microbes in produce samples. In avoiding the use of antibiotics and chemicals, as well as the non-professional harvesting and packaging methods, there is an increased risk of cross-contamination with zoonotic pathogens. In this study, we have evaluated the efficiency of new post-harvest

disinfection processes using natural berry pomace extracts (BPE) as a means to reduce the bacterial load found in two common leafy-greens, spinach and celery. Both spinach and celery were inoculated with a fixed bacterial load of Salmonella Typhimurium (ST) and later soaked in BPE supplemented water (wBPE) for increasing periods of time, and at two different temperatures (24°C and 4°C). The remaining live bacteria were quantified in Log of colony forming units (CFU)/leaf and compared to that of vegetables soaked in only water. The relative expression of virulence genes (hilA1/C1/D1, invA1/C1/E1/F1) of wBPE treated ST was determined. Results show that in the case of spinach, there was a significant (p<0.05) reduction of ST from a range of 0.2 to1.2 log CFU/mL and 0.5 to 5 log CFU/mL at 24°C and 4°C, respectively. For celery there was also a significant (p<0.05) reduction of ST at either 24°C or 4°C. The changes in relative expression of virulence genes of ST isolated from spinach and celery varied depending on the treatment conditions but showed a significant downregulation of inv genes when treated at 24°C for 1440 minutes (p<0.05). After 7 uses of wBPE, concentration of total polyphenolic compounds remains at the effective concentration. This research suggests that soaking these vegetables with BPE containing water at lower temperatures can still reduce load of ST enough to minimize risk of infection and alter the virulence properties. **Publications** - Type: Journal Articles Status: Published Year Published: 2020 Citation: Journal of Food Protection (2020) 83 (8): 14631471 1. Alvarado-Martinez, Z., Tabashsum, Z., Salaheen, S., Mui, C., Lebovic, A., Gaspard, S., Datillio, A., Young, A., Kennedy, N. and Biswas, D.* 2020. Growth inhibition and alternation of virulence genes of Salmonella on produce products treated with polyphenolic extracts from berry pomace. Journal of Food Protection (In Press). doi: 10.4315/JFP-20-038.

PROGRESS

2018/09 TO 2019/08 Target Audience:Target audience are organic integrated crop-livestock farmers (ICLF) specifically in the northeast region. We have started communicating with farmers and are collecting samples from various types of farms. At the same time, we have also started to analyse the sample for pathogens prevalence. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?Personnel involved in this project have all received biological, chemical and hygienic safety training.All were trained in BSL-2 procedures for handling of chick/tissues potentially containing Salmonella and shiga toxin producing E. coli via annual courses approved and directed by the University of Maryland Department of Safety and Occupational Health. How have the results been disseminated to communities of interest? Nothing Reported What do you plan to do during the next reporting period to accomplish the goals?For the next two years of this study, we will evaluate the persistence of Salmonella and siga-toxon producing E. coli in the environment of ICLFs, or in hosts/reservoirs, and the process by which produce becomes contaminated. Depending on the microbiological data of the collected sample, we will also develop effective practices to control contamination of ICLF products by these two enteric pathogens at the pre-harvest stage and assess the role of natural antimicrobial to eliminate these emerging zoonotic agents from ICLF products at the post-harvest level. The main objectives of this project are to identify the ecological factors and paths of major produce-borne microbial pathogens in ICLFs and develop farm-friendly post-harvest processing practices to protect fresh produce from pathogen contamination sold directly or through farmer\'s markets to consumers. We will determine the sources, survival and persistence ability in the various components of the ICLF environment and contamination pathways of SE and STEC. In the 3rd and last year, we will disseminate outcomes of the project and measures for controlling fresh produce contamination through on-farm practices and post-harvest processing to diverse stakeholders.

2019/09 TO 2020/08 Target Audience: Nothing Reported Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?Four graduate students and a postdoctoral fellow are involved in this project and they have learned microbiological techniques including culture, molecular analysis of isolation, identification and 16S sequencing, and data analysis. How have the results been disseminated to communities of interest? Nothing Reported What do you plan to do during the next reporting period to accomplish the goals?We are planning to revisit the farm and collect more produce samples and soil samples for microbiological and molecular analysis including 16S sequencing for comparing soil contamination during \'green\' fertilizer application and while crops are growing. We are also planning to disseminateour research results to the appropriate stakeholders including farmers,food producers, processors, researchers and regulators.

IMPACT

2018/09 TO 2019/08 What was accomplished under these goals? As the Integrated Crop-Livestock Farms (ICLFs) in the USA specifically Maryland-Virginia-Pennsylvania-Delaware mostly grow both components crops and animals together in summer (June to September), we are required to wait to collect the sample from the farms. All key personnel of the project, PI, Dr. Biswas, Co-PIs, Dr. Parveen, Dr. Hashem, Dr. Millner and Postdoctoral fellow, Dr. Pen have met and made the plan for communicating with the targeted farmers and collecting sample in Eastern shore and MD-DC-PA area. Funding was reallocated/distributed to the Co-PIs. We have visited several farms and started to collect the sample for microbiological analysis. Collection of samples from farms and farm workers/machineries will be continued until September 2019 and the repeating of sample collection will be done in summer 2020. **PUBLICATIONS (not previously reported):** 2018/09 TO 2019/08 No publications reported this period.

2019/09 TO 2020/08 What was accomplished under these goals? To achieve goal-1, we have collected800 samples from 4 mixed farms in Maryland and Washington DC areas for determining the prevalence of Salmonella and pathogenic E. coli. The sample categories included water (n=101), feces (n=114), feed (n=95), soil (n=113), compost (n=45), bedding (n=31), grass (n=50), and bedding (n=251). For Salmonella, we are still performing PCR confirmation for the total of 114 (14.25%) presumptive isolates. For pathogenic E. coli, we identified 10.89% positive rate for water samples, 0.88% for fecal samples, 7.37% for feed samples, 1.77% for soil samples, 6.45% for bedding samples, 6.00% for grass samples, and 8.76% for produce samples, which provide a total positive rate of 6.00% among all the samples collected from the mixed farms.Among the total of 70 PCR-confirmed pathogenic E. coli isolates, 14.29% was identified as Enteropathogenic E. coli (EPEC), 24.29% was Shiga toxin-producing E. coli (STEC), 51.43% was Enteroinvasive E. coli (EIEC), 10.00% was Enteroaggregative E. coli (EAEC). We also collected forearm skin swabs from farm workers and the control group, and investigated the environmental influences introduced by livestock/poultry operations on forearm skin microflora of on-site farm workers. A total of 30 human skin swab samples were collected from 20 animal workers in dairy or integrated farms and 10 healthy volunteer controls. The skin microbiome was 16S metagenomics sequenced with Illumina MiSeq system. For skin microbial community analysis, the abundance of major phyla and genera as well as alpha and beta diversities were compared across the groups. We identified distinctive microbial compositional patterns on skin of workers in farm with different animal commodities. Workers in integrated farms containing various animals were associated with higher abundance of epidermal Proteobacteria, especially Pseudomonas and Acinetobacter, but lower Actinobacteria, especially Corynebacterium and Propionibacterium. For those workers with frequent dairy cattle operations, their Firmicutes in the forearm skin microbiota were enriched. Furthermore, farm animal operations also reduced Staphylococcus and Streptococcus as well as modulated the microbial biodiversity in farm workers\' skin microbiome. The alterations of forearm skin microflora in farm workers, influenced by their frequent farm animal operations, may increase their risk in skin infections with unusual pathogens and epidermal diseases. To achieve goal-2, we have developed a washing method for reducing pathogenic microbes in produce samples. In avoiding the use of antibiotics and chemicals, as well as the non-professional harvesting and packaging methods, there is an increased risk of cross-contamination with zoonotic pathogens. In this study, we have evaluated the efficiency of new post-harvest disinfection processes using natural berry pomace extracts (BPE) as a means to reduce the bacterial load found in two common leafy-greens, spinach and celery. Both spinach and celery were inoculated with a fixed bacterial load of Salmonella Typhimurium (ST) and later soaked in BPE supplemented water (wBPE) for increasing periods of time, and at two different temperatures (24°C and 4°C). The remaining live bacteria were quantified in Log of colony forming units (CFU)/leaf and compared to that of vegetables soaked in only water. The relative expression of virulence genes (hilA1/C1/D1, invA1/C1/E1/F1) of wBPE treated ST was determined. Results show that in the case of spinach, there was a significant ($p\<0.05$) reduction of ST from a range of 0.2 to1.2 log CFU/mL and 0.5 to 5 log CFU/mL at 24°C and 4°C, respectively. For celery there was also a significant ($p\<0.05$) reduction of ST at either 24°C or 4°C. The changes in relative expression of virulence genes of ST isolated from spinach and celery varied depending on the treatment conditions but showed a significant downregulation of inv genes when treated at 24°C for 1440 minutes ($p\<0.05$). After 7 uses of wBPE, concentration of total polyphenolic compounds remains at the effective concentration. This research suggests that soaking these vegetables with BPE containing water at lower temperatures can still reduce load of ST enough to minimize risk of infection and alter the virulence properties. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 Type: Journal Articles Status: Published Year Published: 2020 Citation: Journal of Food Protection (2020) 83 (8): 1463&#65533;1471 1. Alvarado-Martinez, Z., Tabashsum, Z., Salaheen, S., Mui, C., Lebovic, A., Gaspard, S., Datillio, A., Young, A., Kennedy, N. and Biswas, D.* 2020. Growth inhibition and alternation of virulence genes of Salmonella on produce products treated with polyphenolic extracts from berry pomace. Journal of Food Protection (In Press). doi: 10.4315/JFP-20-038.

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Transition to Organic in Row-crop Systems: Enhancing Sustainability in Topographically Diverse Midwest Landscapes

Accession No.	1017064
Project No.	MICL05109
Agency	NIFA MICL\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2018-51106-28779
Proposal No.	2018-03546
Start Date	01 SEP 2018
Term Date	31 AUG 2021
Grant Amount	$499,998
Grant Year	2018
Investigator(s)	Kravchenko, A.

NON-TECHNICAL SUMMARY

Transitioning Midwest row crops to organic production will generate valuable environmental benefits; however, there are economic and agronomic challenges during the transition period. The goal of this project is to develop sustainable transition scenarios on topographically diverse agricultural landscapes. We will explore cover crop enhancements of the transition corn-soybean-wheat rotation, the transition system regarded at present as the most profitable. Novel cover crop-based enhancement approaches that will be tested are early cover crop planting in corn, use of cover crop mixtures in corn and wheat, and topography driven site-specific cover cropping. Knowledge gaps that will be filled: mechanisms behind cover crop-driven soil carbon sequestration, magnitudes of soil biodiversity benefits in topographically diverse landscapes, benefits of cover crop mixtures, benefits of site-specific cover crop management as a function of terrain and weather, and improved knowledge of transition economics. Integrated education and extension activities will allow transfer of our findings and recommendations on cover crop optimization during the transition in a corn-soybean-wheat rotation to: (i) farmers, (ii) extension educators, (iii) various agencies involved with cover crop recommendations, and (iv) our students in the classroom.

OBJECTIVES

The overall goal of the proposed project is to develop economically viable, feasible, and environmentally sustainable transition scenarios in row crop systems on topographically diverse agricultural landscapes. The transition scenarios are expected to provide sustainability enhancement via (a) increasing soil organic matter (SOM), (b) maximizing soil biodiversity, (c) maximizing N use efficiency, including reduction in soil N losses and nitrous oxide emissions, (d) controlling weeds, (e) controlling soil erosion, (f) maximizing water use efficiency.

APPROACH

The main methods that will be employed by the project are 1) experimental work at field sites and on-farm and 2) modeling for present/future climate patterns of crop/soil processes and cover crop mixtures in 3D terrain. We will evaluate magnitudes of improvement in ecosystem services of carbon sequestration, soil biodiversity, weed management, and nitrogen use efficiency that can be achieved by cover crop enhanced transitions. **Progress** 09/01/18 to 08/31/22 **Outputs** Target Audience:Researchers in agriculture, agronomy, soil science, weed

science, agricultural economics, farmers interested in sustainable agricultural management and organic farmining, students of agricultural sciences, general public Changes/Problems: As everybody else's work, the progress of the project has been delayed by COVID. However, due to obtained no-cost extension we were able to complete and report all planned experimental work. What opportunities for training and professional development has the project provided? Over its duration the project provided support to two M.S. students and two post-doctoral research associates,and provided work opportunities for 6 undergraduate students helping with field and lab research tasks How have the results been disseminated to communities of interest? The preliminary and then the finalized results of the project were reported at several scientific conferences and described in 4 peer-reviewed manuscripts (three already published and one in preparation for submission). The full list is in the publications section of the project. The producers, crop consultants, and general public were informed about the project's work in the context of the overall importance of organic production and the positive role of cover cropping. That was accomplished via during 2-4 annual extension events taking place throughout the project duration. The specific events are listed in the project's annual reports. Among the information events were field days, in-person and online (during COVID restrictions) extension meetings and webinars, as well as publications ofinformation bulletins. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported **Impacts** What was accomplished under these goals? A three-year study in transitioning to organic production was conducted at 4 experimental fields at Kellogg Biological Station (KBS) (42° 24′N, 85° 24′W), Michigan.The experimentstarted in May 2018 for three of the fields and in May 2019 for an additional field. Prior to the start of the experiment soil samples for baseline analysis were collected from 0-20 cm depth and analyzed for soil texture and total C and N contents.The crop rotation during the three years of the transition consisted of corn in year 1, soybean in year 2, and winter wheat in year 3. Three cover crop systems and control transition scenarios were explored: traditional cover scenario with rye cover following corn harvest and red clover interseeded into winter wheat, a winter hardy cover crop mixture of ryegrass, crimson clover and brassica, and a winter susceptible mixture of oats, winter pea and radish. The cover crop mixtures were first interseeded into young corn during the first year of the transition and then planted again after the winter wheat harvest during the third year. During the experiment we monitored a large number of soil characteristics of potential importance to soil health, including but not limited tosoil organic C, soil total and inorganic N, microbial biomass C, short-term C mineralization, soil particulate organic matter, and soil aggregate size distributions. We also studied greenhouse gas emissions under different cover crop scenarios. We explored the role of cover crops on formation and architecture of soil pores using X-ray computed micro-tomography tools. The results were reported in three published peer-revied manuscripts and at several national conferences. The work on the project was also presented to farmers and general public at several field day and extension events. The project supported two graduate students, two post-doctoral associates and several hourly-paid undergraduate student assistants. **Publications** - Type: Journal Articles Status: Published Year Published: 2022 Citation: Lucas, M., L.T.T. Nguyen, A. Guber, and A. Kravchenko. 2022. Cover crop influence on pore size distribution and biopore dynamics: enumerating root and soil faunal effects. Frontiers in Plant Science-Functional Plant Ecology: doi.org/10.3389/fpls.2022.928569. - Type: Conference Papers and Presentations Status: Other Year Published: 2021 Citation: Singh, Sukhdeep, Linh T.T. Nguyen, Lisa K. Tiemann, Karen T.T. Renner, and A. Kravchenko. 2021. Effects of Inter-Seeded Cover Crop Mixtures on Soil Properties in Topographically Diverse Agricultural Landscapes during Organic Transition Period. Presented at the annual meeting of Soil Science Society of America, Nov 7-10, Salt Lake City, Utah, USA. - Type: Journal Articles Status: Other Year Published: 2022 Citation: Singh, Sukhdeep, Linh T.T. Nguyen, Lisa K. Tiemann, Karen T.T. Renner, and A. Kravchenko. 2022. Organic transition with cover crops: influence on soil properties in topographically diverse agricultural landscapes. (in prep) Agronomy J.

PROGRESS

2018/09 TO 2019/08 Target Audience:Researchers in agriculture, agronomy, soil science, weed science, agricultural economics, farmers interested in sustainable agricultural management andorganic farmining, students of agricultural sciences, general public. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?The project supports one postdoctoral research association (Dr. Linh Nguyen), partially supporst one M.S. student (Mr. Kaleb Ortner), and provides work opportunities for 2-3 undergraduatre students halption with field and lab research tasks. How have the results been disseminated to communities of interest?To research community: The results have been reported at theNorth Central Weed Science Society meeting, Dec 10-13, Columbus, OH. To farming community: The findings and experiences of the first year of the project were reported to the farming commuynity at following extension events: 1) Field Days a) Cover Crop and Cropping Innovation Field Day • Location: MSU W.K. Kellogg Biological Station, Hickory Corners, MI • Date: September 27, 2019 • Organic Transition research plots were toured and the project discussed as part of the Cover Crop and Cropping Innovation Field Day attended by 22 participants including farmers, agribusiness representatives and agency personnel. b) Michigan State University Extension Cover

Crops and Forages Field Day • Location: Richard D\'Arcy Farm, Marlette, MI • Date: September 24, 2019 • Interseeded cover crop plots associated with Organic Transition project were toured and the project discussed as part of Michigan State University Extension Cover Crops and Forages Field Day attended by 150 participants including farmers, agribusiness representatives and agency personnel. 2) Webinars a) MSU Cover Crop Team Webinar Series: Cover Crops in Michigan - Interseeding Cover Crops • Webinar by Co-PI Karen Renner • The first webinar in this series highlights research at MSU on interseeding cover crops into standing corn. • Posted: May 5, 2019 • Available on the web at <https://www.canr.msu.edu/resources/webinar-series1> b) MSU Cover Crop Team Webinar Series: Interseeding cover crops in corn in Michigan • Webinar by Aaron Brooker, graduate student of Co-PI Karen Renner • The second webinar in this series highlights research at MSU on interseeding cover crops into standing corn. • Posted: May 5, 2019 • Available on the web at <https://www.canr.msu.edu/resources/webinar-series2> 3) MSUE News Articles a) MSU Cover Crop Team Webinar Series: Cover Crops in Michigan - Interseeding Cover Crops • Article highlighting the Webinar Series Topic: Cover Crops in Michigan - Interseeding Cover Crops • Posted: May 16, 2019 • Available on the web at <https://www.canr.msu.edu/news/msu-cover-crop-team-webinar-series-cover-crops-in-michigan-interseeding-cover-crops> b) MSU Cover Crop Team Webinar Series: Interseeding cover crops in corn in Michigan • Article highlighting the MSU Cover Crop Team Webinar Series: Interseeding cover crops in corn in Michigan. • Posted: May 21, 2019 • Available on the web at <https://www.canr.msu.edu/news/msu-cover-crop-team-webinar-series-interseeding-cover-crops-in-corn-in-michigan>. c) Attend the Cover Crop and Crop Innovation Field Day at Kellogg Biological Station • First article highlighting the Cover Crop and Cropping Innovation Field Day at the W.K. Kellogg Biological Station. • Posted: September 13, 2019 • Available on the web at https://www.canr.msu.edu/news/attend-the-cover-crop-and-crop-innovation-field-d ay-at-kellogg-biological-station. d) Research and demonstration projects to be highlighted at cover crops and crop innovations field day • Second article highlighting the Cover Crop and Cropping Innovation Field Day at the W.K. Kellogg Biological Station. • Posted: September 23, 2019 • Available on the web at https://www.canr.msu.edu/news/research-and-demonstration-projects-to-be-highlig hted-at-cover-crops-and-crop-innovations-field-day. What do you plan to do during the next reporting period to accomplish the goals?We will continue with the field and lab experimental work, as per time-line of the project. In addition, we will conduct an in-depth analyses of the root patterns of the studied cover crops and their effects on soil pore characteristics using X-ray computed micro-tomography scanning.

2020/09 TO 2021/08 Target Audience:Researchers in agriculture, agronomy, soil science, weed science, agricultural economics, farmers interested in sustainable agricultural management and organic farming, students of agricultural sciences, general public. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?The project supported one PhD student (Mk. Sukhdeep Singh), partially supported one M.S. student (Mr. Kaleb Ortner), and provided work opportunities for 2-3 undergraduate students helping with field and lab research tasks. How have the results been disseminated to communities of interest?To research community: As detailed above, the findings were reported at the European Geophysical Union annual meeting and in the manuscript in review in the journal of Agriculture Ecosystem and Environment. To farming community: The findings from the project have been reported to the farmers at the following events September 2, 2020 - Cover Crop Virtual Field Day. https://www.canr.msu.edu/videos/cover-crops-virtual-field-day-introduction. Video tour and discussing of winter-hardy and winter-kill cover crop mixtures in corn. Roundtable discussion of organic production. 96 participants. July 8, 2021 - Organic Field Day. MSU W.K. Kellogg Biological Station, Hickory Corners, MI. Organic Transition project site was toured and discussed. 70 participants. What do you plan to do during the next reporting period to accomplish the goals?We will continue with the field and lab experimental work, as per time-line of the project. Of particular importance will be collection of the yield data of the first-year organic corn from the 4 experimental sites. Also soil samples at the last year of transition will need to be collected and analyzed from the 5th of the studied fields, followed by its corn yield in 2022.After that, the findings will be summarized in two manuscripts - one devoted to the effects of the transition scenarios on soil characteristics, and one on its effects on corn yields, as well as weeds and cover crop performance.

IMPACT

2018/09 TO 2019/08 What was accomplished under these goals? The first year of organic transition, i.e., corn, was started in three experimental fields in 2018. At each field the experiments followed randomized complete block design with 4-6 replications, with topographical positions and cover crop systems as the studied factors. The cover crop mixtures for interseeding into corn were planted in early summer, while cereal rye cover was planted after corn harvest. Organic soybeans were planted in May and harvested in October, 2019. In spring of

2019 two more experimental fields, one at the MSU research station and one at the participating farmer\'s land, were added to the project, planted with corn and interseeded with cover crops. Thus a total of 5 experimental sites, with three and two representing, respectively, the first and second phases of the 3-year organic transition rotation are now part of the project. During the corn and soybean growing seasons, the plant counts of the cover crop mixtures and weeds were conducted on a regular basis, as well as plant biomass sampling in fall after corn harvest. The preliminary results are reported by Kaleb Ortner, graduate student working on the project at a conference (as listed in the products). Emissions of N2O and CO2 were measured at multiple time points during winter/spring following corn, and continued past cover crop termination, planting of soybeans, as the crop of the second year of transition, and the first month of soybean growth. Dr. Linh Nguyen, the post-doc working on the project, is preparing the manuscript with the results for submission to a peer-reviewed journal. After cover crop termination soil sampling was conducted to assess soil carbon levels, microbial biomass, levels of available nitrogen, and soil aggregation. Analyses of the collected soil data are currently in progress. **PUBLICATIONS (not previously reported):** 2018/09 TO 2019/08 Type: Conference Papers and Presentations Status: Other Year Published: 2019 Citation: Ortner, K.A., K. A. Renner, D.G. Baas, and A.K. Kravchenko. 2019. Weed management in organic soybeans across diverse field topographies. Presented at North Central Weed Science Society meeting, Dec 10-13, Columbus, OH.

2020/09 TO 2021/08 What was accomplished under these goals? The reporting period corresponded to the third year (wheat) of transition in 4 of the experimental sites and to the second year of transition (soybean) in the 5th experimental site. During the reporting period we have completed the main soil sampling effort in the 4 of the sites, assessing the effects of the three years of transition on soil properties. Soil sampling was conducted in spring and soil measurements were completed during the summer. Analysis of the obtained data and preparations for their presentation at the annual meeting of the Soil Science Society of America in Nov 2021 and for publication are in progress. Cover crop biomass, weed biomass, andcrop yields were collected and analyzed during this year as per project\'s timetable. We also collected data on plant height and SPAD leaf readings of the organic corn (the first year after the transition). We have conducted X-ray computed tomography analyses to quantify roots of different cover crops and their effects on soil pore characteristics. The preliminary findings have been reported at the annual meeting of the European Geophysical Union. **PUBLICATIONS (not previously reported):** 2020/09 TO 2021/08 1. Type: Conference Papers and Presentations Status: Other Year Published: 2021 Citation: Lucas, M., T.T.L.Nguyen, A. Guber, A. Kravchenko. 2021. The effect of cover crops on soil structure is mainly driven by root architecture. Presented at the annual meeting of the European Geosciences Union, Apr 19-30, virtual. 2. Type: Journal Articles Status: Under Review Year Published: 2021 Citation: Nguyen T.T.L., L. Tiemann, K. Renner, and A.N. Kravchenko 2021. Effects of cover crops on soil characteristics across topographically diverse agricultural landscapes in corn-soybean-wheat organic transition. Agriculture, Ecosystem, Environment

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Optimization of Bacteriophage for Management of Fire Blight Disease

Accession No.	1017086
Project No.	MICL05110
Agency	NIFA MICL\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2018-51106-28776
Proposal No.	2018-03548
Start Date	01 SEP 2018
Term Date	31 AUG 2021
Grant Amount	$499,996
Grant Year	2018
Investigator(s)	Sundin, G.

NON-TECHNICAL SUMMARY

Organic apple production east of the Mississippi river will not be sustainable without effective management of fire blight disease. The main goials of this project are to develop alternatives to antibiotics for organic fire blight management in humid regions, in particular in the midwestern and eastern United States. Bacteriophage represent a promising tool for the management of bacterial plant diseases; however, phage have not been developed commercially for reasons including reduced efficacy, and environmental issues such as sensitivity to solar UV radiation that impact phage survival. In this project, we will examine the efficacy of phage for the biological control of fire blight in organic orchard systems. Field testing will be conducted in Michigan and North Carolina with goals to optimize implementation. Additives including UV sunscreens and spray adjuvants will be evaluated for effects on increasing phage dispersal and survival. Integrated programs with phage and existing biological control agents will be examined to select the best combinations that maximize disease management efficacy. Larger-scale field testing will be targeted against natural populations of the fire blight pathogen Erwinia amylovora so that we can understand how phage deployment impacts natural pathogen genotypes and ultimately how this affects disease control potential throughout the apple bloom period. Extension activities will include a yearly on-farm field day with demonstration plots available to growers, production and dissemination of scripted videos outlining project research results, regular grower presentations, and site visits.

OBJECTIVES

The major goals of this project are to develop, evaluate, and optimize bacteriophage as biological control agents for management of the disease fire blight. While various bacteriophage have been tested previously for fire blight management, these materials have not been rigorously evaluated and optimized for ultimate use by commercial growers. For example, very little is known about bacteriophage survival on apple flowers following application. In addition, we plan to test optimized phage biocontrols in mixes with other registered biological control agents to maximize the level of efficacy against the blossom blight phase of fire blight. We will also perform detailed economic analyses of phage deployment for commercial organic growers. We have basic research goals in this project as well, and will study the effect of bacteriophage deployment on the apple flower microbiome. Finally, we will utilize our extension programs to educate growers about the use of bacteriophage in fire blight management, proper handling of phage etc to maximize the biocontrol ability of these agents.

APPROACH

In the first set of phage experiments, we will test combinations with phages φ21-4 and φ31-3 deployed either in equal mixtures or at 3:1 ratios in the field at sites in Michigan and North Carolina. The efficacy of these phages in controlling blossom blight will be examined in cocktails, as mixtures of phage have always performed better than single phage treatments in blossom blight tests and in tests conducted on other plant diseases (Schnabel et al. 1999; Nagy et al. 2012). The total phage concentration (overall plaque-forming units (pfu)/ml) will be 1011 pfu/ml, as maximal phage densities are consistently associated with better disease efficacy. In addition to altering phage ratios, we will also examine the effect of addition of two UV sunscreen materials, carrot juice and peptone, on phage survival and efficacy, and will determine if utilization of the OMRI-approved adjuvant Kinetic (Helena Chemical Company; Collierville, TN) improves the efficacy of phage preparations by improving phage distribution among sprayed apple flowers. The experiment will be repeated in Year 2 of the project giving us two years of field data from two locations. Also in Year 2 and 3, we will add treatments with testing with the sunscreens and adjuvant added to the best phage ratio (if different from 50:50) to ensure testing of the best combinations for disease efficacy replicated over two years in the field.We will focus on Serenade Opti (Bayer) and Blossom Protect (Westbridge Biological) as biological control materials that we anticipate would work well in programs with phage for blossom blight control. Experiments will consist of using alternation strategies with phage and Serenade Opti or tank mixes of both materials. Since Blossom Protect is a living yeast biocontrol agent, earlier sprays are required to facilitate flower colonization. Because of that requirement, we will only run one treatment with two applications of phage integrated into a Blossom Protect program.We will scale up the field experiments to perform tests assessing the effect of bacteriophages on managing natural fire blight infections. Experiments will be conducted in the certified organic apple orchard located at the MSU Clarksville Research Station in Clarksville, MI and the transitioning to organic apple orchard at the NCSU MHCRC in Mills River, NC. Tests will be conducted on the highly fire blight susceptible cultivar Gala. We will use a randomized complete block design, 3-tree plots, 6 replicates per treatment. All treatments will be applied to trees using air blast sprayers. We will use the MaryBlyt fire blight disease prediction model to indicate when spray applications are needed targeting fire blight infection during the bloom period; all applications will be applied during evening hours to facilitate phage survival on flower surfaces. We hope to be making a minimum of 2-3 applications during bloom.Field experiments will be conducted in Michigan and North Carolina using the apple cultivar Gala. Phages φ21-4 and φ31-3 will be prepared for field application as described above. Individual flower clusters will be tagged for each treatment (25 flower clusters per tree on each of 2 trees). At two phenological timings (2-3 flowers open \40-60% bloom\ and all flowers open \100% bloom\), phage preparations of φ21-4, φ31-3, or φ21-4 + φ31-3 (each at 1011 pfu/ml) will be applied in the evening. In a second set of treatments, E. amylovora Ea110 will also be applied at a low rate (1 x 105 cfu/ml; 10X lower than normal rate) approximately 2 hr prior to phage application. Samples for microbiome analyses will consist of stigmas from 15 open flowers; microbial cells will be separated from flowers prior to DNA extraction. Extracted microbial DNA will be subjected to 16S rRNA tag-encoded amplicon pyrosequencing using a primer set that inhibits chloroplast amplification. Flowers will be sampled, immediately placed on ice, and processed for DNA extraction within 30 minutes. DNA extractions will be conducted in both Michigan and North Carolina; after extraction, DNA will be stored at -20°C. DNA samples obtained in North Carolina will be shipped overnight to Michigan on dry ice. All sequencing will be conducted at the MSU RTSF sequencing facility. Bioinformatics analysis, including sequence identification, diversity index analyses, and tracking of frequencies of individual species following phage application, will be conducted.We will utilize electronic and printed media outlets as well as presentations and grower field days to extend project results. We will produce a series of scripted videos for YouTube that will detail the concepts and findings of this research for post-antibiotic management of fire blight. Recommendations for organic fire blight management using phage will be incorporated into the MyIPM-NED app, which aids users in fruit disease diagnostics and management and includes organic management recommendations. Project team members will produce a series of one page bulletins that highlight fire blight management tactics using phage that will be updated and distributed online and at grower meetings. **Progress** 09/01/18 to 08/31/21 **Outputs** Target Audience:The target audience for this work is the apple grower community, organic growers, extension agents, representatives for chemical companies and disease scouting consultants, in states where fire blight is a serious problem, especially those east of the Mississippi river. Additional target audience is the research community of plant pathologists that work on bacterial diseases of tree fruit and bacterial pathogens as well as the microbiology research community that works on bacteriophages. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?Training opportunities include mentoring of one Ph.D. student and two M.S. graduate students. How have the results been disseminated to communities of interest?Results have been disseminated to grower audiences, especially organic apple growers, through a presentation at a special grower meeting on fire blight, held in Traverse City, MI in June 2019. Other presentations of this work include to the Great Lakes Fruit and Vegetable EXPO in Grand Rapids, MI in 2020 and to the Michigan Apple Committee in 2019, 2020, and 2021. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported **Impacts** What was accomplished under these goals? We examined the in vitro sensitivity of two

bacteriophage of Erwinia amylovora, phi21-4 and phi31-3. Both of these phage are highly sensitive to UV-C and UV-B wavelengths; these wavelengths cause direct DNA damage. We found that both peptone and kaolinite clay were highly effective as UV-screening agents and conferred significantly-enhanced UVB survival to both phages phi21-4 and phi31-3. In 2019, we tested the efficacy of peptone at 5 mg / ml as a sunscreen additive to phages phi21-4 and phi31-3 and also to a recently-registered new commercial biocontrol product, AgriPhage (Certis Corp.). The addition of peptone resulted in significantly better blossom blight control by AgriPhage, but did not increase control with phi21-4 and phi31-3. In 2020 and 2021, we were forced to work with AgriPhage alone as our cooperator was unable to generate the phages phi21-4 and phi31-3 due to COVID impacts on their laboratory. In 2020, under lower disease pressure, the addition of peptone or of kaolinite clay did not increase the efficacy of AgriPhage in a field experiment. In 2021, the addition of either peptone or of kaolinite clay to AgriPhage resulted in numerically better blossom blight control, but the amount of control was not significantly better. Thus, the importance of adding sunscreen to phage preparations is not clear yet. We also conducted experiments aimed at detemrining the ffect of bacteriophage on the apple flower microbiome. Field experiments using AgriPhage were conducted, samples taken, DNA isolated and was sequenced at a commercial facility. These data are still being analyzed with analysis slated tobe completed by March 2022. **Publications** - Type: Journal Articles Status: Under Review Year Published: 2022 Citation: Dobbins, M.R., Outwater, C.A., Slack, S.M., Nesbitt, D., Svircev, A., Lauwers, E.M., Villani, S.M., and Sundin, G.W. 2022. Assessment of the role of ultraviolet radiation protection in the field efficacy of bacteriophage against fire blight. Plant Dis. (under review)

PROGRESS

2018/09 TO 2019/08 Target Audience:The target audience for this work is the apple grower community, organic growers, extension agents, representatives for chemical companies and disease scouting consultants, in states where fire blight is a serious problem, especially those east of the Mississippi river. Additional target audience is the research community of plant pathologists that work on bacterial diseases of tree fruit and bacterial pathogens as well as the microbiology research community that works on bacteriophages. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?Training opportunities include mentoring of one Ph.D. student, two M.S. graduate students, and one undergraduate student. How have the results been disseminated to communities of interest?Results have been disseminated to grower audiences, especially organic apple growers, through a presentation at a special grower meeting on fire blight, held in Traverse City, MI in June 2019. What do you plan to do during the next reporting period to accomplish the goals?We plan to continue field experiments in Michigan and in North Carolina working with sunscreen additives to either phages phi21-4 and phi31-3 or AgriPhage. We plan to complete the in vitro work we have done to identify the best UV sunscreens for phage protection in the environment. We will also conduct experiments assessing the impact of phage application on the apple flower microbiome.

IMPACT

2018/09 TO 2019/08 What was accomplished under these goals? We examined the in vitro sensitivity of two bacteriophage of Erwinia amylovora, phi21-4 and phi31-3. Both of these phage are highly sensitive to UV-C and UV-B wavelengths; these wavelengths cause direct DNA damage. Since UV-B wavelengths are present in solar radiation, it can be surmised that these phage would be sensitive to inactivation by solar radiation. We tested a variety of materials to utilize as potential sunscreens including peptone (5-50 mg / ml), carrot juice, and kaolinite clay. We found that both peptone and kaolinite clay were highly effective and conferred significantly-enhanced UVB survival to both phages phi21-4 and phi31-3. In 2019, we tested the efficacy of peptone at 5 mg / ml as a sunscreen additive to phages phi21-4 and phi31-3 and also to a recently-registered new commercial biocontrol product, AgriPhage (Certis Corp.). The addition of peptone resulted in significantly better blossom blight control by AgriPhage, but did not increase control with phi21-4 and phi31-3. In a second field experiment using AgriPhage, we observed significantly better blossom blight control (compared to AgriPhage used alone without sunscreens) when Surround (kaolinite clay) was added, but not when peptone was added. In a third field experiment, we added the commercial biocontrol Serenade Opti to AgriPhage, but did not observe significantly better control compared to the untreated control treatment. **PUBLICATIONS (not previously reported):** 2018/09 TO 2019/08 No publications reported this period.

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Manure and Pasture Management to Reduce Swine Parasites in Organic Pastured Pork Production

Accession No.	1017204
Project No.	MIN-02-G05
Agency	NIFA MIN\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2018-51106-28772
Proposal No.	2018-03523
Start Date	01 SEP 2018
Term Date	31 AUG 2021
Grant Amount	$499,542
Grant Year	2018
Investigator(s)	Li, Y.; Carr, RI, .; Hernandez, AL, D..

NON-TECHNICAL SUMMARY

Managing swine intestinal parasites continues to be an obstacle for organic pig farmers because there is a lack of organically-approved options for controlling parasites. This project explores manure and pasture management strategies that would control swine parasites by reducing parasite contamination and transmission in organic pig production. We will evaluate parasite pressures on organic pig farms; determine effectiveness of manure composting on eliminating swine parasites and its underlying mechanisms; assess a novel approach to swine parasite control in pastures via biofumigation; and determine effects of grazing rapeseed by organic pigs on reducing swine parasite contamination in pastures. Field trials will be conducted at the Rodale Institute research farm. Animal trials will be conducted in a bedded, centralized housing facility with pigs fed organic feed according to NOP standards and provided with full-time access to pasture. This project will document the effect of composting swine manure to reduce or eliminate parasites and develop an integrated crop-livestock pasture management strategy to mitigate swine parasites in the field. Results will be disseminated to end-users through field days, workshops, seminars, online materials, and publications. By developing new measures to control swine parasites, this project is expected to support transitioning and organic pig farmers and increase the number of organic pig farmers and the number of pigs raised organically.

OBJECTIVES

The long-term goal of this project is to develop manure and pasture management strategies for organic and transitioning pastured pork producers that mitigate swine parasite contamination and transmission. The specific objectives are: 1. To evaluate parasite prevalence on organic pig farms. 2. To determine effectiveness of manure composting on eliminating swine parasites and its underlying mechanisms.3. To assess biofumigation as an approach to swine parasite control in pastures. 4. To determine effects of grazing biofumigation pastures by organic pigs on reducing swine parasite contamination.

APPROACH

Identify parasite species and quantify parasite infective stages in pig feces and measure the daily variability in parasite stages excreted from pigs.Collect fecal, manure, and soil samples from farm collaborators to assess the severity of swine parasite infection and contamination on organic pig farms.Test swine manure composting as an

effective strategy for eliminating infective stages of swine parasites. This task will be replicated in time during the first two years of the project.Determine the effect of temperature on reducing viability of swine parasites under laboratory conditions. This task will be carried out during the winter of 2018-19. Subsequent assays will be completed during winter of 2019-20 if necessary.Assess the level of field contamination of swine parasites in an organic centralized housing, pastured pork operation prior to establishing experimental biofumigation pastures.Establish experimental pastures of rapeseed (Brassica napus), yellow mustard (Sinapis alba), Alyce clover (Trifolium lappaceum), and Ladino clover (Trifolium repens, control) and evaluate the ability to biofumigate swine parasites after plant residues are mechanically incorporated into the soil.Breed and raise pig for animal trials. Four sows will be bred naturally by two different boars to produce pigs for animal trials. After weaning, pigs will be tagged and weight will be measured weekly for each pig from weaning to finishing weight, which is about 250 lbs. Pigs will be produced for the summer of 2019, spring and summer of 2020.Determine the level of field contamination of swine parasites in an organic centralized housing, pastured pork operation prior to establishing experimental pastures for pig grazing.Establish experimental pastures of rapeseed and Ladino clover in early July of 2019. Pastures will be replicated in time during the spring and summer of 2020.Rotationally graze experimental pastures with pigs after pastures have been established for 6-7 weeks. Two pasture biofumigation techniques will be applied to each pasture: (1) non-mechanically and (2) mechanically incorporated plant residues. All treatments will be replicated in time during the summer of 2019, and spring and summer of 2020.Evaluate the livers of finished pigs for parasite symptoms (i.e. white spots). Symptomatology will be quantified and provide a link between the severity of prevalence parasite infection and field contamination.Extension, outreach and education activities will occur throughout the project period. **Progress** 09/01/18 to 08/25/22 **Outputs** Target Audience:The target audience for this project is organic farmers, their stakeholders, researchers, and agricultural professional. Outcomes of the project have been communicated with the target audience through presentations at conferences, seminars, and stakeholders' meetings; demonstrations at field days, facilities tours, and workshops; and publications in popular press, social media, and peer-reviewed journals. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? The project provided opportunities for the PDs and investigators to interact with organic farmers, researchers and agricultural professionals through conferences, webinars, virtual workshops, field days, and facility tours. A total of 13 undergraduate students, a graduate student, a visiting scholar, a research associate, and several research interns have been trained in organic swine research, on-farm data collection, and lab analysis for internal parasites of pigs. Additionally, Rodale Institute has a farmer training program where more than 40 trainees engaged in daily activities of this project. These activities include animal husbandry, pasture and manure management, and data collection. All these opportunities directly benefit the professional development of PDs and investigators of this project and enhance training students and organic farmers. How have the results been disseminated to communities of interest? Due to the impact of COVID-19 pandemic, conferences, workshops, and field days were held both virtually and in-person throughout the project period. The PDs and investigators were invited to present results of the project at the Midwest Organic and Sustainable Education Service (MOSES) annual conference in 2019, 2021 and 2022. Audience of organic farmers, researchers, extension specialists, and agricultural professionals across the country participated in the conferences and learned management strategies for controlling parasites in organic pigs. Additionally, webinars and field days were organized at Rodale Institute to deliver outcomes of this project to organic farmers and their stakeholders nationwide. Results from this project were shared with audiences in-person at the American Society of Animal Science Midwest Section annual conference, International Conference on Production Diseases in Farm Animals, US Composting Council Conference, PASA Sustainable Agriculture conference, and MOSES annual conference through oral and poster presentations. Progress of the project was communicated with organic communities at each of Rodale Institute's Annual Field Day during the project period with over 400 organic farmers and their stakeholders in attendance. Furthermore, the project was highlighted at several events at Rodale Institute, including the Virtual Field Day, the Media Influencer event, the Media Ambassador event, three Rodale Institute Alumni events, and numerous onsite facility tours conducted annually. Finally, the project was disseminated to broader audiences through publications in peer-reviewed journals, newspapers, newsletters, and social media. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported **Impacts** What was accomplished under these goals? Internal parasite infection is a common problem in organic pig production that can compromise health and growth of pigs, threaten food safety of pork products, and cause economic losses to organic farmers. To understand parasite infection and transmission on organic pig farms, we investigated the infection prevalence of three intestinal roundworm parasites, the large roundworm (Ascaris suum), nodular worm (Oesophagostomum spp.) and whipworm (Trichuris suis) on nine organic and transitioning pig farms across four states. Analysis of pig feces, pasture soil, and bedding samples indicated that all farms were infected with at least one parasite species. A cross-sectional study demonstrated that Ascaris suum eggs were more prevalent in growing-finishing pigs' feces than in sows' feces. Two management strategies, composting manure and biofumigation of pastures, were tested for controlling parasite transmission in organic pigs. Composting manure reduced the number of parasite eggs during the summer, but not during the winter in Pennsylvania. The

effectiveness of biofumigation as an approach to swine parasite control in pastures was not confirmed in this project. The project has successfully completed and produced 28 presentations at conferences, seminars, and stakeholder meetings; 10 extension articles and abstracts; and four manuscripts for peer-reviewed journals (two published or submitted, two in preparation). Eight field days and workshops have been organized to deliver findings of the project to organic farmers and their stakeholders. Results of the project were summarized and presented to organic farmers at the Midwest Organic and Sustainable Education Service Conference, the largest organic conference in the United States, in 2019, 2021, and 2022. Accomplishment of Objective 1): Nine organic and transitioning pig farms across four states (MN, IA, WI, and PA) were visited. Fecal, bedding and pasture soil samples were collected for analysis of the prevalence and intensity of parasite infection. Results indicate that 89%, 56%, and 44% of farms were infected with A. suum, O. spp., and T. suis, respectively. A cross-sectional study indicated that prevalence of infection with A. suum was higher in finishing pigs than in growing pigs (74% vs. 45%; p < 0.001). Parasite infection did not affect growth performance, however, pigs with A. suum worms at slaughter contained many white spots (tissue lesion) on the liver, which is an economic loss to organic farmers due to rejection of the pig liver for human consumption. To control A. suum infection, future research should investigate the efficacy of treating pigs with organically approved anthelmintics during the growing phase of production. Accomplishment of Objective 2): Bedding-packed manure was removed from pig pens and placed in windrows for composting in 3 summer and 2 winter trials. Compost was sampled for analysis of A. suum, O. spp., and T. suis eggs before composting and again weekly for up to eight weeks. Composting temperatures were recorded every 4 hours during the composting period. For summer trials, the composting temperatures remained above 130 F for 4 to 8 weeks. During winter trials, composting temperatures remained above 130 F for less than 10 days. Egg counts of A. suum and O. spp. in compost declined during summer trials, while egg counts of both species remained nearly unchanged during winter trials. Changes in egg count of T. suis was not detected due to the low concentration of T. suis eggs in pig manure used in the study. These results indicate that the effectiveness of eliminating A. suum and O. spp. eggs depended on how long the required temperatures (\>130 F) can be maintained during the composting process. To assess effects of temperatures on parasite egg survival, we conducted an in vitro study using A. suum and T. suis eggs extracted from pigs. A total of 25 Petri dishes per species were cultured in each of five constant temperatures: 39, 57, 72, 99, and 136 F for 24 days. Five dishes were removed from each temperature on days 1, 2, 7, 13, and 24, respectively. Results indicate that eggs of both species were slow to develop at 39 and 57 F. At 99 F, most A. suum eggs were between the 2-cell and morula stage by days 7 and 13, but none developed into a larva. In contrast, approximately 40% of T. suis eggs reached larvae stage at 99 F. At 136 F, all eggs reached morula stage by day 1, and none contained larva on any day during the test period. These results suggest that exposure to a constant temperature of 136 F can interfere with the development of A. suum and T. suis eggs into infective larvae. Accomplishment of Objective 3): Two field trials were conducted in two years to test four pasture crops: yellow mustard and rapeseed as treatment crops with biofumigation properties, and Alyce and Landino clover as control crops. All crop species were established and mechanically incorporated into the soil at the time of flowering to test for biofumigation effects. Soil samples were collected for analysis of parasite eggs before and weekly after crop incorporation for four weeks. Parasite eggs were not detectable either before or after crop incorporation in both trials due to low parasite egg recovery from pasture soil. Experiences gained while working on this objective were beneficial to the accomplishment of Objective 4. Accomplishment of Objective 4): Three animal trials were conducted over three years. In each trial, pigs (n = 26 pigs for Trial 1, n = 32 pigs for Trial 2; and n = 34 pigs for Trial 3) were divided into four treatment groups: grazing biofumigation pastures of rapeseed with mechanical incorporation of plant residues (R1), grazing biofumigation pastures of rapeseed without mechanical incorporation of plant residues (R2), grazing control pastures of Ladino clover with mechanical incorporation of plant residues (C1), and grazing control pastures of Ladino clover without mechanical incorporation of plant residues (C2). Four pastures were planted in each trial, two with rapeseed (R1 and R2) and two with Ladino clover (C1 and C2). Each pasture was split into four 30 ft x 35 ft paddocks, and pigs were given access to each for one week. Fecal samples were collected from all pigs before pigs grazed each pasture and again after two and four weeks. Soil samples were collected from each paddock before and weekly after grazing for four weeks. Biomass yield of Ladino clover was higher (p < 0.01) than that of rapeseed and grazing by pigs reduced biomass in both pastures (p < 0.01). Fecal egg counts for the three parasite species did not differ among pigs that grazed on different pasture crops. After four weeks of grazing, all pigs had lower A. suum (p < 0.01) and T. suis (p < 0.01) egg counts regardless of pasture type. The number of A. suum and T. suis eggs in pasture soil samples were not affected by pasture type. Egg counts of both species were reduced (p < 0.01) three weeks after grazing compared to pre-grazing, and the first two weeks after grazing in all pastures. These results suggest that the number of parasite eggs may decrease over time on pastures, and biofumigation may be not an effective way to control pig parasites on pastures.
Publications - Type: Journal Articles Status: Published Year Published: 2022 Citation: Li, Y. Z., A. D. Hernandez, S. Major, and R. Carr. 2022. Occurrence of intestinal parasites and its impact on growth performance and carcass traits of pigs raised under near-organic conditions. Frontiers in Vet. Sci. 9: 911561. Doi:10.3389/fvets.2022.911561. - Type: Journal Articles Status: Submitted Year Published: 2022 Citation:

Hernandez, A. D., R. Carr, S. Dukes, S. Major, and Y. Z. Li. Intestinal roundworm infection in organic and alternative swine farms in the USA. Submitted to Veterinary Parasitology. - Type: Journal Articles Status: Other Year Published: 2022 Citation: Carr, R. S. Major, A. D. Hernandez, and Li, Y. Z. Effectiveness of manure composting on eliminating swine parasites. In preparation. - Type: Journal Articles Status: Other Year Published: 2022 Citation: Major, S., R. Carr, A. D. Hernandez, and Li, Y. Z. Biofumigation as an approach to swine parasite control in organic pastures. In preparation. - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Li, Y. Z., A. D. Hernandez, S. Major, and R. Carr. 2022. Intestinal parasite infection and its effect on growth performance and carcass traits of organic pigs. 18th International Conference on Production Diseases in Farm Animals (Abstract). Madison, WI. June 15-17, 2022. - Type: Other Status: Published Year Published: 2022 Citation: Li, Y. Z. 2022. Parasite infection in organic pigs. West Central Research and Outreach Center Newsletter. https://wcroc.cfans.umn.edu/research/swine/parasite-infection-organic-pigs. - Type: Conference Papers and Presentations Status: Other Year Published: 2022 Citation: Li, Y. Z. 2022. Intestinal parasite infection and its effect on growth performance and carcass traits of organic pigs. The 18th International Conference on Production Diseases in Farm Animals. Madison, WI. June 15-18, 2022 (Poster). - Type: Other Status: Other Year Published: 2022 Citation: Hernandez, A. D. 2022. Parasite infection patterns in organic pigs. Annual Conference of Midwest Organic and Sustainable Education Service (MOSES). La Crosse, WI. Feb. 25, 2022. Oral Presentation. - Type: Other Status: Other Year Published: 2022 Citation: Carr, R. 2022. Effectiveness of manure composting on eliminating swine parasites and its underlying mechanisms. Annual Conference of Midwest Organic and Sustainable Education Service (MOSES). La Crosse, WI. Feb. 25, 2022. Oral Presentation. - Type: Other Status: Other Year Published: 2022 Citation: Major, S. 2022. Biofumigation as an approach to swine parasite control in pastures. Annual Conference of Midwest Organic and Sustainable Education Service (MOSES). La Crosse, WI. Feb. 25, 2022. Oral Presentation. - Type: Other Status: Other Year Published: 2022 Citation: Li, Y. Z. 2022. Effects of parasite infection on growth performance, carcass traits and liver quality in organic pigs. Annual Conference of Midwest Organic and Sustainable Education Service (MOSES). La Crosse, WI. Feb. 25, 2022. Oral Presentation. **Progress** 09/01/20 to 08/31/21 **Outputs** Target Audience:The target audience reached during this reporting period included organic farmers and their stakeholders, individuals who are interested in organic agriculture, researchers, undergraduate andgraduate students, and agricultural professional. Results and progress of the project have been communicated with the audience through presentations at conferences, publications, and webinars. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?The project provided opportunities for the PDs and investigators to interact with organic farms, researchers and agricultural professionals through webinars and virtual workshops. Three undergraduate students, one visiting scholar, one research associate have been trained in organic swine research, on-farm data collection, and lab analysis for internal parasites of pigs. Additionally, Rodale Institute has a farmer training program where trainees engage in daily activities of our swine parasite project. These activities include animal husbandry, pasture and manure management, and data collection. All these opportunities directly benefit the professional development of PDs and investigators of this project and enhance training of students and organic farmers. How have the results been disseminated to communities of interest?Due to the impact of COVID-19 pandemic, some conferences and workshops/field days were held virtually during this reporting period. A virtual workshop was organized by the Midwest Organic and Sustainable Education Service (MOSES) at their annual conference and Diane DeWitte, a swine extension educator on this project, was invited to present results of parasite prevalence on organic pig farms. Audience of organic farmers, agricultural professionals, scientists and researchers across the country participated in the workshop. Additionally, a webinar was organized by Rodale Institute with Rick Carr and Sara Major as the presenters to deliver outcomes of this project to organic farmers and their stakeholders nationwide. Progress of the project was communicated with organic communities at Rodale Institute's Annual Field Day with over 400 organic farmers and their stakeholders in attendance. Furthermore, the project was highlighted at several events at Rodale Institute, including the Virtual Field Day video highlighting research and findings for Rodale Institute's online campus, the Media Influencer event, the Media Ambassador event, and three Rodale Institute Alumni events. Finally, the project was disseminated to broader audiences through publications in newspapers, newsletters, online publications, and social media. A complete list of publications and outreach events is presented in the Products and Other Products sections. What do you plan to do during the next reporting period to accomplish the goals?During the next reporting period, we will finalize the statistical analysis of all data collected at the University of Minnesota (Objective 1) and write a manuscript for publication in a peer-reviewed journal. A final winter composting trial to complete objective 2 will be conducted during the winter of 2021-22 and if time permits a third summer composting trial will be conducted during the summer of 2022. After completion of these trials, the data will be summarized into a peer-reviewed manuscript for publication. For Objective 3, we will continue our effort to improve parasite egg recovery rate in the greenhouse and in vitro experiments. We intend to finish these trials by the end of this year and then analyze the data for publication. A third run of 32 pigs onto biofumigation pastures will be completed in the fall of 2021 to complete objective 4. After completion of this trial, data collected will be analyzed to evaluate the effectiveness of rapeseed as a biofumigant

to destroy the eggs of three species of swine parasites. Additionally, the change in parasite load in the pastures where plant materials were mechanically incorporated into the soil will be compared to that of the pastures that were not mechanically incorporated to test the efficacy of using pigs' behaviors of rooting, trampling, and foraging as means of \"biological incorporation\" of plant materials into the soil. The data will be summarized into a peer-reviewed manuscript for publication. **Impacts** What was accomplished under these goals? Objective 1: We investigated intestinal parasites in pigs that were raised under near-organic conditions. Fecal samples were collected from 293 pigs in bedded barns, including nursery pigs (n=29), growing pigs (n=91), finishing pigs (n=133), and gestating sows (n=40), for analysis of Ascaris suum (ASC), Trichuris suis (TRI), and Oesophagostomum spp. (OES) eggs. Prevalence (the number of pigs infected as a percent of total pigs sampled) and infection intensity (number of parasite eggs per gram (epg) sample) of each parasite species were evaluated. Preliminary results indicate that ASC was the dominant intestinal parasite in growing and finishing pigs. Seventy-six percent of the growing pigs and 92% of the finishing pigs shed ASC eggs in their feces. For pigs that shed ASC eggs, the average infection intensity in feces was 467 epg for the growing pigs and 2,106 epg for the finishing pigs. Ascaris suum eggs were not detected in the feces of the nursery pigs, and were only detected in the feces of three sows with the infection intensity between 20 and 80 epg. Among the finishing pigs sampled, 103 pigs were individually identified and recorded for growth performance. Correlations of ASC infection intensity with growth performance (initial body weight, average daily gain, and market weight) and carcass traits (hot carcass weight and backfat depth) were estimated. Ninety-three percent of barrows (n = 58) and 98% of gilts (n = 45) shed ASC eggs. The average ASC infection intensity was 2,208 epg for barrows and 2,104 epg for gilts. Neither the difference in ASC prevalence nor the difference in ASC infection intensity was significant (P > 0.10) between sexes. No correlations of ASC infection intensity with growth performance or carcass traits of pigs were detected (all P > 0.33 for Spearman correlation coefficients). These results indicate that ASC prevalence and infection intensity were not associated with sex or growth performance of finishing pigs. A subset of gilts (n = 32) was examined for liver milkspots and ASC worms in the small intestine when the pigs were harvested at market weight (120 kg). All these gilts had milkspots on their livers, and 78% of them had many (more than 10) milkspots on a liver. Among the 32 gilts, 25 (78%) were observed with ASC worms in their small intestines, and 96% of these pigs (24 out of 25) had many milky spots on their livers. These results are consistent with the prevalence and infection intensity of ASC eggs in feces of finishing pigs. Trichuris suis eggs were not detected in any fecal samples in this study. Oesophagostomum spp. eggs were not present in feces of the growing pigs, and were barely present in other fecal samples, with a total of seven pigs (one finishing pig, two nursery pigs, and four sows) being detected for OES. For the seven pigs that were infected with OES, the infection intensity of OES eggs was minimal andranged from 20 to 40 epg. Collectively, results of this study suggest that TRI and OES were not a concern for the herds studied. However, prevalence and infection intensity of ASC in growing and finishing pigs could be a threat to food safety of organic pig products. Thus, management strategies to control ASC infection in growing and finishing pigs are needed for organic pig production. Objective 2: During this reporting period, we completed a third compost trial to investigate efficacy of composting swine manure bedpack to destroy swine parasites during summer months. To date, two summer trials and one winter trial have been completed. We plan to complete another winter trial in 2021-22. The goal of these trials is to determine if composting is an effective manure management strategy to destroy swine parasite eggs across seasons. Based on the results from the first two trials, we predict that composting can destroy parasite eggs effectively during the summer because of the high compost temperatures that are achieved, but not during the winter because compost temperatures fluctuated too low during the trial, which was caused by low ambient air temperatures. In the third trial, as in the first two trials, temperature from ambient air and six different compost pile locations was recorded every four hours using Maxim Integrated Ibutton data loggers. Compost samples were collected each week from six different locations, processed in the lab and then analyzed for parasite eggs. The quantity of three different parasite egg species was recorded as in Objective 1. Data collected from the third trial is still being analyzed. Objective 3: Assessment of biofumigation as an approach to control swine parasites in pastures has been replicated in a controlled greenhouse environment. Three crops (yellow mustard, rapeseed, and Ladino clover as control) were planted in replicated pots in the greenhouse at Kutztown University's Biology Department. The goal of this trial was to mimic how pigs incorporate plant residue into soils and test whether development of parasite eggs was affected. Five replicate pots per treatment were seeded with approximately 2,000 Ascaris suum eggs, and the plant material was then crushed into the soil with a hand garden spade. On Day 10 after seeding with eggs and crushing plant materials, extraction of eggs from soils was done. Recovery of parasite eggs was initially very low (< 1%) and after several trials attempting to improve experimental design egg recovery has improved. In addition, plant metabolites were extracted from all three plant types into aqueous solutions so that replicate in vitro experiments could be run to better assess any effects of plant metabolites on parasite egg development. Experiments are being done in replicate cell culture plates at an optimal constant temperature (\~25°C) for parasite egg development and run for 14 days with sampling on days 0, 1, 2, 7 and 14. Objective 4:During this reporting period, four pastures, two with rapeseed to assess its biofumigation properties and two with Ladino clover as a control, were planted for grazing by pigs. Thirty-two pigs (body weight

= 211 ± 30 lb) that were organically managed were split into four groups of eight pigs each for grazing. Fecal samples were collected from all pigs immediately before pigs grazed each pasture and again after two and four weeks to monitor the level of parasite infection in the herd. Each pasture was split into four 30'x 35' paddocks, which pigs were given access to each paddock for one week. Soil and biomass samples were collected from each paddock prior to and after grazing. For biofumigation purpose, in one clover and one rapeseed pasture, the paddocks were mechanically mowed, spaded, and packed after the pigs' access to the paddock was closed off. The other two clover and rapeseed pastures that were grazed were not mechanically incorporated into the soil and left as is as a control for biological incorporation. After pigs were moved off each paddock, soil samples were collected weekly for four weeks to monitor the parasite load in the pasture. All fecal and soil samples were analyzed for quantity of three different parasite egg species as in Objectives 2 and 3. **Publications** - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: DeWitt, D. 2021 (Invited). Parasites in organic pigs: Lessons learned thus far. Annual Conference of Midwest Organic Service and Education (MOSES). Growing Stronger: Collaborative Conference on Organic & Sustainable Farming. Virtual Workshop. Feb. 23, 2021. - Type: Websites Status: Published Year Published: 2021 Citation: Li, Y. Z. 2021. Reducing piglet mortality in a bedded, group farrowing systems. Presented at the Freedom in farrowing and lactation virtual workshop, Copenhagen, Denmark. Aug. 12-13, 2021.

PROGRESS

2018/09 TO 2019/08 Target Audience:The target audience for this project is existing, transitioningand potential organic farmers, their stakeholders, researchers, andagricultural professionals. Preliminary results of the project have been delivered to the audience through field daysandpresentations at organic conferences. General information about the project has been published in websites, social media, newspapers, and newsletters. Changes/Problems: Personnel changes during the project period. 1. Mr. Ross Duffield, a senior/key investigator of the project from Rodale Institute left his position in 2019. Mr. Duffield was responsible for pasture management. His role has been successfully filled by Ms. Shelby Dukes (a senior/key investigator) and Mr. Rick Carr (Co-PI). It is expected that the departure of Mr. Duffield will not affect the successful completion of the project. Accordingly, funds budgeted for Mr. Duffield will be reallocated to Ms. Dukes (40% of FTE) and Mr. Carr (35% of FTE). 2. Diane DeWitte, a Swine Extension Educator from the University of Minnesota joined the project in 2019. Ms. DeWitte will be responsible for writing extension articles generated from the project, organizing field days and other extension activities, and assisting in data collection at the University of Minnesota and other organic pig farms. There will be no additional funds required for the addition of Ms. DeWitte to the project. Request for one year no-cost extension. Due to the delay in transition of funds from USDA to the main award institution (University of Minnesota), and consequentially from the main award institution to the subaward institutions (Rodale Institute and Kutztown University), the project could not start until January of 2019 (about 4 months later than expected). As a result, all activities proposed for 2018 have to be pushed to 2019. Because organic pig production is affected by seasons, we have to reschedule some activities for a year later than proposed originally in the grant. To complete the project as proposed, we would like to request for one-year extension without additional cost, with anew expiration date ofthe grant being August 31, 2022. The extended period will allow us to collect all data as proposed and complete the project successfully. What opportunities for training and professional development has the project provided? This project provided training opportunites for gradaute students, undergraduate students, organic pig farmers and investigators of the project. Five undergraduate students from Kutztown University have been heavily involved in the project, including one student (Schallmo, Emily) that achieved the award of Honors Independent Research in Biology, Kutztown University of Pennsylvania (Research Title: Effectiveness of composting for parasite management in organic pork production), a student (Holland, Christine) conducted an Independent Research in Biology II (Research Title: Assessing nematode parasite prevalence in organic pig farm enclosures), and three students were trained in parasite laboratory analyses. A graduate student and an undergraduate student at the University of Minnesota were trained and participated in on-farm sample collection. Organic farmers were educated for raising organic pigs on pastures at seminars and conferences given through this project. Participating organic pig farmers learned how to collect fecal samples from pigs for parasite analysis. The project also provided opportunities for investigators to visit organic pig farms to learn about organic pig production, and attend conferences to discuss results of the project with target audiences. How have the results been disseminated to communities of interest? At this point, only preliminary results are available which are not ready for publication in peer-reviewed journals. However, information about project concepts, goals and methods have been widely communicated with organic farmers, researchers and agricultural professionals through presentations at the Midwest Organic and Sustainable Education Service Conference (MOSES), Midwest Organic Pig Conference, U.S. Composting Council Conference (USCC), Rodale Institute\'s Annual Field Day, and publications in newsletter and social media (see Products). What do you plan to do during the next reporting period to accomplish the goals? During the next reporting period, we will collect fecal samples from pigs that are identified by age and sex at the University of

Minnesota\'s research farm (Objective 1) to understand distribution of parasite load among individual pigs. Fecal samples will be analyzed to identify parasite species and to quantify density of each species. We will continue to analyze bedding and soil samples collected from organic pig farms for parasite species and load. All data collected for Objective 1 will be statistically analyzed and prepared for publication. For Objective 2, we will repeat the compost procedure in the fall and spring to obtain enough replicates for statistical analysis. Data of pile temperature, humidity, parasite species and load will be collected and analyzed as described in the proposal. Data will be summarized for publication. For Objective 3, we will repeat the plot trial one more time (the second trial) to confirm what we found in the first trial. Data from two trials will be analyzed and prepared for publication. For Objective 4, four pastures (two pastures with rapeseed as treatment of biofumigation, and two pastures with Ladino clover as control) will be prepared for grazing by pigs. The first group of pigs (approximately 32) will be farrowed and raised organically. The pigs will be tested for grazing on biofumigated pastures versus control pastures. Fecal, bedding and soil samples will be collected and analyzed for parasite species and load as described in the proposal. All activities mentioned above will be communicated with organic farmers, and other interested audience through field days, seminars, conferences, newsletters, social media, and publications in peer-reviewed journals. Two abstracts will be published in the Journal of American Society of Parasitologists, and the Journal of Animal Science as conference proceedings. A presentation with an abstract will be offered at the 2020 MOSES and USCC Conference, respectively. Progress of the project will be updated in newsletters of the University of Minnesota\'s West Central Research and Outreach Center, and on Rodale Institute\'s website.

IMPACT

2018/09 TO 2019/08 What was accomplished under these goals? Managing swine intestinal parasites continues to be an obstacle for organic pig farmers because there is a lack of organically-approved options for controlling parasites. The purpose of this project is to explore manure and pasture management strategies that would control swine parasite contamination and transmission in organic pig production. To achieve Objective 1, fecal, bedding, and soil samples from pastures (when available) have been collected from five organic pig farms and the University of Minnesota\'s West Central Research and Outreach Center as proposed in the grant. Lab analyses to identify parasite species and quantify parasite loads (number of eggs per gram sample \e.p.g\) from feces, bedding, and soil have been conducted at Kutztown University. The preliminary results from 150 fecal samples indicate that parasites are present on all organic pig farms sampled. The load of Ascaris spp. was higher than the load of Trichuris spp. On most farms Oesophagostomum was minimal or undetectable, which may be related to the fact that Oesophagostomum is more common in breeding sows which could be dewormed by the 3rd trimester of gestation. Similar patterns were measured in pen bedding and pasture soil samples as in fecal samples, although recovery of parasite eggs from these types of sample were, as expected, much lower. Preliminary results also indicate a large variation in parasite loads among individual pigs on the same farm and among the participating farms. To achieve Objective 2, two separate trials, one in January and the other in June, were conducted to compost swine bedpack manure from Rodale Institute\'s swine hoop barn. Pre-composted manure samples were collected and analyzed for parasite species and load. Manure was removed from the barn and placed in windrows for composting. Windrows were turned using a Sittler compost turner according to the National Organic Program and then managed to optimize decomposition based on moisture and temperature. Temperature was recorded every 4 hours using I-Button data loggers. Manure was sampled weekly for 4 weeks and analyzed for parasite species and load. Preliminary results indicate great seasonal variation in maximum composting temperatures. Additionally, results indicate composting during the winter does not generate temperatures sufficient enough to destroy parasites but is effective at parasite destruction during the summer. To achieve Objective 3, the first field trial to assess biofumigation as an approach to swine parasite control in pastures has been conducted. Four crops (yellow mustard, rapeseed, Alyce clover, and Ladino clover as control) were planted in the field in March of 2019. One crop (yellow mustard) was ready for biofumigation in May. Replicated plots were artificially inoculated with swine feces with known e.p.g. counts for each of the 3 nematode parasite species. Thereafter, each replicate plot was flail mowed and the remaining plant residues incorporated into the soil using a moldboard plow, disc and culti-packed. Soil samples were collected weekly for parasite analysis. Preliminary results from yellow mustard illustrate variable levels of parasite egg recovery over time for each of the 3 parasite species. Replicated plots for the remaining crop species will be similarly inoculated and biofumigated in August. To achieve Objective 4, field and breeding preparations are underway as described in the proposal. No other activities have been accomplished at this time. **PUBLICATIONS (not previously reported):** 2018/09 TO 2019/08 1. Type: Websites Status: Published Year Published: 2019 Citation: Y. Li, R. Carr, and A. Hernandex. 2019. Research on Parasite Control in Organic Swine Production. Available at: https://wcroc.cfans.umn.edu/wcroc-news/parasite-control 1West Central Research and Outreach Center (WCROC), University of Minnesota, Morris, MN; 2Rodale Institute, Kutztown, PA; 3Kutztwon University, Kutztown, PA. June 2019Y.Z. Li, 2. Type: Conference Papers and Presentations Status: Published Year Published: 2019 Citation: Carr, R. 2019. Composting Part 2: Separating the ﷺFeel Goodﷺ from the ﷺReal

Good.🔲 Pennsylvania Association for Sustainable Agriculture, Lancaster, PA. Feb. 7. 3. Type: Other Status: Other Year Published: 2019 Citation: Carr, R. 2019. Organics recycling and on-farm composting. Field and equipment presentation to delegation from Argentina, Rodale Institute, Kutztown, PA. June 10. 4. Type: Other Status: Other Year Published: 2019 Citation: Carr, R. 2019. Composting and Solid Waste Management. Delaware Valley University Farmer Training Program, Rodale Institute, Kutztown, PA. July 16. 5. Type: Conference Papers and Presentations Status: Published Year Published: 2019 Citation: Carr, R. 2019. On-farm Composting. Northeast Organic Farming Association Conference, Saratoga Springs, NY. January 18. 6. Type: Conference Papers and Presentations Status: Published Year Published: 2019 Citation: Dukes, S. 2019. Pastured pork production. Midwest Organic & Sustainable Education Service Conference. La Crosse, WI. February 22. 7. Type: Conference Papers and Presentations Status: Published Year Published: 2019 Citation: Dukes, S. 2019. Pastured pork production. Midwest Organic Pig Conference, Waterloo, IA. March 8. 8. Type: Other Status: Other Year Published: 2019 Citation: Carr, R. and Dukes, S. 2019. 103rd Pennsylvania Farm Show, Harrisburg, PA. January 5-12. 9. Type: Other Status: Published Year Published: 2019 Citation: Li, Y., L. Johnston, B. Heins, R. Cox, W. Lazarus, J. Tallaksen, W. Martin, F. Forcella, R. Gusch, R. Carr, and A. Hernandez. 2019. Organic Swine Research at the University of Minnesota. Annual Showcase of Department of Animal Science, University of Minnesota, St Paul, MN. April 3. 10. Type: Other Status: Other Year Published: 2019 Citation: Carr, R. and S. Dukes. 2019. Tours of swine facility/pasture and compost site, and poster presentations on organic pig management and manure composting. Rodale Institute Field Day, Kutztown, PA. July 19. 11. Type: Websites Status: Published Year Published: 2018 Citation: Rodale Institute. Researchers to Investigate how to Control Parasites in Organic Pork Production. Rodale Institute Website. Available at: https://rodaleinstitute.org/blog/rodale-institute-partners-with-universities-to -control-parasites-in-organic-swine/. 12. Type: Websites Status: Published Year Published: 2018 Citation: Researchers to Study Controls for Swine Parasites in Organic Pork Production. Lancaster Farming, December 28, 2018: A17 and A20. Available at: https://www.lancasterfarming.com/farming/organic/researchers-to-study-controls- for-swine-parasites-in-organic-pork/article dc746058-8a9a-549e-89f2-40cbb8580c2 1.html Available at: https://www.feedstuffs.com/nutrition-health/project-investigate-parasite-contro l-organic-pork Available at: http://smartsolution247.com/project-to-investigate-parasite-control-in-organic- pork/ Available at: https://americanagnetwork.com/2018/12/researchers-to-study-controls-for-swine-p arasites-in-organic-pork-production/ 13. Type: Other Status: Published Year Published: 2019 Citation: Carr, R., 2019. Kutztown, Pennsylvania: Composting to Manage Swine Parasites. BioCycle: Composting Roundup. May/June, 60:4, 11.

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Harnessing Variation in Vetch and Rhizobia Populations to Optimize Nitrogen Fixation

Accession No.	1017066
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NON-TECHNICAL SUMMARY

Organic farmers identify improved cover crop options as a high priority area for research, and rate symbiotic nitrogen fixation as the most important trait for leguminous species. Leguminous cover crops are the primary source of new nitrogen for organic agriculture and provide a range of ecosystem services valued by organic growers. The performance of vetch cover crops across farmers\' fields is highly variable, making it difficult for organic farmers to manage their N fertility. The overarching goal of this project is to elucidate how interactions among soil fertility, plant genotype, and symbionts drive the N acquisition strategies of hairy vetch (Vicia villosa Roth) in organic systems. We focus our research on hairy vetch because of its importance as a cover crop in organic farming, particularly in colder climates where cover crop species options are more limited. Symbiotic nitrogen fixation is a complex process governed by the three-way interaction involving the plant host, symbionts (rhizobia bacteria and arbuscular mycorrhizal fungi), and the soil environment. We have four objectives. 1) Quantify the relative importance of plant genotype, soil nitrogen availability and genotype x environmental interactions in governing the capacity and plasticity of nitrogen fixation in hairy vetch. 2) Characterize rhizobia populations that reflect different selection pressures and identify strains with improved plant growth benefits for hairy vetch. 3) Quantify differences in AM fungal colonization across hairy vetch breeding lines/populations. 4) Develop curricula modules covering the basics of legume biology and management of legumes for nitrogen supply to crops in organic systems.

OBJECTIVES

Objectives Quantify the relative importance of plant genotype, soil N availability and genotype x environmental interactions in governing the capacity and plasticity of SNF in hairy vetch. Characterize rhizobia populations that reflect different selection pressures including plant genotype, climate, and history of organic management, and identify strains with improved plant growth benefits. Quantify differences in AM fungal colonization across hairy vetch breeding lines/populations. Develop and disseminate curricula modules covering the basics of legume biology and management of SNF for N supply to crops in organic systems.

APPROACH

Our research plan uses a combination of field studies and greenhouse experiments, which will enable us to account for the realistic complexity of interactions occurring in the field while also testing specific hypotheses in controlled environments. We will capitalize on the on-going vetch cover crop breeding efforts by conducting a field experiment comparing the best cold adapted HV populations available to other HV populations currently used by organic farmers. We will conduct several greenhouse experiments targeting key questions about the role of plant genotype, soil N availability and rhizobia communities in governing SNF and vetch N economy.1.1. Nitrogen economy of improved cold adapted HV lines and Objective 3. Characterization of HV AM fungal infections: Experimental sites will be located in MN at the St. Paul Minnesota Agriculture Experiment Station farm and in NY at the Homer Thompson Vegetable Research Farm. In MN, the organic section of the research farm has moderately high fertility due to a history of compost additions. In NY, we will select two fields of Howard gravelly loam with contrasting management histories and N fertility status for our experimental plots. We will select and plant 24 HV vetch lines in mid-August 2019. Following a randomized complete block design, we will have 24 treatments (HV lines) x 6 replications for a total of 144 plots. We will collect the following data on all lines: % emergence, relative vigor at three key time points (fall, spring green-up and 10% flowering), % winter survival, growth stage at a common time point in the spring and collect biomass cuts. We will analyze shoots for N, C, and d15N. We will also collect soil samples from these three fields, measure soil properties and soil N pools. At the time of biomass sampling, we will collect and preserve roots from each plot by placing them in 50% ethanol and storing them at 4oC for later quantification of AM fungi colonization (Objective 3).1.2. Capacity and plasticity of N acquisition strategies in HV lines: For this greenhouse experiment, two factors, vetch genotype and N rate will be tested in a randomized complete block design with ten replications: 8 HV lines x 5 N levels (0N and 4 levels of N additions) x 10 replications for a total of 400 pots. N will be added as (15NH4)2SO4 (5 atom % 15N) to achieve ppm approximating 2, 10, 20 and 40 ppm which will be combined with N-free Hoagland\'s to supply all other nutrients. When at least 50% of the plants have initiated flowering we will destructively sample shoot biomass, and record nodule number and analyze shoots for total N and ?15N. We will calculate SNF rates using two source mixing-models.2.1. Vetch-rhizobia symbiotic performance/nodulation under cold-temperature conditions: We will carry out experiments in specialized growth chamber environments to determine the effect of geographic origin and cold temperatures on nodulation and N fixation capacity. We will simulate temperature conditions encountered in the Upper Midwest/Northeast in the early spring, a critical time for organic farmers wishing to take advantage of legume cover crop capacity to provide sufficient N via SNF. We will use a randomized complete block design with factors of rhizobia inoculant (4 strains) and temperature (4 levels), for 16 distinct treatments. We will include at least 6 replications of each inoculant as blocks in each chamber, totaling 6 nutrient treatments (inoculated plus negative N- and positive N+ controls) x 6 replications x 4 temperatures = 144 plants. We will use 1) three genetically distinct cold-adapted strains from the northern MN research station of Grand Rapids, and 2) a non-cold-adapted species-specific strain from Goldsboro, NC, and 3) controls including non-inoculated plants and N+ plants with no inoculation.2.2. Screening microbial communities from organic soils for HV growth benefits and SNF capacity: We will inoculate four vetch genotypes showing variation in N economy with inoculant soil slurries obtained from 10 farms differing in management histories. We will grow them under sterile, N-limited conditions (4 genotypes x 40 soil dilutions \10 fields x 4 plots/field\ x 10 replications, for 1,600 plants). We will collect soils from farms that have been managed organically for different lengths of time, representing those that have more recently transitioned to organic management (1 - 3 years) and longer-term organic systems (including some with \>20 years of organic management). This will provide a chronosequence of organically managed soils and we know from experience that there will be large differences in SOM and nutrient levels among farms. Fields will be divided equally into 4 plots and composite samples consisting of 20 soil cores (20 cm deep x 1.9 cm diameter) will be collected from each plot giving a total of 40 samples (10 fields x 4 plots/field). This will allow us to have adequate spatial representation of soil abiotic conditions and rhizobia community for each field. We will clean and sterilize (ethanol) soil collection tools between replicate plots/farms to avoid cross-contamination of soil communities. We will measure common soil properties and soil N pools for soils from each of these fields. We will mix 5g of each soil with 50mL sterile diH2O and use these slurries to inoculate HV seeds. We will grow plants in pots as described in the Detailed Analytical Methods section. Plants will be grown for at least twelve weeks. At harvest, along with aboveground biomass measurements, we will count nodule number and after weighing nodules, we will collect pooled nodule samples from a sub-set of plants (3/10 replicate plants from each treatment). We will collect and preserve 2-4 nodules from each of three high vigor plants from all soil inoculant x HV genotype combinations (4 HV genotypes x \10 fields x 4 plots/field = 40 soil inoculants\) for a total of 160 pooled nodule samples.2.3. Effectiveness and efficiency of rhizobia strains isolated from organic farms: Research methods: To meet this objective, we will design and carry out a controlled experiment to understand if organic management history differentially selects for rhizobia with high or low capacity to fix atmospheric N with improved lines of hairy vetch. Using this data, we will identify three farms: two high-performing rhizobia population farms and one low-performing rhizobia population farm. From each of these three farms, we will use 16 pooled nodule samples for strain isolation. Rhizobia will be isolated, cultured and preserved from individual nodules (1 nodule per sample) using standard microbiological protocols. We will select

16 isolates from each of the three farms to be used as inoculants for 48 inoculants derived from organically managed soils. Using a randomized complete block design, we will evaluate SNF attributes with 50 inoculant treatments including vetch that is a) inoculated using each of the 48 rhizobia strain isolates from Exp. 2.2, b) inoculated using two currently recommended commercial rhizobia inoculants.Objective 4: Develop and disseminate curricula modules: We will develop three course modules by drawing on our existing course material, as well as from other experts in the field, to provide rich-picture examples from around the U.S. of farmers actively utilizing SNF in their systems, as well as recent research results. The first teaching module will emphasize practical management of legume cover crops for efficient nutrient management. The second will provide the scientific underpinnings of SNF and basis for management approaches. A final module will consist of a case study and a nutrient management game, developed to help students grapple with the difficult nutrient management and legume cover crop decisions farmers\' face in organic farming systems. **Progress** 09/01/18 to 08/31/23 **Outputs** Target Audience:Our primary target audience is other scientists engaged in agricultural research and extension professionals who will be able to use our findings to develop new cultivars and management strategies that will benefit organic farmers. Specifically, the project will provide new knowledge that legume breeders can use to develop improved green manure species and rhizobia inoculants well-adapted to organic farming systems. We will reach these audiences through articles published in refereed journals and presentations at professional meetings. We will also deliver science-based knowledge about legumes, symbiotic N fixation, and organic N management to undergraduates in the agricultural and environmental sciences through the development of curriculum modules, innovative teaching methods, and formal classroom instruction. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?Over the project period, one postdoc, two PhD students and nineteen undergrads learned about agroecological research encompassing the fields of soil, plant and microbial ecology and biological nitrogen fixation. They all gained knowledge and experience in carrying out agricultural field and greenhouse research and plant/soil laboratory analyses. The postdoc (Cornell: Katherine Muller) and PhD students (Minnesota: Rebecca Fudge and Gaby Hidrobo) deepened their skills in experimental design and execution, mentoring undergrads, and communication with diverse audiences. Fudge received funding for travel to present work at three conferences: the American Geophysical Union, the Australian Conference on Nitrogen Fixation, and Marbleseed. The PhD students and post-doc supervised over 15 undergraduate students across the project\'s life to learn about microbiology techniques, plant growth, and stable isotope analysis. The project has allowed extensive training with two undergraduate students who carried out an independent research project. One student, supervised by the postdoc conducted a greenhouse experiment at Cornell. The other undergrad was supervised by Fudge and secured funding for their independent research project through the University of Minnesota Undergraduate Research Opportunities Program to create growth curves of the six \"successful nodulator\" strains at low temperatures. Muller secured a position with ARS as a research scientist. Fudge secured an AFRI pre-doctoral fellowship and is scheduled to defend her PhD in Spring 2024. How have the results been disseminated to communities of interest?Results from this project have been shared though a diverse array of conference and workshop venues as well as journal articles, a book chapter, and extension articles targeting farmers and extension educators. Early in the project, the pandemic resulted in many cancellations of conferences and workshops. Nevertheless, across the project, we have reached an estimated 1300 individuals through extension and scientific presentations, including invited and plenary presentations and webinars that featured results from the project. In 2023, we presented our results in poster sessions at three conferences (listed under \"Products\"). At the Marbleseed (formerly MOSES) organic farming conference, approximately 80 farmers came to the oral session, many curious about hairy vetch as an annual winter cover crop. Three peer-reviewed publications (a book chapter and two journal articles) have been published, and an additional two will be submitted in the next two months. Two more manuscriptsarebeing prepared for submission in mid-2024. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported **Impacts** What was accomplished under these goals? 1. Quantify the relative importance of plant genotype, soil N availability and genotype x environmental interactions in governing the capacity and plasticity of symbiotic N fixation (SNF) in hairy vetch. Field experiment: We established a common garden experiment in the early fall of 2019 using 30 cold-adapted vetch lines representing the range of genetic diversity available to farmers in our region. These included four improved lines from collaborator Ehlke, four commercial cultivars, nine regional populations (variety-not-stated), and 14 accessions from the USDA-GRIN germplasm collection. All 30 lines were replicated across six randomized blocks and intercropped with triticale and fine fescue to minimize weeds. Before planting, we sampled soil to measure abiotic soil properties and to acquire a sample of resident rhizobia trapped by two contrasting vetch genotypes. We collected data on the emergence and fall vigor, winter survival/vigor, and developmental timing until each line reached 50% flowering when plants were harvested. We harvested \~1000 vetch plants in June 2020. To evaluate SNF rates, we selected a subset of 12 vetch lines for stable isotope analysis. Preliminary analysis shows no apparent correlations among measured traits, including plant size, developmental timing, proportion of N acquired through fixation, and AMF colonization. The final analysis is underway, and we will use multivariate Bayesian models to quantify trait variance and covariance within and

among accessions (manuscript in prep). 15N tracer greenhouse experiment: We investigated genetic variation in SNF traits by comparing clonal vetch populations in a greenhouse experiment June-September 2021. We compared 4 clonal vetch populations propagated from a single parent plant, each representing a distinct vetch accession from the field experiment. We used two sets of treatments. The first set of treatments compares individual clones over a gradient of N availability. The second set compares clones in competition with a non-legume (wheat) at high and medium N levels. Together, the treatments allow us to compare genetic variation in SNF capacity and plasticity and the role of SNF traits in competitive interactions. There was a delay in submitting samples for stable isotope analysis due to the backlog of samples at the stable isotope facility resulting from closure during the pandemic. We now have a complete dataset including shoot, root and nodule biomass, N content and 15N stable isotope data. Papers from both experiments will be submitted this year (2024) even if the PI (LED) must write them herself. 2. Characterize rhizobia populations that reflect different selection pressures including plant genotype, climate, and history of organic management, and identify strains with improved plant growth benefits. These experiments characterize the variability of Rhizobia to function and fix nitrogen under the cold conditions experienced in the northern US and will contribute to the development of effective inocula for organic farmers in this region. A collection of 32 rhizobia strains isolated from Minnesota and North Carolina soils were tested as hairy vetch inoculants. Two cold-hardy ecotypes of hairy vetch (Albert Lea Cold-Hardy VNS and MSP 4045) were inoculated with each rhizobia strain, replicated 4 times. (32 rhizobia strains x 2 hairy vetch ecotypes x 4 replicates = 256 plants) Time to nodulation was higher for Albert Lea plants than MSP 4045 plants ($p = 0.003$). We identified \"consistent nodulator\" strains, determined by selecting strains that nodulated seven or eight of the eight total plants originally inoculated by each strain. This resulted in the selection of 6 of the total 32 strains for further study in experiment 2. A follow-up experiment was conducted with the six \"consistent nodulator\" strains to determine their N-fixation capacity at low temperatures, and the relationship between nodulation speed and fixed nitrogen. Six rhizobia strains were used as inoculant treatments based on their performance in experiment 1, with 3 strains from Minnesota and 3 from North Carolina. We also included two controls: a positive nitrogen control (plant given nitrogen-rich nutrient solution) and a positive nodulation control (plant inoculated with commercial inoculant). Once again, two hairy vetch ecotypes were used \2 hairy vetch ecotypes x 8 treatments (6 rhizobia, 2 controls) x 4 replicates x 2 time points = 128 plants\. The results demonstrated that plant genotype and temperature drove SNF in hairy vetch. Plants fixed dramatically less N and accumulated less shoot biomass under cold conditions than warm conditions ($p \< 0.001$).). Total shoot N is calculated as the %N present in shoot biomass, multiplied by total shoot biomass, to estimate N contributions from hairy vetch plants when incorporated into the soil. Under cold conditions, the hairy vetch genotype AU Merit accumulated more shoot N than any other genotype. Under warm conditions, AU Merit accumulated more shoot N than the genotypes Purple Bounty and MSP 4045, but a statistically equivalent amount to Hungvillosa. While AU Merit is considered \"cold-susceptible,\" (has relatively low winter survival due to low freezing tolerance), it still achieved relatively high N accumulation under cool conditions (15C day/10C night). Agricultural fields can host a broad range of rhizobia, which vary in traits such as N fixation rate and, consequently, benefit the plant. There is evidence that certain plants can selectively reward rhizobia that fix N more efficiently (Kiers et al., 2003), and that particular plant genotypes within a species can select more beneficial rhizobia than other genotypes with the same species (Burghardt et al., 2018). Thus, we hypothesized that plants with greater shoot N associate with superior rhizobia strains than plants with less shoot N. However, nodule population differentiation did not correlate with plant phenotype. Rhizobia population differentiation only varied with soil origin, and soil origin had no effect on shoot biomass or N accumulation. Shoot biomass and N accumulation varied most strongly with temperature and ecotype, which suggests that the most productive path forward to improve N accumulation in vetch may be to focus on plant breeding, rather than harnessing specific associations with rhizobia. Two manuscripts from this work are in preparation and will be submitted within the next four months. 3. Quantify differences in AM fungal colonization across hairy vetch breeding lines/populations. We quantified AMF colonization in \~200 root samples from 12 vetch lines sampled from our field experiment in Freeville, NY (Objective 1). AMF colonization varied greatly across samples, and vetch lines. We are analyzing these data in conjunction with biomass P concentration and vetch P acquisition to determine whether AMF indirectly impacts SNF through their role in P uptake. Preliminary analysis suggests that in this soil, AMF did not play a significant role in facilitating vetch P acquisition or SNF. 4. Develop and disseminate curricula modules covering the basics of legume biology and management of SNF for N supply to crops in organic systems. Two modules were compiled and designed, onecovering the basics of legume biology and a second emphasizing management oflegumes for nitrogen supply to crops in organic systems. They were piloted in existing courses at both institutions, revised, and made available to other universities with an interest in providing student-facing material on legume biology and nitrogen fixation in organic systems. We are working with our respective web designers to post these resources so they will be freely available through open-source repositories to university and extension educators. **Publications** - Type: Book Chapters Status: Published Year Published: 2024 Citation: Schaedel M, Fudge R, Grossman J. Biological Nitrogen Fixation within Agroecosystems and Plant Roots. Biological Approaches to Regenerative Soil Systems, 2nd Edition, edited by Norman Uphoff and Janet Thies, published by

CRC Press. Approaches to Regenerative and Resilient Soil Systems, CRC Press, 2024, pp. 129 - 139. DOI: 10.1201/9781003093718-15 - Type: Conference Papers and Presentations Status: Other Year Published: 2023 Citation: Fudge R and Grossman J. December 2023. Temperature and Host Plant Genotype Drive Nitrogen Fixation Efficiency in a Winter Annual Cover Crop Species (poster). American Geophysical Union Conference. San Francisco, CA. - Type: Conference Papers and Presentations Status: Other Year Published: 2023 Citation: Fudge R. September 2023 Improving nitrogen fixation in a winter annual cover crop. Invited talk. NIBIO Apelsvoll, Kapp, Norway - Type: Conference Papers and Presentations Status: Other Year Published: 2023 Citation: Fudge R. September 2023 Improving nitrogen fixation in a winter annual cover crop. Invited talk. NIBIO Holt, Troms&#65533;, Norway. - Type: Conference Papers and Presentations Status: Other Year Published: 2022 Citation: Fudge R, Denison RF, and Grossman J. December 2022 Nondestructive phenotypic assays for nitrogen fixation research. Australian Conference on Nitrogen Fixation. Fremantle, Western Australia, Australia. - Type: Conference Papers and Presentations Status: Other Year Published: 2023 Citation: Fudge R and Grossman J. December 2023. Temperature and Host Plant Genotype Drive Nitrogen Fixation Efficiency in a Winter Annual Cover Crop Species (poster). American Geophysical Union Converence. San Francisco, CA. - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Drinkwater, L.E. 2022. Using cover crops to optimize nutrient cycling processes and restore soil health. 20th Australian Agronomy Conference, September 2022. Invited plenary presentation and published abstract. **Progress** 09/01/21 to 08/31/22 **Outputs** Target Audience:This year many meetings and extension workshops resumed in person and we reached our primary target audiences (other scientists engaged in agricultural research and extension professionals) through both virtual and in-person venues. We also reached out to farmers through articles and events targeting organic farmers and those interested in transitioning to organic farming. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? This project has allowed one PhD student (Rebecca Fudge) and one post-doc to deepen their skills in experimental design, communication to diverse audiences (funding for travel to three conferences: ASHS, Tri Societies, and MOSES), and mentorship (working with ten undergraduate students total to learn about microbiology techniques, plant growth, and combustion analysis). This experience enabled the post-doctoral researcher to obtain a position as an ARS scientist. The project has allowed extensive training for one undergraduate student (Lou Hoff), culminating in funding for their independent research project through the University of Minnesota Undergraduate Research Opportunities Program to create growth curves of the six \"successful nodulator\" strains at low temperatures. Four additional undergraduate students gained experience in fieldwork and basic laboratory protocols commonly used in agroecological research. How have the results been disseminated to communities of interest? Results from this project have been shared through several venues both virtual and in person over the past year, reaching diverse audiences composed of agricultural researchers, graduate students, extension educators, and farmers. Results from this project were shared at poster sessions at three conferences: American Society for Horticultural Science Annual Conference, Tri Societies International Organic Meeting, and the MOSES Organic Farming Conference. Approximately 50 agricultural professionals viewed this project's poster across the conferences. At the MOSES organic farming conference, approximately 20 farmers came to the poster, many of whom were curious about hairy vetch as a winter annual cover crop. Posters: Fudge, R. and Grossman, J. (2022) Identifying rhizobia partners for cold-hardy hairy vetch. MOSES Organic Farming Conference. La Crosse, WI. Fudge, R. and Grossman J. (2021) Identifying rhizobia partners for cold-hardy hairy vetch. Tri Societies International Annual Meeting. Salt Lake City, UT. Fudge, R., Epstein B., and Grossman J. (2021) Do cold-adapted and cold-susceptible hairy vetch ecotypes differ in their preferences for rhizobia partners? American Society for Horticultural Science. Denver, CO. L.E. Drinkwater. \"Using legumes to supply crop nitrogen while also reaping benefits for soil health\". Webinar sponsored by the Natural Resources Conservation Service. March 2022. Extension educators, grad students and agricultural researchers, 300 attendees. Using Legumes to Supply Crop Nitrogen While Also Reaping Benefits for Soil Health \-- The Webinar Portal (forestrywebinars.net). Two extension articles related to this project have been published. The first article is entitled \"Rhizobia Inoculation for Organic Farming Systems,\" for the University of Minnesota Extension newsletter \"Fruit and Vegetable News.\" The article covers both broad ideas related to biological nitrogen fixation and some information specific to this project. Then a follow-up article was published for a broader audience entitled \"Inoculating Garden Legumes\" for the University of Minnesota Yard and Garden News. Graduate student Rebecca Fudge also hosted an outreach event at the Minnesota State Fair, in August 2022, in collaboration with the Forever Green Project at the University of Minnesota. She discussed winter annual cover crop strategies with over 200 State Fair attendees who visited the booth, about half of whom were farmers. Post-doctoral scientist Katherine Muller presented two virtual talks to the Practical Farmers of Iowa Conference in January 2022. Titles were: \"All About Legumes. Part I: Cover crops. Part II: Nitrogen Fixation.\" What do you plan to do during the next reporting period to accomplish the goals?Our efforts during the next reporting period will largely be devoted to analyzing our experimental results and writing manuscripts to be submitted for publication in referring journals. We aim to submit publications from the vetch field experiment and greenhouse experiment by the end of the 2023 spring semester and the beginning of the 2023 fall semester, respectively. In addition, we will finalize the education modules and disseminate

information about the nutrient budgeting game. **Impacts** What was accomplished under these goals? 1\. Quantify the relative importance of plant genotype, soil N availability and genotype x environmental interactions in governing the capacity and plasticity of SNF in hairy vetch. This work identifies the relationships among traits that are valued by farmers and will speed up developing vetch cultivars that meet the needs of organic farmers. Field experiment: The field experiment evaluated variation in symbiotic N fixation and other key traits within and among 30 vetch accessions (2019-2020). Preliminary analysis shows significant variation across individual vetch plants, but no apparent correlations among measured traits, including plant size, developmental timing, the proportion of N acquired through fixation, and AMF colonization. The final analysis will use multivariate Bayesian models to quantify trait variance and covariance within and among accessions. Greenhouse experiment: The greenhouse experiment compares clonal vetch populations in terms of their regulation of SNF rates in response to varying levels of inorganic N availability (2021). The greenhouse experiment was completed this year, and aside from a few samples in need of re-analysis for stable isotope content, all analytical work has been completed. The experimental design consisted of three crossed treatments: vetch genotype (four clonal families), N level (1, 3, 5, 7, and 9mM N), and competition (vetch + vetch, or vetch + wheat, tested at only two N levels). Response variables include plant size, nodulation, and N acquisition from fertilizer vs. SNF. Once the remaining samples are remeasured, the next steps are to combine and analyze data. 2\. Characterize rhizobia populations that reflect different selection pressures including plant genotype, climate, and history of organic management, and identify strains with improved plant growth benefits. These experiments characterize the variability of Rhizobia to function and fix nitrogen under the cold conditions experienced in the northern US and will contribute to the development of effective inocula for organic farmers in this region. Pilot experiment:A collection of 32 rhizobia strains isolated from Minnesota and North Carolina soils were tested as hairy vetch inoculants. Two cold-hardy ecotypes of hairy vetch (Albert Lea Cold-Hardy VNS and MSP 4045) were inoculated with each rhizobia strain, replicated 4 times. (32 rhizobia strains x 2 hairy vetch ecotypes x 4 replicates = 256 plants) Plants were grown in sterile growth pouches, for 6 weeks in a growth chamber, set at 15C day/10C night to mimic fall planting conditions in the upper Midwest and watered daily with nitrogen-free nutrient solution When comparing number of days to first nodule formation across the two hairy vetch ecotypes, Albert Lea plants developed nodules slightly earlier than MSP 4045 plants. However, a higher percentage of MSP 4045 plants ultimately developed nodules. Time to nodulation was higher for Albert Lea plants than MSP 4045 plants ($p = 0.003$). Results were then combined across hairy vetch ecotype, resulting in 8 inoculated plants for each individual rhizobia strain. Mean days to nodulation ranged from 23.33 to 34 days. However, some strains only nodulated 2 of the 8 plant replicates. We identified \"consistent nodulator\" strains, determined by selecting strains that nodulated seven or eight of the eight total plants originally inoculated by each strain. This resulted in the selection of 6 of the total 32 strains for further study in experiment 2. Interestingly, of the 32 strains tested, 5 of the 6 consistent nodulators were Minnesota-origin strains. Nitrogen Fixation Experiment: A follow-up experiment was conducted with the six \"consistent nodulator\" strains to determine their N-fixation capacity at low temperatures, and the relationship between nodulation speed and fixed nitrogen. Plants were grown in modified Leonard jars for 10 weeks. However, unlike in growth pouches, root systems are not visible in magenta units. Two destructive harvests were carried out: harvest 1 at 6 weeks, and harvest 2 at 10 weeks. I hypothesized that nodule number at 6 weeks would predict biomass at 10 weeks. Six rhizobia strains were used as inoculant treatments based on their performance in experiment 1, with 3 strains from Minnesota and 3 from North Carolina. I also included two controls: a positive nitrogen control (plant given nitrogen-rich nutrient solution) and a positive nodulation control (plant inoculated with commercial inoculant). Once again, two hairy vetch ecotypes were used \2 hairy vetch ecotypes x 8 treatments (6 rhizobia, 2 controls) x 4 replicates x 2 time points = 128 plants\. This experiment sought to address three main questions: 1. How well does early nodule number predict biomass production? At both 6 and 10 weeks, nodule number and plant biomass were highly correlated ($R2 = 0.68$ at 6 weeks, and 0.67 at 10 weeks). However, nodule number at 6 weeks was not correlated with biomass at 10 weeks. These results suggest that growth chamber experiments that run for 6 weeks or less may not give useful information on nitrogen fixation in hairy vetch, especially at low temperatures. 2\. Can cold-adapted rhizobia increase biomass production compared to a commercial inoculant? Rhizobia strain geographic origin did not have a consistent or reliable effect on hairy vetch biomass accumulation. However, the best-performing strain (MNC8) came from Minnesota, and the worst-performing strain (NCC5) came from North Carolina. This suggests the best rhizobia partner for hairy vetch might come from Minnesota soils, but the amount of screening required to find that strain would be extensive. No strain outperformed the commercial inoculant. 3.Does one hairy vetch ecotypes tend to produce more biomass at low temperatures? The two hairy vetch ecotypes, Albert Lea cold-hardy and MSP 4045, performed equally well at 15C day/10C night temperatures. There was no observable difference in their biomass accumulation. Ultimately, rhizobia treatment had a greater effect on biomass accumulation. 3\. Quantify differences in AM fungal colonization across hairy vetch breeding lines/populations. We quantified AMF colonization in \~200 root samples from 12 vetch lines sampled from our field experiment in Freeville, NY. AMF colonization varied greatly across samples, and vetch lines. We are analyzing these data in conjunction with biomass P concentration and vetch P acquisition to determine whether AMF indirectly impact SNF through their

role in P uptake. 4\. Develop and disseminate curricula modules covering the basics of legume biology and management of SNF for N supply to crops in organic systems. We have compiled two sets of PowerPoint slides which will be freely available through open-source repositories to university and extension educators. Each slide set is accompanied by instructions for a hands-on activity that can be used in conjunction with a presentation. The \"Practical Management of Legume Cover Crops\" module provides information about how environmental conditions and management practices impact legume growth and symbiotic nitrogen fixation. A nutrient budgeting exercise accompanies this module. The other module, \"Ecology of the Rhizobia-Legume Symbiosis\" explains the basics of rhizobia biology and ecology and provides practical information about all facets of successful inoculation. This module includes instructions for hands-on activities for viewing nodules and assessing whether there is active nitrogen fixation occurring in nodules. Lastly, we are further developing a nutrient management game that has been used by LED in her agroecology course that can be used to teach principles of organic nutrient management. Using data on nutrient flows in US organic farming systems, we will modify this game to be relevant to these farming systems and make it available to extensionists and university faculty. **Publications** - Type: Journal Articles Status: Accepted Year Published: 2023 Citation: Thurston, Grossman, J. (Corresponding Author), Fudge, R., Maul, J., Mirsky, S., & Weiring. 2023. Cold stress reduces nodulation and symbiotic nitrogen fixation in winter annual legume cover crops. Plant and Soil. - Type: Other Status: Published Year Published: 2022 Citation: Fudge, R (2022). Inoculating garden legumes. University of Minnesota Yard and Garden News. https://extension.umn.edu/yard-and-garden-news/inoculating-garden-legumes - Type: Other Status: Published Year Published: 2022 Citation: Fudge, R (2022). Rhizobia inoculation for organic farming systems. Minnesota Fruit and Vegetable News. <https://blog-fruit-vegetable-ipm.extension.umn.edu/2022/01/rhizobia-inoculation-for-organic.html>. **Progress** 09/01/20 to 08/31/21 **Outputs** Target Audience: Nothing Reported Changes/Problems:Student research assistants were allowed to engage in research on campus starting in January 2021, so we were able to make significant headway on processing a backlog of samples that were at a standstill during fall 2020. We expect that we will need to continue to adjust our plans to accommodate changes in university policies as they are modified to respond to changing infection rates in campus communities. What opportunities for training and professional development has the project provided?One post-doctoral scientist continued to carryout research and develop skills in conducting and analyzing data from field and greenhouse experiments. She also supervised an undergraduate independent research project and developed mentoring skills. We continue to support one graduate student at the University of Minnesota on this project. In addition, undergraduate research assistants (6) supported both the field and lab components of this work and received training in both field and lab methods for agricultural and ecosystem science research. One student completed a research project for course credits. How have the results been disseminated to communities of interest?The pandemic continues to disrupt conferences and workshops. Some that we were scheduled to present at have been postponed to 2022. Nevertheless, we had some presentations to our target audiences during this year. Muller K. and L.E. Drinkwater. Developing Better Green Manures: Harnessing Variation in Vetch Populations. Poster presentation for the Cornell Organic Symposium, April 2019. The audience included researchers, extension professionals, and local farmers. Fudge R, Epstein B, Grossman J. Do Cold-Adapted and Cold-Susceptible Hairy Vetch Ecotypes Differ in their Preferences for Rhizobia Partners? Poster Presented at: American Society for Horticultural Science Annual Conference; 2021 August 5-9; Denver, CO. Drinkwater, L.E. Ecological Nutrient Management: Many Little Hammers. Invited oral presentation at the Ecological Society of America annual meeting, which was held virtually, August 2-6, 2021. This presentation included information supported by this grant. August 31, 2021: Fudge and Grossman held a booth at the Minnesota State Fair, where we educated 50 people on cover crops and biological nitrogen fixation What do you plan to do during the next reporting period to accomplish the goals?The final stages of data collection and analysis for the field and greenhouse experiments performed in New York will be carried out during the winter 2021/spring 2022. We will submit two manuscripts presenting the results from these experiments by the end of 2022. In January 2022, data from the nitrogen fixation growth chamber experiment conducted in Minnesota will be analyzed. Based on those results, 1-3 successful strains from the experiment will be tested under competitive conditions to simulate the likelihood of inoculant success in field conditions. Data will be presented to a farmer/extension audience at MOSES Organic Farming Conference in February 2022. A manuscript describing these results will be submitted for publication by the end of 2022. **Impacts** What was accomplished under these goals? 1. Quantify the relative importance of plant genotype, soil N availability and genotype x environmental interactions in governing the capacity and plasticity of SNF in hairy vetch. We evaluated variation in SNF and other traits within and among 30 vetch accessions in the field. The experiment took place in a moderate-fertility field in Freeville, NY. The field experiment was planted in August, 2019 and plants were harvested in June, 2020. We collected data on winter survival, vigor, developmental timing, and sampled total aboveground biomass. We harvested and processed \~1000 vetch plants from the field. To evaluate symbiotic N fixation rates, we selected a subset of 12 vetch lines for stable isotope analysis and received the data in October 2021. We are in the process of analyzing this data. We investigated genetic variation in SNF traits by comparing clonal vetch populations in a greenhouse experiment that took place between June and September 2021. To prepare for the main greenhouse experiment,

we performed a pilot experiment between March and May 2021. The pilot experiment also contributed to the education of an undergraduate student, who worked on the project for research credits. The main greenhouse experiment compared four clonal vetch populations propagated from a single parent plant, each representing a different vetch accession from the field experiment. The experimental design used two sets of treatments to investigate different questions about SNF plasticity and genetic variation in hairy vetch. The first set of treatments compares individual clones over a gradient of N availability. The second set of treatments compares clones in competition with a non-legume (wheat) at high and medium N levels. Together, the treatments will allow us to compare genetic variation in SNF capacity and plasticity and the role of SNF traits in competitive interactions. We have a complete dataset on plant growth, including shoot biomass (all treatments) and root and nodule biomass (N gradient treatments only). We are processing samples for stable isotope analysis and expect to be finished by March 2022. 2. Characterize rhizobia populations that reflect different selection pressures including plant genotype, climate, and history of organic management, and identify strains with improved plant growth benefits. To characterize rhizobia populations that reflect different selection pressures, we assessed selection pressures created by cold-adapted and warm-adapted vetch ecotypes. The experimental design was described during the last reporting period. Since then, nodule communities were sequenced and analyzed, with allele frequencies calculated at every loci. Pairwise FST tests were calculated across all nodule pools. We hypothesized that if cold-hardy hairy vetch selects for different rhizobia strains than warm-adapted vetch, FST values would be higher across treatments than within treatments. Our results were consistent with that hypothesis. Results were presented at American Society for Horticultural Science Conference and Tri Societies conference. To identify strains with improved plant growth benefits, two experiments were carried out using the strains described above, along with Grossman laboratory collection strains from North Carolina. The first experiment screened 32 total strains, half from Minnesota and half from North Carolina. Two ecotypes of hairy vetch were grown in plastic growth pouches (2 ecotypes x 32 strains x 4 replicates = 256) so that nodule development was visible. The growth chamber was set at 10C day/5C night. We recorded number of days to nodulation of each plant. The three fastest-nodulating and three slowest-nodulating strains were then tested in a follow-up experiment, where we hypothesized that fast-nodulating strains would lead to greater biomass by 10 weeks. Plants were terminated at the end of December and data is currently being processed. 3. Quantify differences in AM fungal colonization across hairy vetch breeding lines/populations. We collected \~200 root samples from 12 vetch lines in our field experiment in Freeville, NY. We finished collecting data on AMF colonization using ink-vinegar staining and the gridline intersect method. We are in the process of analyzing data on AMF colonization along with other traits. 4. Develop and disseminate curricula modules covering the basics of legume biology and management of SNF for N supply to crops in organic systems. We expect to develop teaching modules that can be used either in a classroom with undergraduates or in hands-on outreach events. Examples include curriculum to teach about rhizobia relationships with legumes, inoculation demonstration, plating and growth of rhizobia and visualization using a portable microscope. We started gathering recent papers on legume N supply to use as a basis for the model focusing on legumes and green manure management. **Publications** - Type: Journal Articles Status: Published Year Published: 2021 Citation: Muller, K. E., J. Guinness, M. Hecking, and L. E. Drinkwater. 2021. Estimating agronomically relevant symbiotic N fixation in green manure breeding programs. Crop Science 61: 33143330.

PROGRESS

2019/09 TO 2020/08 Target Audience: Nothing Reported Changes/Problems:The pandemic disrupted our research timeline, and our field season was particularly difficult because we could not hire any student field assistance. As a result, we are behind schedule and will be setting our greenhouse experiment in early March rather than last fall as planned. What opportunities for training and professional development has the project provided?Training one post-doctoral scientist has continued. In carrying out her work on the project she is receiving training in agroecology, field research methods and use of stable isotope methodologies to quantify symbiotic nitrogen fixation. We continue to support one graduate student at the University of Minnesota on this project. In addition, undergraduate research assistants (2 at UM and 2 at CU) supported both the field and lab components of this work and received training in both field and lab methods for agricultural and ecosystem science research. How have the results been disseminated to communities of interest?The pandemic was very disruptive and plans for other presentations were cancelled for this time period other than these three presented below. Guest lecture on rhizobia inoculation strategies in PLPA 8103 - Plant Microbe Interactions. Audience of 15 students (graduate and undergraduate) at UMN. May 2020. Guest lecture on rhizobial ecology at low temperatures in PMB 5802. Audience of 10 students (graduate and undergraduate). July 2020. Shared project goals and information on organic agriculture through the UMN Market Science Program, October 2019. What do you plan to do during the next reporting period to accomplish the goals?Our first priority is to complete data collection and analysis for the 2019-2020 field experiment comparing hairy vetch lines including quantification of AM fungal colonization and symbiotic N fixation rates. These results will be presented in a manuscript and

submitted to a peer-reviewed journal. To continue our work targeting Goals 1 and 3, this spring we will set up a greenhouse experiment comparing nitrogen fertility among lines tested in the field experiment. This will entail running a pilot experiment with one vetch line to help refine methods for the larger experiment comparting multiple lines. In January 2021, experiments will begin to assess Goal 2, identifying rhizobia strains with improved plant growth benefits. A single-strain inoculation experiment will be carried out to determine which rhizobia strains lead to the highest rates of nitrogen (N) fixation with cold-hardy hairy vetch. Newly isolated rhizobia strains from Roseau as well as collection strains from New York, Maryland, and North Carolina will be tested as inoculants. Two cold-hardy ecotypes of hairy vetch will be germinated and planted in magenta units and inoculated rhizobia of interested. Treatment plants will be supplied with N-free nutrient solution and cultivated in a growth chamber for six weeks, at low temperatures to simulate the critical nodulation window in early spring. Following termination, plants will be assessed for nodule number, nodule mass, and nitrogen fixation capacity. The N-difference approach (Unkovich et al. 2008) will be used to quantify SNF, by measuring distinctions in plant N between inoculated and non-inoculated control plants. Since plant N was only obtained via SNF, the N-difference approach will correlate to rhizobia stain capacity to fix N. Information obtained from this experiment will be critical in determining inoculant recommendations for farmers in cold climates. Lastly, once the spring semester ends, the PDs will work on compiling and posting modules to complete our work for Goal #4.

2020/09 TO 2021/08 Target Audience: Nothing Reported Changes/Problems:Student research assistants were allowed to engage in research on campus starting in January 2021, so we were able to make significant headway on processing a backlog of samples that were at a standstill during fall 2020. We expect that we will need to continue to adjust our plans to accommodate changes in university policies as they are modified to respond to changing infection rates in campus communities. What opportunities for training and professional development has the project provided?One post-doctoral scientist continued to carryout research and develop skills in conducting and analyzing data from field and greenhouse experiments. She also supervised an undergraduate independent research project and developed mentoring skills. We continue to support one graduate student at the University of Minnesota on this project. In addition, undergraduate research assistants (6) supported both the field and lab components of this work and received training in both field and lab methods for agricultural and ecosystem science research. One student completed a research project for course credits. How have the results been disseminated to communities of interest?The pandemic continues to disrupt conferences and workshops. Some that we were scheduled to present at have been postponed to 2022. Nevertheless, we had some presentations to our target audiences during this year. Muller K. and L.E. Drinkwater. Developing Better Green Manures: Harnessing Variation in Vetch Populations. Poster presentation for the Cornell Organic Symposium, April 2019. The audience included researchers, extension professionals, and local farmers. Fudge R, Epstein B, Grossman J. Do Cold-Adapted and Cold-Susceptible Hairy Vetch Ecotypes Differ in their Preferences for Rhizobia Partners? Poster Presented at: American Society for Horticultural Science Annual Conference; 2021 August 5-9; Denver, CO. Drinkwater, L.E. Ecological Nutrient Management: Many Little Hammers. Invited oral presentation at the Ecological Society of America annual meeting, which was held virtually, August 2-6, 2021. This presentation included information supported by this grant. August 31, 2021: Fudge and Grossman held a booth at the Minnesota State Fair, where we educated 50 people on cover crops and biological nitrogen fixation What do you plan to do during the next reporting period to accomplish the goals?The final stages of data collection and analysis for the field and greenhouse experiments performed in New York will be carried out during the winter 2021/spring 2022. We will submit two manuscripts presenting the results from these experiments by the end of 2022. In January 2022, data from the nitrogen fixation growth chamber experiment conducted in Minnesota will be analyzed. Based on those results, 1-3 successful strains from the experiment will be tested under competitive conditions to simulate the likelihood of inoculant success in field conditions. Data will be presented to a farmer/extension audience at MOSES Organic Farming Conference in February 2022. A manuscript describing these results will be submitted for publication by the end of 2022.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? 1. Quantify the relative importance of plant genotype, soil N availability and genotype x environmental interactions in governing the capacity and plasticity of SNF in hairy vetch. We evaluated SNF and other traits in 30 vetch accessions within a moderate-fertility field in Freeville, NY. The field experiment was planted in August, 2019 and plants were harvested in June, 2020. We collected data on winter survival, vigor, developmental timing, and sampled total aboveground biomass. We harvested \~1000 vetch plants from the field and are midway through processing biomass samples for analysis of N uptake and symbiotic N fixation. 2. Characterize rhizobia populations that reflect different selection pressures including plant genotype, climate, and history of organic management, and identify strains with improved plant

growth benefits. In this reporting cycle, a comprehensive literature review was conducted to consolidate information about temperature and management effects on soil rhizobia populations, and best approaches to isolate and characterize rhizobia. To identify potentially cold-hardy rhizobia strains, soil samples were collected from the Roseau, MN experimental field station in Northern Minnesota (plant hardiness zone 2b). Soil was collected from two fields, each with a history of hairy vetch cultivation, thus likely to contain naturalized populations of rhizobia well-adapted to climatic conditions. To assess differences in selection on the soil rhizobia population, four different ecotypes of hairy vetch were used to trap rhizobia from the soil (two cold-adapted ecotypes, and two non-cold-adapted ecotypes.) Hairy vetch seeds were surface sterilized and germinated prior to planting in magenta units and each seedling inoculated with diluted soil from the Roseau station. Plant roots were harvested after six weeks cultivation in a growth chamber, total nodule number counted on each root system, and five nodules randomly selected per plant for culturing. Surface sterilized nodules were crushed onto yeast mannitol agar plates and sub-cultured until strains were ready for storage in glycerol. Rhizobia strains were prepared for genetic analysis via Sanger sequencing of the following genes: 16S, nodC, nifH, atpD, recA, and glnII. Nodule occupancy will be assessed across contrasting vetch ecotypes. 3. Quantify differences in AM fungal colonization across hairy vetch breeding lines/populations. We collected \~200 root samples from 12 vetch lines in our field experiment in Freeville, NY. We are in the process of quantifying AMF colonization using ink-vinegar staining and the grid-line intersect method. 4. Develop and disseminate curricula modules covering the basics of legume biology and management of SNF for N supply to crops in organic systems. None to date. We will complete this objecting in the coming year. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 No publications reported this period.

2020/09 TO 2021/08 What was accomplished under these goals? 1. Quantify the relative importance of plant genotype, soil N availability and genotype x environmental interactions in governing the capacity and plasticity of SNF in hairy vetch. We evaluated variation in SNF and other traits within and among 30 vetch accessions in the field. The experiment took place in a moderate-fertility field in Freeville, NY. The field experiment was planted in August, 2019 and plants were harvested in June, 2020. We collected data on winter survival, vigor, developmental timing, and sampled total aboveground biomass. We harvested and processed \~1000 vetch plants from the field. To evaluate symbiotic N fixation rates, we selected a subset of 12 vetch lines for stable isotope analysis and received the data in October 2021. We are in the process of analyzing this data. We investigated genetic variation in SNF traits by comparing clonal vetch populations in a greenhouse experiment that took place between June and September 2021. To prepare for the main greenhouse experiment, we performed a pilot experiment between March and May 2021. The pilot experiment also contributed to the education of an undergraduate student, who worked on the project for research credits. The main greenhouse experiment compared four clonal vetch populations propagated from a single parent plant, each representing a different vetch accession from the field experiment. The experimental design used two sets of treatments to investigate different questions about SNF plasticity and genetic variation in hairy vetch. The first set of treatments compares individual clones over a gradient of N availability. The second set of treatments compares clones in competition with a non-legume (wheat) at high and medium N levels. Together, the treatments will allow us to compare genetic variation in SNF capacity and plasticity and the role of SNF traits in competitive interactions. We have a complete dataset on plant growth, including shoot biomass (all treatments) and root and nodule biomass (N gradient treatments only). We are processing samples for stable isotope analysis and expect to be finished by March 2022. 2. Characterize rhizobia populations that reflect different selection pressures including plant genotype, climate, and history of organic management, and identify strains with improved plant growth benefits. To characterize rhizobia populations that reflect different selection pressures, we assessed selection pressures created by cold-adapted and warm-adapted vetch ecotypes. The experimental design was described during the last reporting period. Since then, nodule communities were sequenced and analyzed, with allele frequencies calculated at every loci. Pairwise FST tests were calculated across all nodule pools. We hypothesized that if cold-hardy hairy vetch selects for different rhizobia strains than warm-adapted vetch, FST values would be higher across treatments than within treatments. Our results were consistent with that hypothesis. Results were presented at American Society for Horticultural Science Conference and Tri Societies conference. To identify strains with improved plant growth benefits, two experiments were carried out using the strains described above, along with Grossman laboratory collection strains from North Carolina. The first experiment screened 32 total strains, half from Minnesota and half from North Carolina. Two ecotypes of hairy vetch were grown in plastic growth pouches (2 ecotypes x 32 strains x 4 replicates = 256) so that nodule development was visible. The growth chamber was set at 10C day/5C night. We recorded number of days to nodulation of each plant. The three fastest-nodulating and three slowest-nodulating strains were then tested in a follow-up experiment, where we hypothesized that fast-nodulating strains would lead to greater biomass by 10 weeks. Plants were terminated at the end of December and data is currently being processed. 3. Quantify differences in AM fungal colonization across hairy vetch breeding lines/populations. We collected \~200 root samples from 12 vetch lines in our field experiment in Freeville, NY. We finished collecting data on AMF colonization using ink-vinegar staining and the

gridline intersect method. We are in the process of analyzing data on AMF colonization along with other traits. 4. Develop and disseminate curricula modules covering the basics of legume biology and management of SNF for N supply to crops in organic systems. We expect to develop teaching modules that can be used either in a classroom with undergraduates or in hands-on outreach events. Examples include curriculum to teach about rhizobia relationships with legumes, inoculation demonstration, plating and growth of rhizobia and visualization using a portable microscope. We started gathering recent papers on legume N supply to use as a basis for the model focusing on legumes and green manure management. **PUBLICATIONS (not previously reported):** 2020/09 TO 2021/08 Type: Journal Articles Status: Published Year Published: 2021 Citation: Muller, K. E., J. Guinness, M. Hecking, and L. E. Drinkwater. 2021. Estimating agronomically relevant symbiotic N fixation in green manure breeding programs. Crop Science 61: 3314&#65533;3330.

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Dynamic Controlled Atmosphere Storage for Non-chemical Maintenance of Apple Quality

Accession No.	1017153
Project No.	NYC-145528
Agency	NIFA NY.C\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2018-51106-28770
Proposal No.	2018-03544
Start Date	01 SEP 2018
Term Date	31 AUG 2020
Grant Amount	$387,916
Grant Year	2018
Investigator(s)	Watkins, C.; Mattheis, JA, .; Rudell, DA, R..; Dando, RO, .

NON-TECHNICAL SUMMARY

Organic apple production continues to increase but requires storage to extend marketing options for growers. Standard controlled atmosphere (CA) storage maintains texture but cannot prevent development of physiological disorders. The goal of this project is to provide growers and storage operators with a new technology, dynamic controlled atmosphere (DCA) storage, extensively used in Europe but still uncommon in the US. DCA allows safe storage of fruit at extremely low oxygen concentrations and thereby control of disorders. While texture of fruit during the marketing chain is not always maintained after DCA storage when compared with effects of the ethylene inhibitor 1-methylcyclopropene (1-MCP), DCA has the potential advantage of greater consumer sensory preference. An interdisciplinary research and extension team with expertise in storage, consumer sensory testing and metabolite analysis will address barriers to the use of DCA technology using six organic apple cultivars over a two year period.Our objectives are to: 1) demonstrate the effect of DCA storage on physical and sensory quality, aroma volatiles, and storage disorders; 2) compare and contrast the effects of DCA against standard CA; and 3) provide confidence in the technology by the organic apple industry.The desired outcome is that each cultivar will have acceptable quality, including superior sensory and flavor volatile composition, after DCA storage compared with CA with or without 1-MCP. An additional benefit will be elimination of postharvest chemicals for non-organic fruit. The proposed project addresses Program priority 4 barrier to organic transition by providing a non-chemical technology to maintain fruit quality.

OBJECTIVES

The goal of this project is to develop the foundation for the safe adoption of a new storage technology known as dynamic controlled atmosphere (DCA) for Northeastern-grown organic apple fruit. DCA technology is used extensively in Europe, but to a very limited extent in the USA; some DCA storage of organic fruit exist in Washington State but there are none in the Northeast mostly because of lack of confidence of growers and storage operators in this new technology. To address this goal we have two objectives. The first objectiveis to develop application of DCA storage technology to address long term storage needs of Northeastern organic growers by defining the tolerances to low O2 of selected cultivars by providing confidence in the technology by the organic apple industry.The second objectiveis to translate research based information to the grower and storage operator community using a variety of extension methodologies - written (print and digital), formal extension programs such as workshops that are designed to measure impact, interaction with non-traditional

audiences, and one-on-one discussion with storage operators and cooperative members.Although it is not a formal outcome within the context of the Organic Transitions program, we also have a further objective that Northeastern conventional growers and storage operators can eliminate all postharvest chemical usage. Northeastern growers are well aware of the increasing need to address consumer demands for organic fruit, but also they are concerned about the long term future of postharvest chemicalregistration, and recognize that postharvest drenching represents a food safety hazard.

APPROACH

This is a multi-disciplinary project that uses the expertise on harvest and storage management with sensory evaluation, and measurement of metabolites and aroma volatiles that are important indicators of apple quality.Six apple cultivars differing in disease resistance, earliness, fruit quality, and shelf life are included in the evaluation to determine cultivar differences in terms of treatment effects: 1) \'Liberty\', selected for scab resistance; 2) \'GoldRush\', a late-maturing yellow apple with excellent fruit quality and long storage ability combined with field immunity to apple scab, a high level of resistance to apple mildew, and moderate resistance to fire blight; 3) RubyFrost\', a new cultivar with excellent shelf life characteristics, but highly susceptible to superficial scald; 4) \'Co-op 39\' (CrimsonCrispTM) is a midseason, high-quality, scab-immune, red apple that has excellent appearance, outstanding flavor, and very crisp flesh; 5) \'Honeycrisp\', a highly profitable apple for US growers, with excellent shelf life characteristics, but susceptible to several physiological disorders; 6) Enterprise\', a late-maturing, attractive, red applewith excellent fruit quality combined with field immunity to apple scab, a high level of resistance to cedar-apple rust, a high level of resistance to fire blight, and moderate resistance to powdery mildew.Fruit of each cultivar will be harvested from two certified organic orchard blocks. Fruit will be stored in refrigerated air storage for 2 and 7 days after harvest. Fruit in each chamber will be untreated or treated with 1-MCP \1 µL L-1 1-MCP (SmartFresh tablets, 0.36% a.i., AgroFresh Co., Spring House, PA) for 24 h in a 4000 L plastic tent using a release and fan system supplied by the manufacturers\ one day after cooling or after 6 days, to provide post-storage contrasts with commonly industry-used procedures. Fruit from each orchard will be stored under standard CA conditions of 2% O2 and 2% CO2 or DCA O2 conditions as determined by fluorescence responses (HarvestWatchTM) in 13 m stainless steel chambers. Each chamber contains up to 24 containers divided by treatment (2 orchards x ±1-MCP x 4 replicates). For assessment of harvest maturity, an additional 10 fruit per replicate will be used for measurement of internal ethylene concentration (IEC), firmness, titratable acidity (TA), soluble solids content (SSC), delta absorbance (IAD), and starch pattern index (SPI) at harvest, and on days 2, 4 and 7, according to standard procedures. For each treatment, four replicates of 70 fruit will be removed from each CA/DCA storage chamber at 4.5 and 9 months of storage. For each cultivar there are two sets of fruit, each representing the delay periods of 2 and 7 days. Ten fruit will be used for assessment of IEC, firmness, SSC, IAD reading and TA on days 1, 4 and 7 at 20 oC. The remaining 60 fruit per replicate will be used for sensory panels after 4 days at 20 oC.Sensory analysis of three cultivars per year will assay consumer response to appearance, texture, flavor, aftertaste/residual, purchase intent, and the respondents\' overall liking of the samples. Samples will be tested on 2 separate removal dates, for a total of 12 sensory tests. Samples will be assessed using the Quartermaster corps. 9-point hedonic scale, for overall and attribute liking. After liking questions, JAR scaling of flavor and texture attributes will be combined with overall liking of the samples to generate penalty analysis graphs for all treatments, thus determining the impact of any off-flavors or textural deficits on consumers\' purchase intent. 120 consumers of the cultivar in question will be recruited from the Cornell sensory panelist listserv, and screened for defects in taste or olfactory ability, and apple consumption habits. Data will be collected in individual booths using RedJade sensory analysis software on touch screen interfaces at the Cornell Sensory Evaluation facility, a state of the art sensory evaluation facility routinely running N \> 200 consumer studies. Panelists will assess whole apples for their visual characteristics, before tasting segments, and reporting overall liking, flavor, texture and aftertaste/residual qualities. Data will be analyzed both using repeated measures ANOVA with GraphPad Prism (GraphPad Software, San Diego, CA), and via the building of a linear mixed model IBM SPSS (IBM Corp, Endicott, NY), with panelists serving as a random effect, and treatment conditions and demographics as fixed effects/covariates. Interaction terms will be tested for significance, and maintained in the model to control for their respective variation if tested under a level of $p < 0.1$, while significance of main treatment effects will be ascribed at $p < 0.05$. Finally, data will be analyzed along with demographic information for any evidence of sensory segmentation.On day 4, subsamples of 10 fruit also will be used to obtain composite samples of juice for each replication. Fruit will be extracted using a commercial juicer and immediately poured into polyethylene sample vials and submerged in LN2 to flash freeze. Samples will remain at -80oC and transported on dry ice to ARS-Wenatchee for volatile analysis. Volatile metabolites present in the fruit juice will be assessed using a GC-MS volatile headspace sampling system. For the analysis, 500 µL of juice will be pipetted into 20 mL headspace sampling vials containing 500 µL frozen NaCl solution (saturated) and sealed immediately. Samples will then be thawed and then sonicated for 5 min. Vial headspace will be analyzed using an Agilent 6890N gas chromatograph coupled with a 5975B mass selective detector and an automated

Gerstel multipurpose sampler equipped with a dynamic headspace sampler. Mass spectra ranging from m/z 30 to 600 will be recorded. Volatile metabolites will be identified using authentic standards, comparison with our in-house mass spectra library, and comparison with the NIST12 library. Metabolite concentration will be calculated from standard curves generated using the standard addition method to account for matrix effects.The approaches described are divided into three separate but interrelated areas: analysis of physical fruit quality including disorder evaluation, sensory analyses, and aroma volatile measurements. As assessment of fruit quality is typically complex, we have chosen to evaluate using three techniques based on instrumental and human evaluation. The influence of each fruit quality variable on the resultant consumer liking from sensory evaluations will be analyzed using principal components analysis, first to establish the dimensionality of the data set, and then, partial least squares-discriminate analysis using the fruit quality variables as responses and sensory variables as predictors. Aroma volatile results will be analyzed separately using principal components analysis to indicate volatile components associated with each cultivar/storage duration/treatment combination. Comparison of model results will reveal quality and aroma characteristics associated with taste panel quality choices and treatment and storage durations most reflecting high-quality fruit and a good eating experience. The consensus of quality data using multiple points of evaluation is expected to more accurately reflect not only comparisons among cultivar/treatment combinations, but also towards optimum quality among treatments for any one cultivar.The advisory committee will inform strategies to involve stakeholders for maximum impact as described in the outcomes section. **Progress** 09/01/18 to 08/31/21 **Outputs** Target Audience:The target audience for this project is first and foremost, Northeastern organic growers and storage operators. Even though growing organic fruit in the eastern environment is difficult in comparison with desert-like environments in Washington State, growers here are increasingly exploring expansion of organic production. There are notable examples such as the largest orchard in the east (Fowler Brothers), which is transitioning blocks over to organic. While this project is forward focused because of the limited organic production in the east, the results provide confidence that non-chemical postharvest technologies can provide storage performance that is equivalent to that currently obtained with postharvest chemical use. This is critical to ensure that organic growers and storage operators are not disadvantaged in the marketplace. The second audience is the national organic community including the Washington State industry. The third audience, although not a formal one within the context of the Organic Transitions program, is Northeastern conventional growers and storage operators as we can provide a means of eliminating all postharvest chemical usage. Northeastern growers are well aware of the increasing need to address consumer demands for organic fruit, but also they are concerned about the long term future of postharvest chemicalregistration, and recognize that postharvest drenching represents a food safety hazard. Last but not least, are the consumers, who are increasingly concerned about the use of postharvest chemicals. Changes/Problems:Year 2, originally the final year of this project, was affected by COVID-19. Although a complete study of the physiological fruit responses to CA and DCA was completed as planned, of the three cultivars harvested that year, only two cultivars were subjected to sensory analysis and volatile measurement (4.5 months) because of the shutdown of facilities that involved interaction with panelists. Following a no-cost extension by NIFA, research on the three cultivars was reported in full, plus an additional cultivar (Honeycrisp) for which a high incidence of physiological disorders was encountered in year 1. The sample sizes for sensory analysis were limited slightly due to building density requirements, although not to a degree that would put inferences in any doubt. Overall, the expectations of the project, namely the effects of DCA storage on three organic cultivars for each of the two years was met in full. However, the second objective of this project that concerns dissemination of the results of this project has been delayed. First, because results from the 2020 harvest season have only recently been completed given the 9-month storage season and subsequent volatile analyses, extension of this research to the grower community is only now being initiated. Also, the NOFA meeting (January 2022) in which interactive participation of the organic community was expected has been limited by an ongoing virtual format. Most emphasis, therefore, will be on written formats. What opportunities for training and professional development has the project provided?Presentations to grower audiences. 2019Organic Apple Field Day. Cornell Orchards. Ithaca, NY. 1 Aug. 'The potential of dynamic controlled atmosphere storage for the eastern organic apple industry. 2022 New York State Fruit School, Zoom, January 18. Dynamic controlled atmosphere (DCA) for storage of organic and traditional apple varieties. How have the results been disseminated to communities of interest?Through one organic field day, and now that the project is completed through Fruit Schools, a popular article and two papers in refereed scientific journals (in preparation). What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported **Impacts** What was accomplished under these goals? The overall approach taken in this project was to access organically grown fruit from New York orchard. Fruit of six cultivars were accessed from two major orchards, the first focusing on disease-resistant traditional organic cultivars, and the second from a large-scale operation where they have transitioned blocks of several mainstream cultivars such as Golden Delicious, Jonagold, Fuji and Honeycrisp to organic growing systems. The effects of dynamic controlled atmosphere (DCA) and controlled atmosphere (CA) storage were investigated, with the non-organic, but widely used inhibitor of ethylene production, 1-methycyclopropene (1-MCP), being used as a comparison for effects on post-storage quality. In year 1, two disease resistant cultivars

(Gold Rush and Enterprise) and a mainstream cultivar (Honeycrisp) after storage for 4.5 and 9 months were investigated. Sensory and volatile analyses were completed in full for Gold Rush and Enterprise but restricted to only one analysis time (6 months) for Honeycrisp because of unacceptable incidences of physiological storage disorders. In year 2, Golden Delicious, Jonagold and Fuji were stored for 6 and 9 months and quality assessments made in full. However, COVID-19 restrictions limited sensory analyses and volatile assessments of Golden Delicious and Jonagold to 6 months only, with no sensory evaluation of Fuji (see section on changes). In year 3, full analyses of Golden Delicious, Jonagold and Fuji were carried out. In addition, we repeated work with Honeycrisp given the issues of disorders encountered in year 1. In terms of fruit quality, the results vary by cultivar type. Two groups of response were identified. First, for those cultivars (Gold Rush, Enterprise, and Honeycrisp) that ripen very little after harvest, e.g., limited loss of texture and softening, few effects on firmness, soluble solids contents, titratable acidity and background color were detected, even when ethylene production was inhibited. Second, Golden Delicious and Jonagold, and to a lesser extent, Fuji, softened after CA storage. Although the residual effect of DCA on slowing softening was not as marked at 1-MCP, the residual effect of DCA resulted in a firmer apple than that found for CA storage alone. The comparison of CA/DCA, with and without 1-MCP are being written up for publication in a refereed publication and in a grower magazine (Fruit Quarterly) A major part of this project was the multidisciplinary approach taken to integrate physiological data with sensory and volatile production by fruit. In contrast with the results above, we took the approach of examining all aspects on a specific time after removal from storage (day 4). Different cultivars produce different volatile profiles with ripening and, consequently, are impacted differentially. DCA influence on volatile production followed established patterns where lower oxygen levels led to diminished alcohol, ester, ketone, and sesquiterpene production. Likewise, when tested 1-MCP reduced production of these classes of volatiles alone or additively with reduced O2. Aldehyde levels were differentially influenced with trans-2-hexenal and cis-3-hexenal levels remaining higher in apples stored in lower oxygen storage environments for most cultivars. Some cultivars, primarily Honeycrisp and Fuji, produced elevated levels of ethanol and derived esters when stored at or following removal from storage under the lowest oxygen atmospheres. Differing cultivars also produced different patterns of liking with storage, with Jonagold and Golden Delicious being more vulnerable to decreases in liking with CA storage, when compared to either DCA or treatment with 1-MCP, which appeared on the whole to produce similar levels of liking after prolonged periods of storage. Decreases in liking were less prominent at the first removal time than the second longer one, as would be expected. Storage condition was a significant effect in the models measuring overall liking, appearance liking, texture liking, aroma liking and mealiness, with CA storage scoring the least favorably in most cases, and texture liking and mealiness rating (although logically correlated) showing the strongest effect. Interaction effects between storage condition and cultivar were also significant effects in all cases above, indicative of differential effects by cultivar. One notable exception was in aroma liking, where CA scored higher than DCA, and may be illustrative of volatile production patterns. Our ongoing analyses are focused on asking questions such as does aroma really have any impact on product preferences by the average consumer, and in relation to acidity and firmness. The results have met the first objectiveof the project; to develop application of DCA storage technology to address long term storage needs of Northeastern organic growers by defining the tolerances to low O2of selected cultivars byproviding confidence in the technology by the organic apple industry.Tolerances to low oxygen have been identified, while also revealing limitations for cultivars that are particularly susceptible to storage disorders. The results have also revealed that DCA storage is not necessarily an advantage for cultivars that maintain fruit quality through extended storage periods. The second objective focused is focused on translation of research-based information to the grower and storage operator community using a variety of extension methodologies - written (print and digital), formal extension programs such as workshops that are designed to measure impact, interaction with non-traditional audiences, and one-on-one discussion with storage operators and cooperative members. This objective has not yet been met because results from the 2020 harvest season have only recently been completed given the 9-month storage season and subsequent volatile analyses. Therefore, extension of this research to the grower community is only now being initiated. Because of the limitations of virtual formats in prime extension vehicles such as the NOFA meetings most emphasis is on providing translation via written formats. Third, the informal objective of extending this knowledge to Northeastern conventional growers and storage operators with the objective of eliminating all postharvest chemical usage will be shared at the upcoming Fruit Schools in New York early in 2022. The demand for information about DCA is emerging rapidly, and the research funded by this program is serving as powerful resource for northeastern growers. **Publications** - Type: Journal Articles Status: Other Year Published: 2022 Citation: Al Shoffe, Y, Rudell, D.R., Dando, R., Park, D.S., Watkins, C.B. Dynamic controlled atmosphere (DCA) for storage of Eastern organically grown apple cultivars. HortScience - Type: Journal Articles Status: Other Year Published: 2022 Citation: Al Shoffe, Y, Rudell, D.R., Dando, R., Park, D.S., Watkins, C.B. Fruit quality and volatile production effects on sensory of organically-grown apple cultivars. Postharvest Biology and Technology - Type: Other Status: Submitted Year Published: 2022 Citation: Al Shoffe, Y, Rudell, D.R., Dando, R., Park, D.S., Watkins, C.B. Maintaining quality of organically-grown apple varieties with dynamic controlled atmosphere (DCA) storage. Fruit Quarterly 30 (1) Spring 2022.

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Breaking Down the Barriers to Organic No-till Soybean and Dry Bean Production Through Improved White Mold Management

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NON-TECHNICAL SUMMARY

Diseases often limit organic crop production and organic farmers consistently rank disease management as a top research priority. Robust disease management systems are needed that complement other production priorities such as weed management. This project directly addresses program priorities 1 (Documenting and understanding effects of organic practices) and 4 (Developing practical information and tools to help producers overcome barriers to organic transition). We build on recent success with cover crop-based, organic rotational no-till soybean production and focus on white mold and weed management. White mold is caused by the fungus Sclerotinia sclerotiorum and is a devastating disease of soybean and dry bean. Our research shows excellent weed suppression and consistently high yields of organic soybean that are no-till planted in rolled-crimped cereal rye. Preliminary results from research conducted in 2017 on using cereal rye mulch for white mold suppression are also promising. However, high planting rates that are recommended for weed suppression might facilitate pathogen spread by creating a more favorable habitat. Therefore, we will evaluate the impact of rolled-crimped cereal rye and crop planting rate on S. sclerotiorum carpogenic germination and ascosporic inoculum. This approach represents a transformative change in disease management and has potential to improve soil health and reduce labor and fuel requirements. Our extension and farmer engagement program will include farmer-to-farmer learning and an on-farm demonstration trial. We will also develop and disseminate threshold-based cover crop management and planting rate recommendations for soybean and dry bean based on white mold incidence and weed abundance.

OBJECTIVES

Cover crops can improve soil health, suppress diseases, weeds and pests, and overcome a variety of challenges associated with the transition to certified organic crop production. Cover crops such as cereal rye can be used as mulch, thereby reducing soil tillage and cultivation. Our preliminary research shows that organic soybean and dry bean that are no-till planted into rolled-crimped cereal rye can produce yields that are as good or better than traditional tillage-based organic management. Although researchers and organic farmers tend to understand the importance of cereal rye residue for weed suppression, the effects of mulch on plant diseases has received relatively little attention. Moreover some practices that have been encouraged in organic no-till soybean production for enhancing weed suppression, such as increasing crop seeding rates, may enhance disease by creating more conducive conditions for pathogen growth. Therefore an integrated systems approach is needed to

examine multi-trophic pest interactions and improve organic no-till crop production.White mold caused by the fungus, Sclerotinia sclerotiorum is amongst the most devastating and recalcitrant plant diseases worldwide, and results in substantial annual losses in crop production. White mold is difficult to manage due to long-lived primary inoculum (sclerotia), the broad host range of the fungus, and the absence of appreciable host resistance in many crops. Dry beans and soybeans are amongst the most susceptible crops to white mold. White mold is particularly problematic for organic farmers because of the important soil improvement role that legume crops such as soybean and dry bean play in their crop rotations, and because these crops are highly profitable. Cultural practices such as extended crop rotations and decreased crop planting rates are recommended in organic production but are often insufficient for the control of white mold. Moreover, because conventional soybean and dry bean farmers often rely heavily on fungicides for control, ineffective white mold management is one of the major barriers for soybean and dry bean farmers who are interested in transitioning to organic production.Goal: Facilitate the transition to organic by providing cost effective suppression of white mold and weeds in organic soybean and dry bean.Objectives:Quantify the efficacy of cereal rye mulch on white mold and weed suppression in organic soybean and dry bean production.Examine the effects of increasing soybean and dry bean seeding rates on white mold and weed suppression and test for tradeoffs.Disseminate research findings including refined seed rate recommendations that account for site-specific disease management to farmers considering organic production and existing organic farmers through farmer-to-farmer learning, on-farm demonstrations, newsletter articles, research bulletins, and presentations at extension meetings.

APPROACH

Objective 1. Integrated Cover Crop Experiment. The experimental design is a split-block randomized complete block with four replicates. Main plot treatments will becrop and sub plot treatments will include: 1) no cover crop, 2) cereal rye with tillage, and 3) cereal rye mulch. The experiment will be repeated twice in different positions of the field. Cereal rye will be drill seeded in late August at 200 kg seed ha-1 in year 1, which will be used for no-till soybean and dry bean production in year 2. The remaining portion of the trial area will not have a cover crop planted and weeds will be controlled using shallow tillage as required. We will plant a black bean (var. Eclipse) that can be harvested with a combine. Prior to planting the dry bean and soybean seed in spring, we will mechanically terminate the cover crop with either tillage or a roller-crimper. Plowing in the no cover and tilled cereal rye treatments will occur prior to stem elongation to match standard practices used by organic farmers.Sampling and Data Collection. Cereal Rye Biomass. The dry weight of the cereal rye will be quantified prior to rolling and crimping, and the resulting mulch will be measured at crop harvest.Plant Density.The effectof cereal rye mulch on crop establishment will be assessed by counting the number of soybean and dry bean plants in each plot following emergence within two randomly selected 2 m within row transects.Carpogenic Germination of S. sclerotiorum. The effect of rolled cover crop on S. sclerotiorum apothecia will be tested in two complementary approaches in the dry bean and soybean crops. First, we will monitor the number of apothecia produced in two pre-marked one meter transects within the center rows of each plot. The number of apothecia will be counted at three-day intervals from pre-flowering to two days prior to harvest. In addition, eight sclerotia conditioned in the laboratory (Pethybridge et al. 2015) will be placed in sand within each of six pots/plot (three in each of the two center rows). The number of functional (stipe + disc) and nonfunctional (stipe only) apothecia produced within these pots on a plot basis will also be counted at each assessment. Data from the pots will be used to verify and further understand data from transect-based monitoring of disease incidence.Microclimatic conditions. Watchdog data loggers (Spectrum Technologies) will be placed within the canopy at a standard height (10 cm) to measure temperature and relative humidity at hourly intervals. To test the effect of the cereal rye mulch on light interception, a line quantum meter (LI-191R; Li-Cor) will be used to measure light (photosynthetic photon flux density; µmol s-1 m -2) under the canopy and the cereal rye mulch at ten arbitrarily selected locations within each plot at three times each year: early (at planting), mid (July) and late (August) season.Sclerotinia sclerotiorum Ascosporic Inoculum Density. The effect of rolled cover crop on release of S. sclerotiorum ascospores into the dry bean and soybean canopies will be quantified using the placement of semi-selective media and a PCR developed for ascosporic inoculum of Sclerotinia spp. by the group of PD Pethybridge (Jones et al. 2015). We will place three rotation arm impaction (GRIPST-2009, Aerobiology Research Laboratories) samplers in the cover crop and no cover cropped areas. Traps will positioned approximately 30 cm above the ground. The ascosporic monitoring period will be measured pre-flowering to 3 days before harvest. Each sampler will be fitted with pairs of stainless steel rods covered with a thin coating of silicone grease and collected at 72 h intervals with sterile forceps. The rotorods will then be placed into 60 µL of MO BIO extraction solution S1 and 200 µL of MO BIO Solution IRS and vortexed at maximum speed for 10 min. Tubes will then be placed into boiling water for 2 min before centrifugation at 10,000 g for 30 sec. DNA will then be extracting according to the manufacturer\'s recommendations (PowerSoil DNA Isolation Kit). Each sample will be tested using the primers developed to the intergenic spacer region, which amplifies a 170-bp product (Jones et al. 2015). Ascosporic inoculum will also be quantified using the blue plate technique (Ben-Yephet & Bitton, 1985).White Mold Disease

Incidence.The incidence of white mold on plants and pods will be assessed within the entire length of the two center rows of each plot at the end of flowering and at harvest.Weed Suppression. Weed biomass will be quantified in late August by clipping all weed biomass at the soil surface from four 0.5 m2 quadrats that are randomly positioned within each plot when the dry bean and soybean crops reach maturity. Weed samples will be separated by hand to quantify biomass of individual species, dried, and weighed. Weed density of individual species will also be recorded.Cash Crop Performance. Cash crop biomass will be evaluated at mid-season (mid to late July) at before harvest (late August) by removing all crop biomass from each of four 0.5 m2 quadrats within each plot. Yield will be determined by harvesting the center two rows of each plot using a plot combine with a calibrated yield monitor.Statistical Analysis. We will use a variety of univariate and multivariate statistics to analyze the data collected in this objective.Economic Analysis. We will conduct a partial budget analysis to quantify the costs and benefits of the management systems in the ICCE.Objective 2. Trade-offs between weed and disease suppression for increasing seeding rates in organic no-till soybean.An experiment will be conducted in each of the two years at two locations (Musgrave Research Farm in Aurora, New York, and Cornell University in Geneva, New York). Soybean will serve as a model system as it is highly susceptible to white mold and the variables associated with optimization of this cropping system are better understood in comparison to dry bean. Similar to the experiment associated with Objective 1, cereal rye will be drill seeded in late August at 200 kg seed ha-1. Cereal rye will be terminated mechanically using the roller-crimper prior to no-till planting of soybean to provide a uniform layer of mulch. The dry weight of the cereal rye will be quantified prior to rolling and crimping and at harvest.Using a randomized complete block design with at least four replications, we will compare five different soybean seeding rates ranging from 195,000 to 915,000 seeds ha-1. The experiment in Aurora will enable comparisons in the absence of white mold, which has the potential to cause complete crop loss at high seeding rates. Each plot will be 3 m wide × 10 m long.Variables to be assessed on a plot basis for analysis of treatment effects will be as described within the experiments planned for Objective 1 with the exceptions of light interception and ascosporic inoculum quantification.Statistical Analysis. The effect of seeding rate (five different levels) on weed population densities, and crop yield will be analyzed using generalized linear modeling, including ANOVA, non-linear regression, and partial correlation analysis.Objective 3. Extending research findings.On-farm trial. An on-farm demonstration trial will be conducted in Years 2 and 3 in collaboration with organic grower, Kreher Family Farms.Research Bulletins. Four research bulletins based on successful organic cropping practices that decrease losses associated with weeds and diseases will be developed.Field Days. We will highlight project activities in one field day each season in New York at one of the research farms (Geneva in Year 1; Aurora in Year 2).Analyses. The adoption rate of new practices will be analyzed through on-farm workshops, workshop surveys, and a post-program evaluation six months after the educational events. Data will be analyzed to determine the impact of new organic crop production recommendations. **Progress** 09/01/18 to 08/31/23 **Outputs** Target Audience:The target audience reached during this project period were field crop farmers considering transitioning from conventional to organic production in New York and the Northeastern USA and other areas with similar precipitation patterns. Findings have also been extended to industry stakeholders, dry bean and soybean processors, crop scouts and others that provide advice on crop management to these farmers (e.g. extension personnel and agronomists) through multiple avenues via our multimodal extension program.Current organic growers of soybeans and dry beans were also highly engaged in the project to learn of the benefits and optimization of their production system, to prevent a transition back to conventional agriculture, and due to their keen interest in the value of cover crops in their production systems. The stakeholder advisory panel, consisting of members of the NYS Dry Bean Council and extension educators met for the final time with the project team during this reporting period (August 2023). The scientific community has also been an important audience for this information in the form of scientific refereed journal articles. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?This studypartially supported the graduate studies of Ashley Jernigan (Objective 1) and Uriel Menalled (Objective 2). Ashley Jernigan has nowtaken up a position as an Assistant Professor of Organic Agriculture at Virginia Tech. Uriel Menalled has also graduated with his PhD and is continuing in the Ryan lab (Cornell University, Ithaca) in a postdoctoral role. An undergraduate student, Adam Sharifa, has also been involved in this project (Objective 2). He is now a senior undergraduate at Cornell University and is considering graduate studies in organic agriculture in 2024. The students have been involved in all aspects of the project including field trial design and establishment, data collection, analysis, and writing of scientific journal articles and extension outputs. How have the results been disseminated to communities of interest?In 2023, results were disseminated to communities of interest through three main extension events at the Maine Organic Growers Conference, two events of the NYS Dry Bean Industry Council,including a twilight grower walk of the trials (August 2023). During the twilight grower meeting, topics such as practical issues associated with crop establishment in a rolled-crimped cereal rye cover crop and advantages for weed suppression and disease control were discussed. Background information on the biology and epidemiology of white mold were also presented in a handout including the efficacy of OMRI-listed biopesticides for disease control. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported **Impacts** What was accomplished under these goals? Objective 1: Data from multiple

years of small plot, replicated field trials has been collated and analyzed. Treatments in these trials were: (i) cereal rye cover crop established at two different rates (1 and 3 bu/A); and (ii) no rye (tillage) control. The main crops of dry bean and soybean were then planted into each treatment in spring. Findings have demonstrated that increasing the rate of cereal rye cover crop significantly decreased the frequency of sclerotial germination, likely a function of the reduction in light, that is responsible for inducing the production of apothecia. Using a \'blue plate\' bioassay, the frequency of S. sclerotiorum ascospores in each plot was also monitored in each year. This data has showed that sclerotial germination was associated with a significant reduction in ascospore abundance and correlated with reductions in white mold in both soybeans and dry bean. Permutations of these treatments were then further evaluated in subsequent field trials to determine if the effect of rye was a barrier or allelopathic. These trials found that the effect on white mold control was related to the physical barrier and not the allelopathic chemicals known to exude from roots of cereal rye. Trade-offs in agronomic factors and weed suppression in the two main crops associated with the cereal rye cover crop were also evaluated. The higher rate of cereal rye cover crop was associated with a significant reduction in dry bean pod and bean yield, and substantial weed suppression. In soybean, weed suppression was also achieved in the higher rate of cereal rye cover crop but no significant reductions in soybean pod yield were identified. These findings suggest that increasing the rate of cereal rye cover crop planted in fall may be beneficial for white mold control and weed suppression in soybean with no deleterious lag in yield. However, further optimization of the cereal rye cover crop for dry bean is required. Concepts associated with weed-crop competition were also investigated and found that differences in weed-crop competition intensity were driven by crop yield potential. These findings may also guidesoil and crop management, especially in conservation agriculture where soil tillage and its microbial legacy reducing effects are minimized. Objective 2: A second year of the direct-harvested dry bean and soybeanseeding rate trial was conducted in 2023. To prepare for the trial, cereal rye was planted at a consistent rate of 2 bu/A in September 2022. The cereal rye was terminated with a roller crimper at 75% anthesis in June 2023 and in a second operation, black bean and soybean was planted in 30-in. rows with a no-till planter to a depth of 2 in., at five different seeding rates and three different nitrogen rates. The data wascombined with previous year data and published in the journal, Frontiers of Agronomy. Overall, work in this objective address the paradox between no-till planting crops into rolled-crimped cover crops can improve soil health while reducing labor and fueld requirements compared with traditional tillage-based production. Results were more consistent in soybean. The lowest weed biomass occurred at the highest soybean density in the lowest soil nitrogen environment. An interaction was also observed between soybean seeding rate and nitrogen treatments on weed communities. An interaction was also observed between soybean seeding rate and nitrogen treatments on weed communities. Soybean yield increased asymptotically with crop density and was not affected by nitrogen or site treatments. When pooled over nitrogen treatments, partial returns to the soybean seeding rates were maximized at \$2,238/ha with 527,800 seeds/ha. Results suggested that crop density is an important factor for optimizing weed suppression and crop yield in organic no-till soybean and dry bean, and that managing for low soil nitrogen conditions may further enhance weed suppression while maintaining high yields. Objective 3: Findings from this research have been disseminated to a broad target audience include scientists (four refereed journal articles), to the stakeholder advisory panel for this project, and organic dry bean and soybean growers. In 2023, findings were also presented at the Maine Organic Growers Conference and several extension publications (see list of products). **Publications** - Type: Journal Articles Status: Published Year Published: 2023 Citation: Menalled, U. D., Smith, R. G., Cordeau, S., Di Tommaso, A., Pethybridge, S. J., and Ryan, M. R. 2023. Phylogenetic relatedness can influence cover crop-based weed suppression. Scientific Rep. 13:17323. - Type: Journal Articles Status: Published Year Published: 2024 Citation: Pethybridge, S. J., Murphy, S., Lund, M., and Kikkert. J. R. 2024. Survival of Sclerotinia sclerotiorum sclerotia in central New York. Plant Disease. PDIS-10-23-2126-SC. Accepted 6 November 2023. On First Look 9 November 2023. Proofs returned 26 February 2024. https://doi.org/10.1094/PDIS-10-23-2126-SC. **Progress** 09/01/21 to 08/31/22 **Outputs** Target Audience: The target audience for this project was field and specialty crop farmers navigating the process to transition to organic crop production in the Northeastern United States and similar ecoclimatic regions. Those involved in providing farmers with recommendations for this transition period and organic crop production were also targeted with our research findings. These communities included crop scouts extension educators, and purveyors of organic produce. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?This study has partially contributed to the graduate studies of two PhD students (Ashley Jernigan \Objective 1\ and Uriel Menalled \Objective 2\, and undergraduate student, Adam Sharifa. These students obtained individual training in organic agriculture and crop production, data analysis, field trial planning, agronomic considerations, planning for pitfalls and limiations and extension of findings to the communities of interest, and listening to feedback. How have the results been disseminated to communities of interest?Results of the project were extended to the communities of interest (organic farmers, extension educators and industry personnel) through two primary extension events. The first was a presentation in the Northeast Cover Crop Meeting through a virtual event attracting over 100 people. The second was an all-day, in-person event at the Hudson Valley Farm Hub focusing on organic no-till crop production. During this meeting,

discussions and feedback from organic farmers were conducted on the results of our trials. Trials on organic no-till crop production were also observed through field works. This event attracted over 60 organic growers and materials from the presentations were also distributed to others unable to attend through the Farm Hub network. What do you plan to do during the next reporting period to accomplish the goals?The next reporting period is the final NCE. Activity in this period will focus on compilation of research results over the three years of field trials to additional scientific journal articles from work conducted in Objectives 1 and 2. The key messages for organic and transitioning growers will be identified and these will be diffused to the communities of interest to ensure broad engagement and understanding of the findings. **Impacts** What was accomplished under these goals? Objective 1: Cereal rye mulch for white mold and weed suppression. A replicated trial was conducted at the Research North facility (of CornellAgriTech, Geneva, NY. The trial was approximately 3 ha in size. The trial design was a randomized, complete block with three main crop within each of the treatments. Treatments (established in fall 2021) were: (i) a cereal rye cover established at two different planting rates (1and 3bu/A); and (ii) no rye (tillage) control. The main crops were soybean, dry bean, and sunflower planted perpendicular to the cereal rye rolling-crimping direction in spring. Plots were 60 feet long and wide and crops were planted at a 30 inch row spacing. There was a 50 foot buffer on tilled and no-rye cover crop between each of the four blocks. a split-block, randomized, complete block design with four replicates and around 2.5 ha in size. Evaluations during the main cropping season included aboveground biomass, root disease incidence and severity, weed populations (density and diversity), white mold incidence, and agronomic yield components at harvest (plant population, pod and bean/sunflower seed yield). The effect of treatment on white mold incidence was also evaluated. The dry bean crop from this trial has been harvested, and the sunflower and soybeans will be harvested in late October. Data will then be analyzed and compared from different years for scientific journal publications and identification of the extension message for dissemination to our communities of interest. Objective 2:Direct-harvested dry (black) bean has shown promise for incorporation into rolled-crimped cereal rye mulch cropping systems. However, in past trials, aboveground biomass production and yield have been suboptimal compared to crops planted into tilled soil, when the same seeding rates were used. This trial was conducted to determine if dry bean performance may be enhanced in the rolled-crimped cereal rye mulch system by increasing dry bean seeding rate, as we have previously quantified in soybean. The hypotheses were: (i) higher seeding rates of dry bean will improve dry bean yield as well as profitability; and (ii) higher seeding rates of dry bean will reduce weed biomass. Cereal rye (cv 'ND Gardner') was established in September 2021 with a grain drill planted at 2 bu/A; with row spacing of 7.5 inches; at a depth of 2 cm. The entire field at the Research North Facility was planted. The cereal rye was terminated with a roller crimper at 50% anthesis. Rolling-crimping of cereal rye was conducted before dry bean planting but the two operations were conducted on the same day and in the same machine pass. The dry beans (cv. 'Zorro') were planted in 30-inch rows with a no-till planter at a depth of 1.5-2.5 inches, at five treatment rates (75,000; 150,000; 225,000; 300,000; and 375,000 seeds/A). The experimental design was a completely randomized block with four replications. Each plot had four rows and were 90 feet long x 10 feet wide. Cereal rye biomass was quantified at termination and at dry bean harvest on 14 September 2022. Weed biomass was evaluated in mid-August and at harvest. White mold incidence was evaluated at harvest. Pods from this trial harvest are currently being dried and will be threshed to calculate bean yield. Data will then be analyzed to quantify the effect of dry bean planting rate on the various response variables and to examine trade-offs. Objective 3:Our research findings have been published in a scientific journal and to organic growers at two extension events (Northeast Cover Crop Meeting and Hudson Valley Farm Hub Extension Meeting). **Publications** - Type: Journal Articles Status: Published Year Published: 2021 Citation: Menalled, U., Pethybridge, S. J., Pelzer, C., Smith, R. G., DiTomasso, A., and Ryan, M. R. 2021. High seeding rates and low soil nitrogen optimize weed suppression and profitability in organic no-till planted soybean. Front. Agron. 3:678567. **Progress** 09/01/20 to 08/31/21 **Outputs** Target Audience:Field crop farmers considering transitioning from conventional to organic production are the predominant target audience for the outcomes from this research. Whilst the focus of the project outcomes are directly transferable to farmers in New York, the findings have also been extended to farmers with similar objectives in the Northeastern US and other areas with similar precipitation patterns and therefore fit cover crops into their rotations. Findings have also been extended to industry stakeholders, crop scouts and others that provide advice on crop management to these farmers (e.g. extension personnel and agronomists). The scientific community has also been an important audience for this information (see list of outreach and extension products). Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?This study has partially contributed to the graduate studies of two PhD students (Ashley Jernigan \Objective 1\ and Uriel Menalled \Objective 2\. A Postdoctoral Research Associate (Daniel Heck) has also been involved in Objective 1. These early career scientists have received training in research and extension skills ranging from extending results to farmers and the scientific community,conducting field trials, practical agronomy, organic agriculture, data analysis. How have the results been disseminated to communities of interest?Results were extended to farmers considering organic transition and industry personnel including crop scouts and extension educators through virtual and in-person field days and extension events, ranging from the American Farmland Trust to the Indiana

Certified Crop Adviser program. Virtual offerings of these kind enabled us to reach audiences outside our region that we had not thought we are able to connectIn-person events were also held at three locations around New York to reach local transitioning farmers. At the in-person events, over 250 participants were reached. Results were also presented at the premiere national agronomy scientific conference (ASA) . A production guide on organic no-till soybean production was also produced and published (<https://bit.ly/ONTPSguide>). What do you plan to do during the next reporting period to accomplish the goals?This next reporting period is the final no-cost extension of this project. Data will be combined from all experiments including information on soil insect populations and disease for an additional two scientific publications. The data will also be distilled into farmer-friendly information for dissemination across a broad range of farmer audiences this winter including extension events and fact sheets. **Impacts** What was accomplished under these goals? Objective 1.White mold caused by the fungus, Sclerotinia sclerotiorum is a devastating disease of soybean (Glycine max) and other leguminous crops, including dry bean (Phaseolus vulgaris). Previous research has demonstrated that no-till planting soybean into rolled-crimped cereal rye residue can enhance weed management, improve soil health, and reduce labor requirements in organic production. However, there are limited data on the effects of cereal rye residue on white mold suppression in no-till planted soybean and dry bean. Two field trials were conducted in 2016-2017 (Year 1) and repeated in 2017-2018 (Year 2) to evaluate the potential of cereal rye cover crop residue to suppress white mold in these crops. In each trial (soybean and dry bean), the experimental design was a randomized complete block with two treatments: 1) rolled-crimped cereal rye residue and 2) no cover crop control. Treatment effects on plant population, biomass, and yield components varied between the main crops. Compared to the control treatment, cereal rye residue reduced the incidence of white mold in soybean in both years and in dry bean in Year 2. The reduction in white mold in cereal rye residue plots was due to a combination of 1) decreased sclerotial germination (no stipes formed) and 2) increased nonfunctional sclerotial germination defined here as sclerotia that germinated but produced stipes without the expanded cup where asci containing ascospores are formed. Weed density and biomass were lower in cereal rye residue plots in soybean and dry bean, except in year 1 in soybean when weed biomass was low in both treatments. Our findings indicate that cereal rye residue could help organic and conventional farmers manage white mold in no-till planted soybean and dry bean. Germination of sclerotia resulting in nonfunctional apothecia could potentially exhaust soilborne inoculum in the upper soil profile and reduce infections in subsequent crops. Objective 2.No-till planting crops into rolled-crimped cover crops can improve soil health while reducing labor and fuel requirements compared with traditional tillage-based production. However, little information is available to help farmers optimize the management of organic no-till planted crops. Weed suppression, crop yield, and profitability were assessed across soybean \Glycine max(L.) Merr.\ seeding rates and soil nitrogen environments in an experiment conducted at two sites in central New York. Soybeans were no-till planted into rolled-crimped cereal rye (Secale cerealeL.) at 0, 185,000, 371,000, 556,000, and 741,000 seeds ha−1. Three rates (0, 63, or 125 kg ha−1) of sodium nitrate (15-0-2) were applied across seeding rates to create different soil nitrogen environments. When pooled over sites, the lowest weed biomass occurred at the highest soybean density in the lowest soil nitrogen environment. An interaction was observed between soybean seeding rate and nitrogen treatments on weed communities. Soybean yield increased asymptotically with crop density and was not affected by nitrogen or site treatments. When pooled over nitrogen treatments and sites, partial returns to the soybean seeding rates were maximized at \$2,238 ha−1with 527,800 seeds ha−1. Results suggest that crop density is an important lever for optimizing weed suppression and crop yield in organic no-till soybean, and that managing for low soil nitrogen conditions may further enhance weed suppression while maintaining high yields. Objective 3. Our findings in the last reporting period have been extended to a broad audience focusing on field crop farmers and their stakeholders considering transitioning from conventional to organic production. These venues have included extension presentations (6), training events (2), and a production guide (1). Two refereed journal articles were also published and results presented at an international meeting (ASA). **Publications** - Type: Journal Articles Status: Published Year Published: 2020 Citation: Pethybridge, S. J., Brown, B., Kikkert, J. R., and Ryan, M. R. 2020. Systems benefits and trade-offs of rolled-crimped cereal rye mulch for white mold control and weed suppression in no-till soybean and dry bean production. Ren. Agric. Food Sys. 35:599-607. - Type: Journal Articles Status: Published Year Published: 2021 Citation: Menalled, U., Pethybridge, S. J., Pelzer, C., Smith, R. G., DiTomasso, A., and Ryan, M. R. 2021. High seeding rates and low soil nitrogen optimize weed suppression and profitability in organic no-till planted soybean. Front. Agron. 3:678567. - Type: Books Status: Published Year Published: 2021 Citation: Ryan, MR, BA Caldwell, K Crowley, JA Liebert, U Menalled, CJ Pelzer, L Pickard, and S Wayman. 2021. Organic No-Till Planted Soybean Production. Sustainable Cropping Systems Lab. Cornell University, Ithaca, NY. https://bit.ly/ONTPSguide **Progress** 09/01/19 to 08/31/20 **Outputs** Target Audience:The target audiences for this research are field crop farmers considering transitioning from conventional to organic production in New York. Farmers with similar objectives across the Northeastern United States in regions with similar precipitation patterns and in which cover crops are particularly popular already are also recipients of these findings. Industry stakeholders, extension personnel and crop scouts that provide advice to these growers are also the target audience. The scientific community is another target audience to hear of the

research outcomes which are extended agroecosystem and agroecological foundational research knowledge. Results are being prepared in manuscripts to be submitted to scientific journals and to be presented at scientific conferences. The broader field crop and vegetable farmers in the region have also been informed of the outcomes of this research through annual research and extension commodity events in New York and Pennsylvania (see list of outreach and extension products). Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?This study has partially contributed to the graduate studies of two PhD students (Ashley Jernigan \Objective 1\ and Uriel Menalled \Objective 2\. Students have received advanced training in conducting field trials, practical agronomy, organic agriculture, data analysis and writing of papers, and extension of findings to diverse audiences. How have the results been disseminated to communities of interest?Results of the project were extended to growers and industry personnel including crop scouts and extension educators through virtual field days and extension events. Over these four events, at least 150 participants received information from this study. Results were also presented at the premiere national agronomy scientific conference to extend our findings to the agronomy and crop science research community. A white mold fact sheet and extension bulletin was also developed and published in the Cornell Cooperative Extension weekly update (VegEdge) distributed to over 400 growers. What do you plan to do during the next reporting period to accomplish the goals?The next reporting period will be spent completing the yield component data collection from Objective 1. This data will then be combined with that obtained from Year 1, and form the basis of a partial budget analysis. This information will then be prepared for submission to a scientific journal and distilled for key messages to grower and industry stakeholders in extension events during this period. Data from the trials conducted in Objective 2 is also being finalized and analyzed for publication and extension presentations. We will continue to work for a broad range of extension agents to ensure visibility of the results to a broad range of grower audiences. **Impacts** What was accomplished under these goals? Objective 1: Cereal rye mulch for white mold and weed suppression. A replicated trial was conducted at the Research North facility (Field RN25) at Cornell AgriTech, Geneva, NY. The trial was a split-block, randomized, complete block design with four replicates and around 2.5 ha in size. Treatments were established in fall 2019 and were a direct replicate of the trial conducted in 2018/19 to evaluate the robustness of our findings across years. Treatments included either soybean or dry black bean as the main crop. Subplot treatments were: (i) no cover crop - left fallow and shallowly tilled prior to main crop planting; (ii) cereal rye with tillage prior to planting; and (iii) cereal rye mulch rolled and crimped before planting. Evaluations during the main cropping season included aboveground biomass, root disease incidence and severity, weed populations (density and diversity), white mold incidence, and agronomic yield components at harvest (plant population, pod and bean yield). The effect of treatment on S. sclerotiorum apothecia and airbone ascosporic inoculum was also evaluated. This data will be combined with that obtained from the previous trial. Outcomes will be used for a partial budget analysis of the different management systems and extension of findings to the scientific community, and growers. Objective 2:Seeding rate trad-offs.Fuel and labor costs from tillage-based weed management challenge organic soybean production. Organic rotational no-till farming is an alternative practice with lower labor and fuel inputs than traditional organic production and can be accomplished through seeding a cash crop into a rolled cover crop mulch. However, cover crop residue alone is inadequate for successful weed suppression across the entire growing season. High crop density and low nitrogen availability may improve weed management in organic no-till soybean. Weed suppression, weed communities, crop yields, and partial returns were assessed across a gradient of soybean (Glycine max (L.) Merr.) density and nitrogen fertilization in a rolled mulch system. Soybeans were seeded into a cereal rye (Secale cereale L.) mulch at 0, 185,000, 371,000, 556,000, and 741,000 seeds ha-1, and 0, 63, or 125 kg ha-1 of 15-0-2 starter sodium nitrate were applied to each seeding rate. Weed biomass was reduced by soybean density but increased by nitrogen fertilization. In the absence of soybeans, fertilization with 125 kg N ha-1 resulted in a 135% increase in weed biomass than the 0 kg N ha-1 treatment. Nitrogen fertilization also changed weed communities with increased annual grass weeds in high nitrogen treatments. Soybean yield increased asymptotically with crop density and was not affected by nitrogen fertilization. Given the cost of nitrogen and its neutral effects on yield, partial returns for organic feed-grade soybean were maximized at \$3,120 ha-1 in the 0 kg N ha-1 treatment at 493,734 seeds ha-1. Thus, high crop density and low nitrogen availability are economically viable weed management strategies for organic no-till soybean. Objective 3: Dissemination of research findings. Our research findings to date have been presented to a broad range of stakeholders including the scientific community (ASA/CSA virtual meeting; August 2020) and to growers and crop scouts through extension presentations (four in this reporting period), fact sheets (white mold), and newsletter articles (VegEdge article). **Publications** - Type: Journal Articles Status: Awaiting Publication Year Published: 2020 Citation: Pethybridge, S. J., Brown, B., Kikkert, J. R., and Ryan, M. R. 2020. Systems benefits and trade-offs of rolled-crimped cereal rye mulch for white mold control and weed suppression in no-till soybean and dry bean production. Ren. Agric. Food Sys. <https://doi.org/10.1017/S174217051900022X>. - Type: Journal Articles Status: Under Review Year Published: 2020 Citation: Menalled, U., Pethybridge, S. J., Pelzer, C., Smith, R. G., DiTomasso, A., and Ryan, M. R. 2020. Organic no-till soybean seeding rate and nitrogen fertilization effects on weed suppression, crop yield and profit. - Type: Conference Papers and Presentations Status: Published Year

Published: 2020 Citation: Menalled, U., Ryan, M. R., and Pethybridge, S. J. 2020. Organic No-till Soybean Seeding Rate and Nitrogen Fertilization Effects on Weed Suppression. ASA, CSSA & SSSA International Annual Meeting. ASA Section: Agronomic Production. Virtual event. PROGRESS: 2018/09 TO 2019/08 Target Audience:The target audiences for this work include field crop farmers interested in transitioning to organic productionin western and central New York, and field and vegetable crop farmers in the Northeast and other regions with similar precipitation patterns and cropping systems (organic or conventional)county and regional agricultural extension agents, industry stakeholders, and members of the scientific community. The advisory group for this project consists of key organic and conventional field crop growers (innovators), industry stakeholders responsible for the agronomic production of field crops within New York, and field crop extension personnel. The broader field crop community have also been informed of the research program at annual research and extension commodity events in collaboration with Cornell Cooperative Extension (see list of outreach and extension activities). Results and findings from these studies have been presented at scientific conferences to the broader agricultural science community (see list of presentations), and will be included in manuscripts to be submitted to scientific journals. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?Two graduate students have been involved in this project (Ashley Jernigan wthin Objective 1; Uriel Menalled within Objective 2). Both students are receiving training in field trials, organic agriculture, data compilation and statistical analysis, and extension and outreach of findings. Several undergraduates were also immersed in data collection of trials at Musgrave and Geneva in 2019. How have the results been disseminated to communities of interest? Field crop growers, extension personnel and industry stakeholders have received progress of the project to date and will continue to be integrally involved in the extension and outreach of the findings through participation in regular educational events. As outlined in the products section, information and research was summarized in extension publications (e.g. VegEdge), and workshops and field days throughout the first year of this project. Together, over 100 growers and extension personnel were reached to date. Results have also been disseminated to the scientific cmmunity through a peer-reviewed journal article and presentation at an academic conference. What do you plan to do during the next reporting period to accomplish the goals?The project is proceeding as planned in the next reporting period within each of the objectives. IMPACT: 2018/09 TO 2019/08 What was accomplished under these goals? Objective 1.A trial was conducted at the Research North field facilities of Cornell AgriTech at The New York State Agricultural Experiment Station, Geneva, NY with a split-block randomized complete block design with four replicates in fall 2018 (\~ 2 ha in size). Main plot treatments were soybean or dry bean and subplot treatments were: no cover crop, cereal rye with tillage, and cereal rye mulch. Cereal rye was drill seeded in early September in the selected plots at approximately 200 kg seed/ha. The remaining plots were left fallow and weeds were controlled using shallow tillage. The cereal rye crops were terminated in spring according to their treatment allocation, and main crops were planted using a precision planter using varieties typical of those selected by organic growers in central NY. The cereal rye biomass prior to rolling and crimping in spring and that remaining at harvest was quantified. Throughout the cropping season, agronomic yield attributes (plant population, aboveground biomass, and pod and bean yield), weed populations, and white mold incidence were quantified. The effect of the rolled cover crop on *S. Sclerotiorum* apothecia and airborne ascospores was also evaluated. Harvest of this trial was completed one month ago, and data is in the process of being collated and analyzed for the effect of treatment. A repeat of this trial was planted in fall 2019 and will continue throughout 2019/20. Data from both trials will be collected and form the basis of a partial budget analysis to quantify the costs and benefits of the different management systems included in this objective. Objective 2. Seeding rate tradeoffs. Two small plot, replicated trials were established at the research facilities of Cornell University (Musgrave Farm, Aurora, NY, and Research North, Geneva, NY) aiming to test for tradeoffs between weed and disease suppression from increasing seeding rates in organic no-till soybean. In each field, cereal rye was drill-seeded in fall 2018 and the main soybean crop no-till planted in spring 2019 after termination of the cereal rye by a roller-crimper. The experimental design was a randomized complete block with at least four replications of five different soybean seeding rates (195,000 to 915,000 seeds/ha) and two nitrogen rates provided by hand-application of sodium nitrate (OMRI-approved source). These trials have also been harvested recently and data is being compiled for analysis. Preliminary analysis has indicated that nitrogen and soybean seeding rate has influenced soybean light reflectance and interception dynamics. For example, across all growth rates, increased nitrogen fertilization and seeding rate increased the interception of far-red light. Nitrogen fertilization also influenced tissue composition of the soybean, and chlorophyll index light reflectance values indicate that nitrogen fertilization increased soybean leaf chlorophyll content. Light interception also increased throughout the season and was greater in high nitrogen and seeding rate plots. Preliminary weed community analyses suggests significant site and nitrogen rate effects on weed community composition. This data will be interrogated further to explore weed-crop competition through structural equation modeling, and modeling light dynamics. Objective 3. Disseminate research findings.The goals and objectives of this projecthave been presented to our target audience over the first cropping season and findings will continue to be disseminated through the winter meetings of 2019/20 and future years within our field crop extension meetings and extension newsletters, and through

direct discussions with members of the advisory group. PUBLICATIONS (not previously reported): 2018/09 TO 2019/08 1\. Type: Journal Articles Status: Published Year Published: 2019 Citation: Vincent-Caboud, L, M Casagrande, C David, MR Ryan, E Silva, and J Peigne. 2019. Can mulch cover no tillage be integrated into organic farming practices for arable crop production? - A review. Agronomy for Sustainable Development 39(5), p.45. https://link.springer.com/article/10.1007/s13593-019-0590-2 2\. Type: Journal Articles Status: Published Year Published: 2019 Citation: Ryan, MR, MG Ball, AB Jernigan, CJ Pelzer, S Wayman, and CL Mohler. 2019. Lessons from the Cornell organic grain cropping Systems experiment. ASA, CSSA & SSSA International Annual Meeting. ASA Section: Agronomic Production Systems 60-3.

PROGRESS

2019/09 TO 2020/08 Target Audience:The target audiences for this research are field crop farmers considering transitioning from conventional to organic production in New York. Farmers with similar objectives across the Northeastern United States in regions with similar precipitation patterns and in which cover crops are particularly popular already are also recipients of these findings. Industry stakeholders, extension personnel and crop scouts that provide advice to these growers are also the target audience. The scientific community is another target audience to hear of the research outcomes which are extended agroecosystem and agroecological foundational research knowledge. Results are being prepared in manuscripts to be submitted to scientific journals and to be presented at scientific conferences. The broader field crop and vegetable farmers in the region have also been informed of the outcomes of this research through annual research and extension commodity events in New York and Pennsylvania (see list of outreach and extension products). Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?This study has partially contributed to the graduate studies of two PhD students (Ashley Jernigan \Objective 1\ and Uriel Menalled \Objective 2\. Students have received advanced training in conducting field trials, practical agronomy, organic agriculture, data analysis and writing of papers, and extension of findings to diverse audiences. How have the results been disseminated to communities of interest?Results of the project were extended to growers and industry personnel including crop scouts and extension educators through virtual field days and extension events. Over these four events, at least 150 participants received information from this study. Results were also presented at the premiere national agronomy scientific conference to extend our findings to the agronomy and crop science research community. A white mold fact sheet and extension bulletin was also developed and published in the Cornell Cooperative Extension weekly update (VegEdge) distributed to over 400 growers. What do you plan to do during the next reporting period to accomplish the goals?The next reporting period will be spent completing the yield component data collection from Objective 1. This data will then be combined with that obtained from Year 1, and form the basis of a partial budget analysis. This information will then be prepared for submission to a scientific journal and distilled for key messages to grower and industry stakeholders in extension events during this period. Data from the trials conducted in Objective 2 is also being finalized and analyzed for publication and extension presentations. We will continue to work for a broad range of extension agents to ensure visibility of the results to a broad range of grower audiences.

2020/09 TO 2021/08 Target Audience:Field crop farmers considering transitioning from conventional to organic production are the predominant target audience for the outcomes from this research. Whilst the focus of the project outcomes are directly transferable to farmers in New York, the findings have also been extended to farmers with similar objectives in the Northeastern US and other areas with similar precipitation patterns and therefore fit cover crops into their rotations. Findings have also been extended to industry stakeholders, crop scouts and others that provide advice on crop management to these farmers (e.g. extension personnel and agronomists). The scientific community has also been an important audience for this information (see list of outreach and extension products). Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?This study has partially contributed to the graduate studies of two PhD students (Ashley Jernigan \Objective 1\ and Uriel Menalled \Objective 2\. A Postdoctoral Research Associate (Daniel Heck) has also been involved in Objective 1. These early career scientists have received training in research and extension skills ranging from extending results to farmers and the scientific community,conducting field trials, practical agronomy, organic agriculture, data analysis. How have the results been disseminated to communities of interest?Results were extended to farmers considering organic transition and industry personnel including crop scouts and extension educators through virtual and in-person field days and extension events, ranging from the American Farmland Trust to the Indiana Certified Crop Adviser program. Virtual offerings of these kind enabled us to reach audiences outside our region that we had not thought we are able to connectIn-person events were also held at three locations around New York to reach local transitioning farmers. At the in-person events, over 250 participants were reached.Results were also presented at the premiere national agronomy scientific conference (ASA) . A production guide on organic no-till soybean

production was also produced and published (https://bit.ly/ONTPSguide). What do you plan to do during the next reporting period to accomplish the goals?This next reporting period is the final no-cost extension of this project. Data will be combined from all experiments including information on soil insect populations and disease for an additional two scientific publications. The data will also be distilled into farmer-friendly information for dissemination across a broad range of farmer audiences this winter including extension events and fact sheets.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? Objective 1: Cereal rye mulch for white mold and weed suppression. A replicated trial was conducted at the Research North facility (Field RN25) at Cornell AgriTech, Geneva, NY. The trial was a split-block, randomized, complete block design with four replicates and around 2.5 ha in size. Treatments were established in fall 2019 and were a direct replicate of the trial conducted in 2018/19 to evaluate the robustness of our findings across years. Treatments included either soybean or dry black bean as the main crop. Subplot treatments were: (i) no cover crop - left fallow and shallowly tilled prior to main crop planting; (ii) cereal rye with tillage prior to planting; and (iii) cereal rye mulch rolled and crimped before planting. Evaluations during the main cropping season included aboveground biomass, root disease incidence and severity, weed populations (density and diversity), white mold incidence, and agronomic yield components at harvest (plant population, pod and bean yield). The effect of treatment on S. sclerotiorum apothecia and airbone ascosporic inoculum was also evaluated. This data will be combined with that obtained from the previous trial. Outcomes will be used for a partial budget analysis of the different management systems and extension of findings to the scientific community, and growers. Objective 2:Seeding rate trad-offs.Fuel and labor costs from tillage-based weed management challenge organic soybean production. Organic rotational no-till farming is an alternative practice with lower labor and fuel inputs than traditional organic production and can be accomplished through seeding a cash crop into a rolled cover crop mulch. However, cover crop residue alone is inadequate for successful weed suppression across the entire growing season. High crop density and low nitrogen availability may improve weed management in organic no-till soybean. Weed suppression, weed communities, crop yields, and partial returns were assessed across a gradient of soybean (Glycine max (L.) Merr.) density and nitrogen fertilization in a rolled mulch system. Soybeans were seeded into a cereal rye (Secale cereale L.) mulch at 0, 185,000, 371,000, 556,000, and 741,000 seeds ha-1, and 0, 63, or 125 kg ha-1 of 15-0-2 starter sodium nitrate were applied to each seeding rate. Weed biomass was reduced by soybean density but increased by nitrogen fertilization. In the absence of soybeans, fertilization with 125 kg N ha-1 resulted in a 135% increase in weed biomass than the 0 kg N ha-1 treatment. Nitrogen fertilization also changed weed communities with increased annual grass weeds in high nitrogen treatments. Soybean yield increased asymptotically with crop density and was not affected by nitrogen fertilization. Given the cost of nitrogen and its neutral effects on yield, partial returns for organic feed-grade soybean were maximized at \$3,120 ha-1 in the 0 kg N ha-1 treatment at 493,734 seeds ha-1. Thus, high crop density and low nitrogen availability are economically viable weed management strategies for organic no-till soybean. Objective 3: Dissemination of research findings. Our research findings to date have been presented to a broad range of stakeholders including the scientific community (ASA/CSA virtual meeting; August 2020) and to growers and crop scouts through extension presentations (four in this reporting period), fact sheets (white mold), and newsletter articles (VegEdge article). **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 1. Type: Journal Articles Status: Awaiting Publication Year Published: 2020 Citation: Pethybridge, S. J., Brown, B., Kikkert, J. R., and Ryan, M. R. 2020. Systems benefits and trade-offs of rolled-crimped cereal rye mulch for white mold control and weed suppression in no-till soybean and dry bean production. Ren. Agric. Food Sys. https://doi.org/10.1017/S174217051900022X. 2. Type: Journal Articles Status: Under Review Year Published: 2020 Citation: Menalled, U., Pethybridge, S. J., Pelzer, C., Smith, R. G., DiTomasso, A., and Ryan, M. R. 2020. Organic no-till soybean seeding rate and nitrogen fertilization effects on weed suppression, crop yield and profit. 3. Type: Conference Papers and Presentations Status: Published Year Published: 2020 Citation: Menalled, U., Ryan, M. R., and Pethybridge, S. J. 2020. Organic No-till Soybean Seeding Rate and Nitrogen Fertilization Effects on Weed Suppression. ASA, CSSA & SSSA International Annual Meeting. ASA Section: Agronomic Production. Virtual event.

2020/09 TO 2021/08 What was accomplished under these goals? Objective 1.White mold caused by the fungus, Sclerotinia sclerotiorum is a devastating disease of soybean (Glycine max) and other leguminous crops, including dry bean (Phaseolus vulgaris). Previous research has demonstrated that no-till planting soybean into rolled-crimped cereal rye residue can enhance weed management, improve soil health, and reduce labor requirements in organic production. However, there are limited data on the effects of cereal rye residue on white mold suppression in no-till planted soybean and dry bean. Two field trials were conducted in 2016-2017 (Year 1) and repeated in 2017-2018 (Year 2) to evaluate the potential of cereal rye cover crop residue to suppress white mold

in these crops. In each trial (soybean and dry bean), the experimental design was a randomized complete block with two treatments: 1) rolled-crimped cereal rye residue and 2) no cover crop control. Treatment effects on plant population, biomass, and yield components varied between the main crops. Compared to the control treatment, cereal rye residue reduced the incidence of white mold in soybean in both years and in dry bean in Year 2. The reduction in white mold in cereal rye residue plots was due to a combination of 1) decreased sclerotial germination (no stipes formed) and 2) increased nonfunctional sclerotial germination defined here as sclerotia that germinated but produced stipes without the expanded cup where asci containing ascospores are formed. Weed density and biomass were lower in cereal rye residue plots in soybean and dry bean, except in year 1 in soybean when weed biomass was low in both treatments. Our findings indicate that cereal rye residue could help organic and conventional farmers manage white mold in no-till planted soybean and dry bean. Germination of sclerotia resulting in nonfunctional apothecia could potentially exhaust soilborne inoculum in the upper soil profile and reduce infections in subsequent crops. Objective 2.No-till planting crops into rolled-crimped cover crops can improve soil health while reducing labor and fuel requirements compared with traditional tillage-based production. However, little information is available to help farmers optimize the management of organic no-till planted crops. Weed suppression, crop yield, and profitability were assessed across soybean \Glycine max(L.) Merr.\ seeding rates and soil nitrogen environments in an experiment conducted at two sites in central New York. Soybeans were no-till planted into rolled-crimped cereal rye (Secale cerealeL.) at 0, 185,000, 371,000, 556,000, and 741,000 seeds ha−1. Three rates (0, 63, or 125 kg ha−1) of sodium nitrate (15-0-2) were applied across seeding rates to create different soil nitrogen environments. When pooled over sites, the lowest weed biomass occurred at the highest soybean density in the lowest soil nitrogen environment. An interaction was observed between soybean seeding rate and nitrogen treatments on weed communities. Soybean yield increased asymptotically with crop density and was not affected by nitrogen or site treatments. When pooled over nitrogen treatments and sites, partial returns to the soybean seeding rates were maximized at \$2,238 ha−1with 527,800 seeds ha−1. Results suggest that crop density is an important lever for optimizing weed suppression and crop yield in organic no-till soybean, and that managing for low soil nitrogen conditions may further enhance weed suppression while maintaining high yields. Objective 3. Our findings in the last reporting period have been extended to a broad audience focusing on field crop farmers and their stakeholders considering transitioning from conventional to organic production. These venues have included extension presentations (6), training events (2), and a production guide (1). Two refereed journal articles were also published and results presented at an international meeting (ASA). **PUBLICATIONS (not previously reported):** 2020/09 TO 2021/08 1. Type: Journal Articles Status: Published Year Published: 2020 Citation: Pethybridge, S. J., Brown, B., Kikkert, J. R., and Ryan, M. R. 2020. Systems benefits and trade-offs of rolled-crimped cereal rye mulch for white mold control and weed suppression in no-till soybean and dry bean production. Ren. Agric. Food Sys. 35:599-607. 2. Type: Journal Articles Status: Published Year Published: 2021 Citation: Menalled, U., Pethybridge, S. J., Pelzer, C., Smith, R. G., DiTomasso, A., and Ryan, M. R. 2021. High seeding rates and low soil nitrogen optimize weed suppression and profitability in organic no-till planted soybean. Front. Agron. 3:678567. 3. Type: Books Status: Published Year Published: 2021 Citation: Ryan, MR, BA Caldwell, K Crowley, JA Liebert, U Menalled, CJ Pelzer, L Pickard, and S Wayman. 2021. Organic No-Till Planted Soybean Production. Sustainable Cropping Systems Lab. Cornell University, Ithaca, NY. https://bit.ly/ONTPSguide

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Does Organic Farming Promote Soil Microbial Communities with High Nitrogen Use Efficiency in Acidic Southeastern Us Soils?

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Project No.	NC09855
Agency	NIFA NC.\
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Investigator(s)	Hu, S.
Performing Institution	WEST VIRGINIA UNIVERSITY, 886 CHESTNUT RIDGE RD RM 202, MORGANTOWN, WEST VIRGINIA 26505-2742

NON-TECHNICAL SUMMARY

Agricultural soils in the southeastern U.S., marked by high rainfall and warm temperatures, are often characterized with low organic C and soil pH (i.e., acidic soil). Organic farming relies on organic inputs and fundamentally alters the sources and forms of reactive carbon (C) and nitrogen (N) inputs. It also induces changes in soil physical and geochemical properties that may profoundly affect the abundances, composition and activities of soil microbes, including nitrifiers (i.e., ammonia-oxidizing bacteria and archaea) and diverse types of denitrifiers. However, there is still a lack of a holistic picture of organic farming effects on N-cycling microbes and their activities. We hypothesize that organic farming, if properly adopted, may induce consistent and predictable changes to foster the development of microbial communities for N retention and mitigation of emissions of trace gas, N2O. We plan to test our three specific hypotheses on long-term (19-yr) cropping systems at the Center for Environmental Farming Systems (Goldsboro, NC) as well as on multiple organic farms across North Carolina. Three major questions will guide our proposed research: 1) Does long-term organic farming cause consistent, predictable shifts in N-cycling microbes across different soils? 2) What are the primary drivers and/or environmental factors that affect the composition and activities of N-cycling microbes? 3) To what extent do changes in N-cycling microbes alter N retention in plant-soil systems and N2O emissions? Answering these questions will advance understanding of N transformations in organic systems and facilitate development of management regimes that enhance N use efficiency and ecosystem N retention, while reducing N2O emissions.

Small ruminants provide a profitable niche for organic growers. However, certain intestinal parasites, specifically gastrointestinal nematode (GIN) infections, are the major factor limiting organic production. Consumption of condensed tannin (CT) containing forages aids in suppressing GIN infections, however, the bulk of the research on GIN control has been on forages adapted for warm climates. Our previous work showed that a temperate forage, birdsfoot trefoil (BFT), has anthelmintic activity, however efficacy varies with the amount of CT-containing forage in the diet. Because birdsfoot trefoil is slow to establish, improved methods to assure high levels in pastures or in ruminant rations are needed to realize the potential for GIN control. This proposal represents the next logical steps in research, by establishing a multi-state, multi-disciplinary project to enhance productivity and profitability of organic livestock production in the Northeast. Specifically, commercially-available cultivars of BFT will be evaluated by: 1) comparing agronomic pasture establishment practices, 2) determining efficacy of GIN

suppression using in vitro and in vivo methods, including grazing trials and experimental infections, 3) demonstrating these improved pasture management practices in on-farm trials and 4) extending these practices to organic and transitioning growers. Outreach activities will include grower trials, IPM workshops, field days, and will also explore use of on-line services such as eOrganic and eXtension. Growers will realize increased animal productivity, reduced numbers of animals removed from organic status due to dewormer intervention, and will have increased confidence in coping with GIN during transition from conventional to organic farming. This project addresses OREI goals one, three, and four.

OBJECTIVES

The overarching goal of this integrated research, education and extension project is 1) to understand the mechanisms that control soil N transformations and greenhouse gas (particularly N2O) emissions in organic systems, and 2) to educate stakeholders and students about optimizing management for mitigation of greenhouse gas emissions. Our interdisciplinary team will undertake four specific objectives using both field and greenhouse experiments, as well as field-scale demonstrations using replicated certified organic plots at our existing long-term experimental site at the Center for Environmental Farming Systems (CEFS), Goldsboro, NC. Objective 1. Characterize the effects of organic farming on N-cycling microbes both in field plots at CEFS and on the shareholders\' organic farms. Objective 2. Identify the primary driving factors that modulate the effect of organic farming on N-cycling microbes Objective 3. Validate the relationships between N transformations and abundances, composition and activities of N-cycling microbes both in field and controlled environments. Objective 4. Educate the next generation of organic agriculture researchers and farmers through student training and an active, multi-pronged outreach program.

This proposed project would enable us to continue studying the potential of birdsfoot trefoil (BFT) to combat two major barriers to organic small ruminant production: gastrointestinal parasite infections and the costs of organic concentrate feed that is often necessary to obtain sufficient growth rates in lambs and kids compromised by parasite infection. With this new project, our research objectives include evaluation of: agronomic practices to improve BFT establishment in existing pastures; the ability of protein supplementation to substantially increase parasite tolerance of organically managed lambs to gastrointestinal nematodes; and the effect of feeding BFT processed as either hay, high-moisture hay or pellets on experimental and natural gastrointestinal nematode infections in lambs. Outreach objectives include: teaching regional workshops on parasite management in small ruminants; conducting on-farm grazing trials with four goat or sheep farms (two certified organic and two still lacking confidence to transition) to demonstrate effective BFT management practices; organizing field days and pasture walks to demonstrate improved practices; preparing extension fact sheets to translate research results into practical management suggestions; and evaluating the impact of the field days, workshops and on-farm trials on grower adoption of best practices.Our overall goal is to develop organic grazing practices that suppress GIN parasites in sheep and goats while maintaining organic integrity. We propose new methods to validate harvesting methods that preserve the anthelmintic activity of the forage and develop best management practices for the establishment of BFT. This project will integrate three research and three outreach objectives (described below).Research Objectives:1) Evaluate agronomic practices to improve BFT establishment in existing pastures.2) Compare efficacy of BFT pastures and supplemental feed in field-scale grazing trials to evaluate whether the use of BFT in the feeding program of sheep and goats can increase tolerance to gastrointestinal nematodes as well as replace or minimize grain supplementation needed for optimum growth.3) Determine the effect of feeding BFT processed as either hay, high- moisture hay or pellets on experimental and natural gastrointestinal nematode infections in lambs. Outreach and Extension Objectives:4) Demonstrate research results in on-farm trials with select growers.5) Extend research results to organic and conventional sheep and goat producers throughout the Northeast, via Extension fact sheets, workshops, pasture walks, field days, and continuation of the web page.6) Evaluate success of outreach activities through surveys of growers and participants at workshops, as well as economic performance analyses of growers participating in on-farm trials, or other adoption of management recommendations.

APPROACH

Diverse methodologies will be applied to approach our objectives. Objective 1. Characterize the impact of organic farming on N-transforming microbes. Method overview: Our aim is to obtain the essential data of the N-cycling microbes so that the relationships between the functions (nitrification and denitrification potentials, N2O

emissions) and the abundances and community composition of N-cycling microbes can be explored. Soil sampling: We will use a 2.5-cm diameter soil corer to take 8-10 cores to 20 cm depth at the selected sampling dates three times per year. Soil cores will be immediately separated into 0-10 and 10-20 cm depth sections and then pooled by the depth section per plot for analyses of soil physical, chemical, and microbial parameters (see below).Abundances of N-cycling microbes will be measured by Real-time PCR quantification of the amoA (AOA), amoA (AOB), nirS, nirK and nosZ genes following DNA extraction from soil. Objective 2. Identify the primary driving factors that modulate the effect of organic farming on N-cycling microbes. We will conduct extensive analyses of soil physical, chemical and microbial parameters to examine the relationships between soil properties and N-cycling microbe. We will record all the major farming activities and material inputs (such as manures and lime) both in our experimental plots at CEFS and on farms. Subsamples of organic inputs will be collected and analyzed for C, N and elemental contents. Structural equation model analysis to identify the primary driving factors that control the abundance of N-cycling microbes and ratios of different denitrifier groups: We will use structural equation modeling (SEM) to identify potentially causal linkages between soil variables and N-cycling microbial parameters. Based on the linear regression results, we will estimate the strength of direct and indirect relationships among the considered variables and then check the bivariate relationships between variables to ensure that linear models are appropriate. Objective 3. Validate the relationships between N transformations and abundances, composition and activities of N-cycling microbes both in field and controlled environments. Field assessment of relationships between communities of N-cycling microbes and N2O emissions: We use a combination of static and continuous chambers to characterize N2O emissions. Continuous measurements provide a temporal index of the intensity of emissions between treatments for a given event. We will use this advantage to characterize the community of nitrifiers, denitrifiers and other heterotrophs present during bursts of N2O emissions especially after major events: e.g. fertilization. Soil samples used to describe the microbial community will be referenced to the start of an event as well as the relate intensity of emissions at the time of sampling. We anticipate sampling \~ 4 events per crop. Determination of the impact of community shifts in N-cycling microbes on soil N retention and plant N uptake: Microcosm experiments will be conducted in greenhouse to examine whether the presence of growing plant roots significantly alter the responses of N-transforming microbes to alterations in relative C to N availability. Stable carbon and nitrogen isotopes will be used to enhance the sensitivity of detection. Objective 4. Educate the next generation of organic agriculture researchers and farmers through student training and an active, multi-pronged outreach program. Training of individuals directly funded by this project will include a postdoc, a PhD student and one undergraduate student (one per year), In addition, educational modules will be developed based on novel research findings derived from the research projects described in this proposal. CEFS has developed a variety of well-structured training programs that are available online (<http://www.cefs.ncsu.edu/whatwedo.html>). Outreach and provision of faculty-mentored research experiences for undergraduate students will also occur through the CEFS Sustainable Agriculture Internship Program. Reaching Farmers: We will communicate our research to farmers through field days and on-farm meetings.Project Evaluation: In addition to recruiting farmers for soil testing, Rural Advancement Foundation International (RAFI) will serve as the outside evaluator for the project. They will determine whether farmers find the soil testing we provide to the farmers is of value to them and whether our research findings are important. The PIs will provide the RAFI evaluators the progress reports so that they can assess whether the project proceeds on schedule. The evaluators will also collect attitudes toward information on soil health that we can incorporate annually to our outreach efforts. Also, results from this work and our protocols will be evaluated through the scientific review process via manuscripts submitted for publication and presentations at scientific meetings. In addition, we are members of the Center for Environmental Farming Systems at NCSU team and as such we have an internal evaluation process among the main faculty members and with stakeholders. **Progress** 09/01/18 to 08/31/23 **Outputs** Target Audience:Students: Undergraduate students, graduate students, postdoctoral scholars and visiting scientists, as well as technicians, who are associated with the PI and co-PIs programs. Also, students who enroll in PI\'s classes. The scientific community in general, particularly ecologists, soil scientists, and biologists and graduate and undergraduate students. Results obtained from our project have been partially presented in the scientific meetings and five papers have been published in peer-reviewed journals. Farmers and land managers. The field experiment at the Center for Environmental Farming at NCSU, the nitrous oxide CEFS study, was presented at the North Carolina State University Organic Field Day, August 2022, and field days each year. Work from this project has also been presented at a researcher and farmer focused Webinar titled \"Farming for Ecological Outcomes\". Changes/Problems:The COVID 19 pandermic did affect our research, but we did our best to approachour objectives and achieved what we wanted. What opportunities for training and professional development has the project provided? Three postdoctoral scholars (Drs. Arjun Pandey, Sean Bloszies and Henrique Da Ros Carvalho) participated in this project at different phases of research. Two graduate students (PhD student Christopher Gillespie) and MS student (Miss Bolin Dong) did their thesis research, partially supported by this project. Two other graduate students worked on the field experiment associated with this project, although they were not financially supported by this project. Four undergraduate students (Miss Juhi Dattani Miss Brooke Matusko, Misss Ayanna Hatton and Mr. Nicholas Terwilliger) finished

their independent research projects associated with this project and received training on greenhouse experiments, greenhouse gas sampling, and soil chemical and molecular analyses. Multiple other undergraduate students received training in field greenhouse gas sampling (static chamber) and automatic monitoring under the supervision of co-PI (Dr. Alexander Woodley). Three visiting scholars, including one USDA-FAS scholar from Nigeria (Dr. Isalar Oluwatoyin from Nigeria) received training through this project. This project has been introduced to graduate students who took the Ecology of Soil Ecosystems, taught by the PI (Shuijin Hu) in 2000 and 2002. How have the results been disseminated to communities of interest?Our field site at CEFS has hosted a Center for Environmental Farm Systems farm tour which included an information session on this innovative design (\~30ppl), the purpose of the tour was for interested faculty at NCSU not familiar with CEFS to come and potentially find collaborators for future research at CEFS. In addition, this site has hosted interested researchers from the Nature Conservancy and the Environmental Defense Fund. Also, our field projects and results were introduced to students of a class entitled Ecology of Soil Ecosystems in Spring, 2020, and again in the Spring, 2022. In addition, the field experiment (the nitrous oxide CEFS study) was presented at the North Carolina State University Organic Field Day August 2021 (75 people). Work from this project was also presented at a researcher and farmer focused Webinar titled \"Farming for Ecological Outcomes\". Results from our study have been presented at annual meetings of Ecological Society of America (2023) and of Association of Applied Biologists. Rothamstead Research, UK (2023). Lastly, five papers about organic farming effects on soil microbes, C and N cycling, and N2O emissions have been published in peer-reviewed journals during the project period. Three manuscripts have been submitted or are under review. Three additional manuscripts are expected to be submitted in 2024. What do you plan to do during the next reporting period to accomplish the goals?Thisproject was officially completed on August 31, 2023. One PhD student (Mr. Christopher Gillespie) who had been supported by this project, plans to graduate in Spring 2024. He is currently working on his dissertation. He is working on multiple manuscripts, and we plan to submit them to peer-reviewed journals in 2024. Also, results from other participants (postdocs and undergraduate students) are being analyzed and prepared to be published in peer-reviewed journals. Finally, our field long-term continuous N2O monitoring study and greenhouse experiments, and the results obtained will be introduced to students of an advanced course entitled Ecology of Soil Ecosystems, taught by the PI in Spring, 2024. **Impacts** What was accomplished under these goals? ? Agricultural soils in the southeastern U.S., marked by high rainfall and warm temperatures, are often characterized with low organic C and soil pH (i.e., acidic soil). Organic farming relies on organic inputs and fundamentally alters the sources and forms of reactive carbon (C) and nitrogen (N) inputs. It also induces changes in soil physical and geochemical properties that may profoundly affect the abundances, composition and activities of soil microbes, including nitrifiers (i.e., ammonia-oxidizing bacteria and archaea) and diverse types of denitrifiers that critically control plant N use efficiency and the environmental consequences (such as N leaching to water systems and greenhouse gas (N2O) emissions) of chemical N fertilizers and organic manures. However, there is still a lack of a holistic picture of organic farming effects on N-cycling microbes and their activities. We conducted multiple field and greenhouse experiments for this project, and we have found some very exciting results. These results have been or will be disseminated to communities of interest mainly through publications in peer-reviewed journals and presentations to scientific meetings, as well as to farmers and land managers through field demonstrations and field days. The knowledge generated from our research may also help us design new N management regimes under future climate change scenarios. Five papers have been published in peer-reviewed journals and three manuscripts have been submitted or are under review. Three more manuscripts are expected to be submitted in 2024. Major accomplishments can be summarized into four aspects below. Abiotic controls over N2O emissions: Our on-farm sampling study showed that N2O emissions are highly variable among the farms across different areas of North Carolina. In general, soil available N, organic C and soil pH appear to be the major abiotic factors controlling soil N2O emissions. Our global synthesis of N2O emissions found that soil pH critically affects the emission factor (i.e., the percentage of N input emitted as N2O). Soil pH has a nonlinear relationship with N2O emissions and highest N2O emissions occur in moderately acidic soils. Although soil mineral N, organic C and pH have been known to affect soil N2O emission, our finding of nonlinear relationship between soil pH and soil N2O emission is novel. This finding significantly advances our general understanding of abiotic controls over soil N2O emission, and has significant implications for N fertilization in managed ecosystems, as well as in designing liming practices in cropping systems on acidic soils. Biotic controls over soil N2O emissions: Our results from both field and greenhouse experiments showed that plant N uptake and microbial N immobilization are the major biotic factors controlling soil N2O emissions. In particular, we found that arbuscular mycorrhizal fungi significantly affected soil N2O emissions in both conventional and organic farming systems. They increased plant N uptake and suppressed N2O emissions mainly through altering the community composition of the denitrifying microbes that are responsible for denitrification. AM fungi have been believed to have little impact on plant N uptake in agroecosystems with high N input. However, our results demonstrated that AMF significantly increased plant N acquisition and reduced soil N2O emissions under both conventional and organic farming systems with high N input through altering the community composition of denitrifying microbes. These results suggest that the role of AMF in the N cycling in agroecosystems may have been under-

appreciated. Effects of organic farming and tillage on N2O emissions: Our results showed that tillage and organic input interactively affected soil N-cycling microbes and N2O emissions. N2O emissions in soils under the conventional tillage were significantly higher than in systems of organic with conventional tillage or conventional fertility with reduced tillage. Tillage and synthetic N input also produced the lowest MBN, suggesting that reduced N immobilization may be largely responsible for the high N2O emission in conventional tillage system. Our findings suggest that combination of organic fertilization and reduced tillage may offer an effective approach for reducing N2O emissions, while maintaining sufficient productivity for farmers\' profits. Effects of biochar on N cycling genes, cumulative emissions of N2O and CO2. While organic agroecosystems contained higher abundances of N cycling genes, cumulative emissions of N2O and CO2 were low compared to conventional agroecosystems. Biochar did not mitigate differences in the abundance of N-transforming microbes, but biochar tended to reduce the difference in the microbiomes between conventional and organic farming system soils and did slightly decrease N2O emissions across all agroecosystems. Moreover, despite the differential impacts of soil organic carbon (SOC) on N-cycling gene abundances, reductions in N2O emission were associated with increasing C content, higher soil pH, and a decreasing ratio of (nirK+nirS)/nosZ. These results advance our understanding of how biochar application may modify soil N2O emissions. **Publications** - Type: Journal Articles Status: Published Year Published: 2021 Citation: Qiu, Y., Guo, L., Xu, X., Zhang, L., Zhang, K., Chen, M., Zhao, Y., Burkey, K.O., Shew, H.D., Zobel, R.W. and Zhang, Y., 2021. Warming and elevated ozone induce tradeoffs between fine roots and mycorrhizal fungi and stimulate organic carbon decomposition. Science Advances, 7(28), p.eabe9256. - Type: Journal Articles Status: Published Year Published: 2022 Citation: Zhang X, Qiu Y, Gilliam FS, Gillespie CJ, Tu C, Reberg-Horton SC, Hu S. Arbuscular Mycorrhizae Shift Community Composition of N-Cycling Microbes and Suppress Soil N2O Emission. Environmental Science & Technology. 2022 Aug 30;56(18):13461-72. - Type: Journal Articles Status: Published Year Published: 2022 Citation: Bloszies, S.A., Reberg?Horton, S.C., Heitman, J.L., Woodley, A.L., Grossman, J.M. and Hu, S., 2022. Legume cover crop type and termination method effects on labile soil carbon and nitrogen and aggregation. Agronomy Journal, 114(3), pp.1817-1832. - Type: Journal Articles Status: Published Year Published: 2023 Citation: Li, H., Yu, G.H., Hao, L., Qiu, Y. and Hu, S., 2023. Mycorrhizae enhance reactive minerals but reduce mineral-associated carbon. Global Change Biology, 29(20), pp.5941-5954. - Type: Journal Articles Status: Published Year Published: 2023 Citation: Zhang, P., Zhang, W. and Hu, S., 2023. Fungivorous nematode Aphelenchus avenae and collembola Hypogastrura perplexa alleviate damping-off disease caused by Pythium ultimum in tomato. Plant and Soil, 482(1-2), pp.175-189. - Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Gillespie, C.J., A. Woodley, and S. Hu. 2023. Linking N-transforming microbes to N2O emissions in Southeastern US conventional and organic agroecosystems. 2023 Ecological Society of America Conference. Portland, OR. Abstract and poster presentation. - Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Woodley A.L., Franzluebbers A., Reberg-Horton, C., Hu S., Schroder-Moreno M. 2023. A 20-Year Retrospective of the Centre for Envrionmental Farming Systems large-scale field experiment in the Southeast USA. Long Term Experiments: Meeting future challenges. Association of Applied Biologists. Rothamstead Research, West Common, Harpenden, UK. June 2023. - Type: Journal Articles Status: Submitted Year Published: 2023 Citation: Bloszies, S., Reberg-Horton, C., Heitman, J., Woodley, A., Grossman, J., and Hu, S. 2023. Tillage differentially affects affect soil N2O emissions in conventional and organic farming systems. Soil Tillage Research. Under revision. - Type: Journal Articles Status: Submitted Year Published: 2023 Citation: Qiu, Y., Zhang, Y., Zhang, K., Xu, X., Zhao, Y., Bai, T., Zhao, Y., Wang, H., Sheng, X., Bloszies, B., Gillespie, C.J., He, T., Wang, Y., Chen, H., Guo, L., Song, H., Ye, C., Wang, Y., Woodley, A., Guo, J., Cheng, L., Bai, Y., Zhu, Y., Hallin, S., Firestone, M.K., Hu, S. 2023. Intermediate soil acidification induces highest nitrous oxide emissions. Nature Communications. Under revision. - Type: Journal Articles Status: Submitted Year Published: 2023 Citation: Gillespie, C.J., Sit, T.L., Loucanides, A.J., Hu, J., Matusko, B.E., Terwilliger, N.R., Shi, W., Steffen, S.M., Woodley, A.L., Hu, S. 2023. Multi-Amplicon Nitrogen Cycling Gene Standard: An Innovative Approach for Absolute Quantification of N-transforming Soil Microbes in Terrestrial Ecosystems. Soil Biology and Biochemistry. Under internal review. **Progress** 09/01/21 to 08/31/22 **Outputs** Target Audience:Students: One PhD student received training from this project to conduct field, greenhouse and lab experiments to assessing the effects of different organic management practices on soil C and N dynamics, soil microbial community, N-cycling microbes and greenhouse gas emissions. One postdoctoral scholar has been working on the project. Four undergraduate summer students received training on greenhouse gas static chamber sampling and soil analyses. Also, a second postdoc received training in the automated chamber design and installation. The scientific community in general, particularly ecologists, soil scientists, and biologists and graduate and undergraduate students. We published three papers in scientific journals that reach to the scientific community in general. Farmers and land managers. The field experiment was presented the nitrous oxide CEFS study at the North Carolina State University Organic Field Day, August 2022. Work from this project has also been presented at a researcher and farmer focused Webinar titled \"Farming for Ecological Outcomes\" Changes/Problems:Because of the COVID panermic, the project did not progress as planned in 2020 and 2021. Therefore, we have to extend our field sampling and greenhouse experiments through summer, 2023.

However, we will not change our goals and approaches. What opportunities for training and professional development has the project provided? Two postdoctoral scholars, one technician, one PhD student, three undergraduate students received training through this project. Tom Stanton (a research technician) and Scott Holden set up the field gas emission experiment and have been working on this project since. Three undergraduate students received training in soil trace gas collections. In particular, Miss Brooke Matusko (NC State) received training as an ASPIRE intern and Miss Ayanna Hatton (Bowdoin College) received training as a Kelman Scholar, and their projects were supported by this project. The postdoctoral scholar who is financially supported by this project (Dr. Sean Bloszies) has been responsible for field sampling and analyses both in the long-term farming systems in Goldsboro, NC, and farms across the region. How have the results been disseminated to communities of interest? Our field site at CEFS has hosted a Center for Environmental Farm Systems farm tour which included an information session on this innovative design (\~30ppl), the purpose of the tour was for interested faculty at NCSU not familiar with CEFS to come and potentially find collaborators for future research at CEFS. In addition, this site has hosted interested researchers from the Nature Conservancy and the Environmental Defense Fund. Also, our field projects and results were introduced to students of a class entitled Ecology of Soil Ecosystems in Spring, 2020, again in the Spring, 2022. In addition, the field experiment was presented the nitrous oxide CEFS study at the North Carolina State University Organic Field Day, August 2022. Work from this project was also presented at a researcher and farmer focused Webinar titled \"Farming for Ecological Outcomes\". Lastly, three papers about organic farming effects on soil microbes, C and N cycling, and N2O emissions have been published in peer-reviewed journals during this year. What do you plan to do during the next reporting period to accomplish the goals? Research Program: We will continue to carry out the multiple experiments initiated recently to address our three long-term goals in understanding the effects of organic systems and tillage on soil C and N dynamics, N-cycling microbes, and greenhouse gas emissions. Objective 1. Characterize the impact of organic farming on N-transforming microbes. Our aim is to obtain the essential data of the N-cycling microbes so that the relationships between the functions (nitrification and denitrification potentials, N2O emissions) and the abundances and community composition of N-cycling microbes can be explored. Because of the COVID-19 cancellations in 2020, we had to extend our final sampling in field to September 2021 and conduct analyses of soil physical, chemical, and microbial parameters, including soil properties (The texture, pH and moisture; Total soil C and N; Microbial biomass C and N, Extractable N and C), abundances of N-cycling microbes and their activities: Realtime PCR quantification of the amoA (AOA), amoA (AOB), nirS, nirK and nosZ genes, Nitrification potential and Denitrification potential. We found there were high variations in some parameters and plan to sample soils from long-term conventional and organic farming systems at CEFS, Goldsboro in the fall, 2022. Objective 2. Identify the primary driving factors that modulate the effect of organic farming on N-cycling microbes. We will continue to conduct extensive analyses of soil physical, chemical and microbial parameters to examine the relationships between soil properties and N-cycling microbe for the samples collected through November, 2022. We will start to establish structural equation models (SEM) to identify the primary driving factors that control the abundance of N-cycling microbes and ratios of different denitrifier groups. Objective 3. Validate the relationships between N transformations and abundances, composition and activities of N-cycling microbes both in field and controlled environments. Our major task will be field assessment of relationships between communities of N-cycling microbes and N2O emissions. Because of the COVID pandemic and our PhD student has some chronic respiratory disease history, we had to postpone our greenhouse (mesocosm) experiments to March, 2022 to validate results obtained from the field experiments. Another mesocosm experiment will be conducted in March 2023. We had used and will continue to use stable isotope-labelled fertilizers to trace the movement of chemical N through quantifying plant N utilization, N2O emissions and N remained in soil. Objective 4. Educate the next generation of organic agriculture researchers and farmers through student training and an active, multi-pronged outreach program. Students training: Training of individuals directly funded by this project includes one postdoc, a PhD student and two undergraduate students in 2022-2023. Multiple other students will be also trained through this project, although they are supported by other funding sources. Outreach and provision of faculty-mentored research experiences for undergraduate students will also occur through the CEFS Sustainable Agriculture Internship Program. The PIs will direct two student mentored research projects in this coming year. Outreach program: Reaching Farmers: Co-PIs, Chris Reberg-Horton and Alex Woodley will actively participate in numerous extension-oriented activities, including Field Days at our field site (CEFS, Goldsboro). We planned to introduce our project to a broad audience (>500). We will continue to our field activities, including Spring field day at CEFS and summer field day at cooperating organic farms on soil differences and sampling methods. **Impacts** What was accomplished under these goals? Agricultural soils in the southeastern U.S., marked by high rainfall and warm temperatures, are often characterized with low organic C and soil pH (i.e., acidic soil). Organic farming relies on organic inputs and fundamentally alters the sources and forms of reactive carbon (C) and nitrogen (N) inputs. It also induces changes in soil physical and geochemical properties that may profoundly affect the abundances, composition and activities of soil microbes, including nitrifiers (i.e., ammonia-oxidizing bacteria and archaea) and diverse types of denitrifiers that critically control plant N use efficiency and the environmental consequences (such as N leaching

to water systems and greenhouse gas (N2O) emissions) of chemical N fertilizers and organic manures. However, there is still a lack of a holistic picture of organic farming effects on N-cycling microbes and their activities. We have multiple ongoing experiments for this project and we have found some very exciting results. These results have been or will be disseminated to communities of interest mainly through publications and presentations to scientific meetings, as well as to farmers and land managers through field demonstrations and field days. The knowledge generated from our research may also help us design new N management regimes under future climate change scenarios. 1. Field trace gas emissions. At the Center for Environmental Farming Systems (CEFS) in Goldsboro, North Carolina, a field study was established in a long-term rotational study in March of 2019. Four rotational treatments were selected including: 3yr organic (corn, soybean and sunflower), a 6-yr organic production/forage (corn, soybean, sunflower, hay, hay, hay), a 3yr conventional no-till (corn, double crop wheat/soybean, sorghum) and a 3 yr conventional till (corn, double crop wheat/soybean, sorghum). In 2019, all phases of the rotation are in corn. In April of 2019, a system for semi-continuous measurement of nitrous oxide (N2O) was established in 3 locations within each cropping rotation treatment. This continuous measurement is achieved by using LI-Cor 8100-104 Long-Term Soil Gas Flux Automatic Chamber System. Soil temperature and soil moisture content is also being collected at each chamber for further interpretation of the soil microbial results (see more details below). Field gas sampling were continuing in 2021-2022. 2. Effects of biochar and compost on N2O emissions and N-cycling microbes: In early 2020, we set up a long-term field experiment at CEFS that assesses the impact of biochar, chemical N fertilizer, and compost on N-cycling microbes and N2O emissions. The field continuous monitoring of N2O emissions has been ongoing since Spring 2020. We have been collecting soil samples once every three months for chemical and microbial analyses. 3. Soil carbon and nitrogen dynamics and N-cycling microbes in the long-term farming systems at CEFS and on farms of four organic farmers: Soil sampling: Soil samples were collected from 0-10 cm and 10-20 cm at five georeferenced sampling points at each sampling time. Soils were sampled twice in the fall at or after soybean maturity on October 20, 2020, November 10, 2020, June 7, 2021. Collaborators at RAFI provided a list of 12 organic farmers. Soil chemical and microbial analyses: All samples from on-farm and the research station have been processed for pH, microbial biomass C and N, potentially mineralizable C and N, K2SO4-extractable C and NO3- and NH4+, and incubation N2O emissions potential. In general, higher microbial biomass N and potentially mineralizable C was enhanced in organic relative to conventional research station plots. The quantification of N-cycling microbes is underway. 4. Mesocosm experiments examining the effects of organic and conventional farming soils on N2O emissions. We have conducted two experiments examining the effects of organic and conventional farming on soil microorganisms and N2O emissions. The first experiment was conducted with loamy soils from organic and conventional systems at CEFS. Our results showed that the presence of arbuscular mycorrhizal fungi (AMF) and roots critically reduced N2O emissions through affecting the composition of denitrifying microbes, which has been recently published in journal Environmental Science & Technology. The second experiment was conducted with a clayey soil from western North Carolina to examine how biochar affects soil N-cycling microbes and N2O emissions. We completed this experiment in the past summer and are in the process of analyzing the abundances and composition of the soil microbial community. **Publications** - Type: Journal Articles Status: Published Year Published: 2022 Citation: Bloszies SA, Reberg?Horton SC, Heitman JL, Woodley AL, Grossman JM, Hu S. Legume cover crop type and termination method effects on labile soil carbon and nitrogen and aggregation. Agronomy Journal. 2022 Apr 13. - Type: Journal Articles Status: Published Year Published: 2022 Citation: Zhang X, Qiu Y, Gilliam FS, Gillespie CJ, Tu C, Reberg-Horton SC, Hu S. Arbuscular Mycorrhizae Shift Community Composition of N-Cycling Microbes and Suppress Soil N2O Emission. Environmental Science & Technology. 2022 Aug 30;56(18):13461-72. - Type: Journal Articles Status: Accepted Year Published: 2022 Citation: Zhang P, Zhang W, Hu S. Fungivorous nematode Aphelenchus avenae and collembola Hypogastrura perplexa alleviate damping-off disease caused by Pythium ultimum in tomato. Plant and Soil. 2022 Sep 7:1-5. Abstract{.underline} (Zhang et al., 2022) Mycorrhizae are ubiquitous symbiotic associations between arbuscular mycorrhizal fungi (AMF) and terrestrial plants, in which AMF receive photosynthates from and acquire soil nutrients for their host plants. Plant uptake of soil nitrogen (N) reduces N substrate for microbial processes that generate nitrous oxide (N2O), a potent greenhouse gas. However, the underlying microbial mechanisms remain poorly understood, particularly in agroecosystems with high reactive N inputs. We examined how plant roots and AMF affect N2O emissions, N2O-producing (nirK and nirS) and N2O-consuming (nosZ) microbes under normal and high N inputs in conventional (CONV) and organically managed (OM) soils. Here, we show that high N input increased soil N2O emissions and the ratio of nirK to nirS microbes. Roots and AMF did not affect the (nirK + nirS)/nosZ ratio but significantly reduced N2O emissions and the nirK/nirS ratio. They reduced the nirK/nirS ratio by reducing nirK-Rhodobacterales but increasing nirS-Rhodocyclales in the CONV soil while decreasing nirK-Burkholderiales but increasing nirS-Rhizobiales in the OM soil. Our results indicate that plant roots and AMF reduced N2O emission directly by reducing soil N and indirectly through shifting the community composition of N2O-producing microbes in N-enriched agroecosystems, suggesting that harnessing the rhizosphere microbiome through agricultural management might offer additional potential for N2O emission mitigation. **Progress** 09/01/20 to 08/31/21 **Outputs** Target Audience:Students: One PhD student received training from this project

to conduct field and lab experiments to assessing the effects of different organic management practices on soil C and N dynamics and greenhouse gas emissions. One postdoctoral scholar has been working on the project. Four undergraduate summer students received training on greenhouse gas static chamber sampling and soil analyses. Also, a second postdoc received training in the automated chamber design and installation. The scientific community in general, particularly ecologists, soil scientists, and biologists and graduate and undergraduate students. We published papers in scientific journals that reach to the scientific community in general. Farmers and land managers. The field experiment was presented the nitrous oxide CEFS study at the North Carolina State University Organic Field Day August 2021 (75 people). Work from this project was also presented at a researcher and farmer focused Webinar titled \"Farming for Ecological Outcomes\" Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?Two postdoctoral scholars, one technician, one PhD student, four undergraduate students received training through this project. Tom Stanton (a research technician) and Scott Holden set up the field gas emission experiment and have been working on this project since. Three undergraduate students received trains The postdoctoral scholar who is financially supported by this project (Dr. Sean Bloszies) joined the team in November, 2019 and has been responsible for field sampling and analyses. 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Work from this project was also presented at a researcher and farmer focused Webinar titled \"Farming for Ecological Outcomes\" What do you plan to do during the next reporting period to accomplish the goals?Research Program: We will continue to carry out the multiple experiments initiated recently to address our three long-term goals in understanding the effects of organic systems and tillage on soil C and N dynamics, N-cycling microbes, and greenhouse gas emissions. Objective 1. Characterize the impact of organic farming on N-transforming microbes. Our aim is to obtain the essential data of the N-cycling microbes so that the relationships between the functions (nitrification and denitrification potentials, N2O emissions) and the abundances and community composition of N-cycling microbes can be explored. Twelves (12) organic farms and their adjacent conventional farms have been sampled through our collaboration with RAFI_USA and we plan to collect more samples from a few farms based on our results. Because of the COVID-19 cancellations in 2020, I had to extend our final sampling in field to September 2021 and conduct analyses of soil physical, chemical, and microbial parameters, including soil properties (The texture, pH and moisture; Total soil C and N; Microbial biomass C and N, Extractable N and C), abundances of N-cycling microbes and their activities: Real-time PCR quantification of the amoA (AOA), amoA (AOB), nirS, nirK and nosZ genes, Nitrification potential and Denitrification potential. Objective 2. Identify the primary driving factors that modulate the effect of organic farming on N-cycling microbes. 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Outreach program: Reaching Farmers: Co-PIs, Chris Reberg-Horton and Alex Woodley will actively participate in numerous extension-oriented activities, including Field Days at our field site (CEFS, Goldsboro). We planned to introduce our project to a broad audience (>500). We planned to have our on-farm meeting in this past spring, but it had to be canceled. We will continue to our field activities, including Spring field day at CEFS and summer field day at cooperating organic farms on soil differences and sampling methods. **Impacts** What was accomplished under these goals? Agricultural soils in the southeastern U.S., marked by high rainfall and warm

temperatures, are often characterized with low organic C and soil pH (i.e., acidic soil). Organic farming relies on organic inputs and fundamentally alters the sources and forms of reactive carbon (C) and nitrogen (N) inputs. It also induces changes in soil physical and geochemical properties that may profoundly affect the abundances, composition and activities of soil microbes, including nitrifiers (i.e., ammonia-oxidizing bacteria and archaea) and diverse types of denitrifiers that critically control plant N use efficiency and the environmental consequences (such as N leaching to water systems and greenhouse gas (N2O) emissions) of chemical N fertilizers and organic manures. However, there is still a lack of a holistic picture of organic farming effects on N-cycling microbes and their activities. We have multiple ongoing experiment for this project and we have found some very exciting results. These results have been or will be disseminated to communities of interest mainly through publications and presentations to scientific meetings, as well as to farmers and land managers through field demonstrations and field days. The knowledge generated from our research may also help us design new N management regimes under future climate change scenarios. Multiple field experiments are underway. Field trace gas emissions. At the Center for Environmental Farming Systems in Goldsboro, North Carolina, a field study was established in a long-term rotational study in March of 2019. Four rotational treatments were selected including: 3yr organic (corn, soybean and sunflower), a 6-yr organic production/forage (corn, soybean, sunflower, hay, hay, hay), a 3yr conventional no-till (corn, double crop wheat/soybean, sorghum) and a 3 yr conventional till (corn, double crop wheat/soybean, sorghum). In 2019, all phases of the rotation are in corn. In April of 2019, a system for semi-continuous measurement of nitrous oxide (N2O) was established in 3 locations within each cropping rotation treatment. This continuous measurement is achieved by using LI-Cor 8100-104 Long-Term Soil Gas Flux Automatic Chamber System. Soil temperature and soil moisture content is also being collected at each chamber for further interpretation of the soil microbial results (see more details below). Field gas sampling were continuing until March 2020 when the COVID-19 field travel was halted. In early 2020, we set up a long-term field experiment at CEFS that assesses the impact of biochar, chemical N fertilizer, and compost on N-cycling microbes and N2O emissions. The field continuous monitoring of N2O emissions have been ongoing since Spring 2020. We have recently collected soil samples for chemical and microbial analyses. Soil carbon and nitrogen dynamics and N-cycling microbes in the long-term farming systems at CEFS and on farms of four organic farmers: Soil sampling: Soil samples were collected from 0-10 cm and 10-20 cm at five georeferenced sampling points at each sampling time. Soils were sampled twice in the fall at or after soybean maturity on October 20, 2020, November 10, 2020, and June 7, 2021. Collaborators at RAFI provided a list of 12 organic farmers. All 12 were contacted by NCSU researchers and assisted with soil sampling on their farms, beginning with farms one and two on October 28, 2020 and ending with farm twelve on January 29, 2021. Soils were sampled from the 0-20 cm depth at five georeferenced points within each field on the farms. Soil chemical and microbial analyses: All samples from on-farm and the research station have been processed for pH, microbial biomass C and N, potentially mineralizable C and N, K2SO4-extractable C and NO3- and NH4+, and incubation N2O emissions potential. In addition, processing has begun for N cycling gene quantification, with four clades of nirK and two clades of nirS genes quantified for the June 7, 2021 soil using qPCR. Preliminary findings reveal a trend of higher N2O emissions potential among organic than conventional management soils in the research station samples, especially when complete denitrification to N2 is experimentally halted via acetylene. In addition, Fall 2020 emissions seem to have been elevated by the senescence of soybean root nodules, with roughly half the emissions of October 19 occurring on November 10, capturing when soybeans had passed from R7 to R8. Higher microbial biomass N was observed in organic soils than conventional at the June 2021 research station sampling, likely due to poultry litter applications to the 2021 sunflower crop. Under reduced tillage, potentially mineralizable C was enhanced in organic relative to conventional research station plots. Within the on-farm soil samples, dairies' soils were notable for their elevated potentially mineralizable C and N, and microbial biomass C and N. Neither comparisons of organic versus conventional nor high versus low yield historical yield seem to have driven noticeable differences in N2O emissions among on-farm samples. **Publications** - Type: Journal Articles Status: Published Year Published: 2021 Citation: Qiu, Y., Guo, L., Xu, X., Zhang, L., Zhang, K., Chen, M., Zhao, Y., Burkey, K.O., Shew, H.D., Zobel, R.W. and Zhang, Y., 2021. Warming and elevated ozone induce tradeoffs between fine roots and mycorrhizal fungi and stimulate organic carbon decomposition. Science Advances, 7(28), p.eabe9256. - Type: Journal Articles Status: Under Review Year Published: 2021 Citation: Xuelin Zhang , Yunpeng Qiu, Frank S. Gilliam , Cong Tu , S. Chris Reberg-Horton, Shuijin Hu. 2021. Arbuscular mycorrhizae shift community composition of N-cycling microbes and suppress soil N2O emissions. Environmental Science and Technology - Type: Journal Articles Status: Under Review Year Published: 2021 Citation: Sean A. Bloszies, S. Chris Reberg-Horton, Joshua L. Heitman, Alex L. Woodley, Julie M. Grossman, and Shuijin Hu. 2021. Tillage differentially affects soil N2O emissions in conventional and organic farming systems. Agriculture, Ecosystems & Environment

Obj. 1. Evaluate agronomic practices to improve BFT establishment in existing pastures. Plots (3 by 8 m) will be established at two pasture sites (both Westmoreland silt loams with 8-15 % slopes) differing in levels of soil phosphorus at the WVU Organic Research Farm. Half of the plots in the low-P site will be amended with cal-phos

to match levels of available P in the high-P site. Cal-phos will also be applied to half of the plots at the high-P site. Three CT-containing forage legumes (Bruce and Pardee BFT, and sainfoin cv. Delaney) will be established in August 2019 via strip tillage with a split-hood rototiller or by using a no-till drill planting into strips from which the sod layer has been excised. Each plot will receive BFT or sainfoin alone, plus within-row mixtures with oats cv. Reins at 25% of total seed count or density. Additional untilled plots will be mowed at 5 cm in Fall 2019 whenever sward height exceeds 10 cm, then frost seeded with Pardee BFT (without oats) in February 2020. Three replicates of each treatment combination (2 phosphorus fertility levels times 3 seeding methods times 3 legume cultivars) will be arranged in randomized complete blocks in each site (108 plots total). The experiment will be repeated in 2020 and 2021. Data will be collected on legume emergence rate, and density of legume seedlings and established plants in Fall 2019, botanical composition and productivity of pastures at beginning and end of each growing season thereafter, pest damage to legume seedlings, degree of light interception or shading in seeded rows and adjacent canopies, and diet selection of grazing animals. Condensed Tannin concentration will be assessed via near-infrared reflectance spectroscopy.Obj. 2. Compare efficacy of BFT pastures and supplemental feed in field-scale grazing trials to evaluate whether the use of BFT in the feeding program of sheep and goats can increase tolerance to gastrointestinal nematodes as well as replace or minimize grain supplementation needed for optimum growth. A trial at WVU will compare performance and GIN infection of lambs on BFT pastures with lambs on conventional pasture with or without supplemental protein. One-acre plots will be established as a randomized complete block design with three replicates of BFT and six replicates of orchardgrass/red clover pastures. Forage samples will be clipped at 2-week intervals, sorted as legumes, grasses and weeds, and dried at 70oC to estimate pasture composition, and forage consumption. Lambs born in April will be weaned at \~ 60 days and placed on pastures in mini-flocks of six lambs each. Lambs will be rotationally grazed for 90 days using a \"challenge\" grazing regime of 7 days on a paddock, followed by 21 days rest for pasture regrowth, before lambs return to that paddock. Lamb weight, fecal egg counts, FAMACHA© and Body Condition Index scores will be determined at 2-week intervals. The experiment will be repeated in 2021 and 2022. Data will be analyzed using GLM procedures in SAS and unpaired Student t-test as appropriate.Obj. 3. Determine the effect of feeding BFT processed as either hay, high- moisture hay or pellets on experimental and pasture gastrointestinal nematode infections in lambs.Infective larvae will be obtained from donor lambs experimentally infected with H. contortus. BFT dry hay and high-moisture sweet hay will be grown and harvested from a 12-acre hay field, managed organically, at the University of Rhode Island\'s Peckham Farm. Pellets will be produced from the BFT:grass dry hay. Twenty-four 3-month-old lambs born and housed indoors, under conditions excluding nematode infection, will be administered 5,000 infective H. contortus larvae. After 4 weeks, lambs will be stratified into dietary treatment groups, balanced for FEC, sex and weight. Lambs will be fed for 8 weeks with: 1) BFT:grass dry hay, 2) BFT:grass high-moisture sweet hay, or 3) alfalfa:grass hay. Fecal egg counts, packed cell volume, FAMACHA score and weight will be measured weekly. In Year 2, 32 lambs will be administered 5,000 infective H. contortus larvae and the infection allowed to mature for four weeks. Lambs will be fed for 8 weeks with: 1) BFT:grass dry hay, 2) BFT:grass dry hay pellet, 3) alfalfa:grass hay and 4) pelleted alfalfa:grass hay. Fecal egg counts, packed cell volume, FAMACHA score and weight will be measured weekly. In Year 3, 32 lambs will graze GIN-infected pastures for a minimum of 4 weeks. Sixteen lambs will be removed from pasture and maintained indoor and fed either BFT-pellet or Control-pellet at 2.5% of body weight for 8 weeks. The remaining 16 lambs will continue to graze the GIN pasture during the day for 8 weeks and will be provided free choice access to BFT-pellets or Control-pellets when brought indoors overnight. Fecal egg counts, packed cell volume, FAMACHA score and weight will be measured weekly. At the end of each experiment, lambs will be slaughtered and worm numbers, stage of development and fecundity will be determined. Rectal fecal samples will be collected and FEC will be determined. Fecal samples during YR3 will also be stained using a fluorescein-label PNA (peanut agglutinin) procedure to identify H. contortus eggs versus other GIN species that will be present on pasture. Data will be analyzed using GLM procedures in SAS and unpaired Student t-test.Obj. 4 . Demonstrate research results in on-farm trials with select farmers. On-farm grazing trials will compare the parasite load and weight gains of weaned lambs and goat kids grazing pastures with ≥ 25% BFT versus conventional pastures. Farmers will establish a 2-3 acre grazing paddock of BFT on each farm during 2019. Eight-week grazing trials will occur at the farmsduring the summer/fall of 2019 or 2020. Weaned lambs or goat kids will graze the BFT pasture or conventional pastures with low tannin legumes and grasses. At 2-week intervals, weanlings will be monitored for GIN infection. Weanlings will be weighed at the beginning and end of the 8-week trials.Obj. 5. Extend research results to organic and conventional sheep and goat producers throughout the Northeast via Extension fact sheets, workshops, pasture walks, field days, and maintenance of the project website. A minimum of12 regional FAMACHA© certification workshops will teach the FAMACHA© anemia scoring technique, parasite life cycles, dewormer options and methods, parasite monitoring techniques, and the principles of IPM, forage-based parasite control, dewormer resistance, and refugia maintenance. Each study farmer will host a field day to share their on-farm trial experience with the BFT pastures and the forage/parasite management practices. Additional field days will be hosted at the WVU Organic Research Farm and the URI farms for pasture walks and to demonstrate research results. Results will also be presented at a minimum of eight sheep, goat and organic

farming conferences and at statewide veterinary association annual meetings. Factsheets outlining results from our research studies will be developed. The primary website used to report results will be at: <https://web.uri.edu/sheepngoat/>. In addition, Cornell University has created a Small Ruminant Parasite Research website: <http://blogs.cornell.edu/smallruminantparasites/>. Obj. 6. Evaluate success of outreach activities through surveys of farmers and participants at workshops, as well as economic performance analyses of farmers participating in on-farm trials.Surveys will be developed and distributed to workshop participants and other appropriate audiences to evaluate the impact of the training workshops on farmers, as well as to collect economic benefit data for the project methods. Long-term follow-up of producers is planned to assist farmers in determining whether increased earnings have been realized. Participants in workshops and field days and on-farm studies will receive follow-up surveys to measure adoption rates. **Progress** 09/01/20 to 08/31/21 **Outputs** Target Audience:The Target Audiences are sheep and goat producers in the Northeast US. Grower trials are planned to demonstrated effectiveness of birdsfoot trefoil to suppress intestinal parasites, and parasite workshops and grower presentations will be held, including one focused on veterinarians. Field days and pasture walks focusing on organic management of sheep and goats and forage management to reduce parasite infection in sheep and goats will be held, as well as workshops on alternative forages with potential to assist in parasite management for participating researchers and farmers. Changes/Problems:At WVU, drought conditions in 2020 slowed plant growth in birdsfoot trefoil pastures, resulting in a shift to lower-than-planned stocking rates. In addition, the COVID-19 epidemic prevented us from staffing the project at expected levels, thus the planned fall-2020 planting at WVU was not done, and while forage samples were collected for condensed tannin analyses, these have not yet been processed. An additional set of laboratory experiments was added to assess allelopathy of pasture grasses against birdsfoot trefoil. Trays (30-50-cm) were planted with eight common pasture grass species (tall fescue, meadow fescue, timothy, orchard grass, blue grass, reed canary grass, perennial ryegrass, and smooth brome grass) and maintained in the greenhouse. Shoot tissues were harvested after 60 days and dried, and ground. Extracts were prepared by mixing 0, 1.875, 3.75, 7.5 and 15 g in 25 ml distilled water. Three ml of each extract was placed on filter papers in petri dishes, and 20 birdsfoot trefoil seeds were added to assess effects of germination. Germination of birdsfoot trefoil was inhibited at the highest concentration of extracts of all grasses. Kentucky bluegrass, orchard grass, smooth brome and timothy were significantly less inhibitory than the other grasses at lower concentrations. Additional work is planned to evaluate allelopathy of root extracts as well as shoot extracts. Changes in land use and COVID-19 also precluded work at University of Rhode Island (URI). A change of objectives was approved by USDA in July, 202 to change Objective 3. In lieu of forage trials, URI is evaluating the Condensed Tannin profiles of birdsfoot trefoil forage preserved via freezing versus drying, and will characterize in vitro anti-parasitic activity of these samples. Persistence of anti-parasitic activity in storage will also be assessed using laboratory assays of the effects of birdsfoot trefoil extracts on egg hatch, larval motility, exsheathment, and growth. Goats in the control flock in Jefferson County, NY became heavily infected with multiple parasites in 2021, exacerbated by limited nutrition and previous contamination of their pastures. Affected animals were dewormed but two goats died. Additional efforts to improve animal care and use will include instructions to growers on appropriate de-worming methods. What opportunities for training and professional development has the project provided?Training opportunities were limited by on-going COVID restrictions. An independent study course was offered for one undergraduate, and six undergraduate interns also participated in hands-on learning with the project in 2021. WVU hosted a virtual NE-SARE Farm Tour on July 29, 2021 with a short video describing the sheep project and an on-line interactive tour. How have the results been disseminated to communities of interest?Objective 5. Extend research results to organic and conventional sheep and goat producers throughout the Northeast via Extension fact sheets, workshops, pasture walks, field days, and maintenance of the project website. Parasite Mgmt./FAMACHA© section of Cornell Sheep Management Class, Ithaca, NY. Mar 30 through Apr 2, 2021. Lecturer (tatiana Stanton) and hands-on lab instructor (Janice Liotta). 8 hrs. 42 university animal science students completed all activities to be certified in FAMACHA. ZOOM Mini-Conference for \"Overcoming barriers to transitioning small ruminants to organic production: effects of feeding birdsfoot trefoil on parasite control, nutritional status and profitability\". Apr 26, 2021. 1 ½ hr. Advisory committee of 4 goat and sheep farmers enrolled in the planting and grazing study and 6 researchers, extensionists and graduate students. IPM/ FAMACHA© Training Workshop for Madison County CCE, Morrisville, NY. Aug 17, 2021. tatiana Stanton and Janice Liotta, instructors, 5 hrs. 10 farmers certified in FAMACHA. Pasture Walk at Peaceful By Nature Farm: Pasture reclamation & small ruminant grazing Norwich, NY, Aug 27, 2021. Karen Hoffman, Jack Salo, tatiana Stanton, presenters, 2 hr. \-\--experienced and prospective goat and sheep farmers. Ongoing on-farm grazing study was discussed and observed in depth. Northeast Organic Farmers Assoc.-NY Pasture Walk at Cross Island Farms Experiences establishing birdsfoot trefoil in goat and beef pastures using small tractor implements and hand tools. Co-hosted by CCE North Country Regional Ag Team, Wellesley Island, NY, Aug 30, 2021. David Belding, tatiana Stanton, Kitty O'Neil, presenters, 2 hr. 7 experienced and prospective goat and sheep farmers. Terminating on-farm grazing study was discussed and observed in depth. WVU hosted the annual Organic Farm Field Day on Aug. 28, 2021, with approximately 85 people in attendance. An on-line version was also provided (available at:

https://fieldday.wvu.edu/), with the website recording 26 visitors on that day, and dozens more since. URI continued to provide online trainings and maintain the project homepage and events page. https://web.uri.edu/sheepngoat/orgbft/ Objective 6. Evaluate success of outreach activities through surveys of farmers and participants at workshops, as well as economic performance analyses of farmers participating in on-farm trials, or other adoption of management recommendations. Farmers at the IPM/ FAMACHA© Training on Aug 17, 2021 returned pre and post quizzes and answered questions on their plans. Scores were 40.3 % for pre-quizzes and 61.2% for post quizzes, respectively. When asked about parasite management changes, five, four, two and one respondents, respectively, indicated that they planned to 1) make improvements in pasture and/or barnyard and kidding pasture management, 2) start or make improvements in multispecies (cattle or horses with small ruminants) grazing management, 3) explore the use of copper oxide wire particles as part of their barber pole worm control strategy or 4) explore herbal dewormer options. What do you plan to do during the next reporting period to accomplish the goals? WVU will determine stand density and overwintering survival in established pastures and will repeat grazing trials in high-tannin forage pastures. Small plots will assess allelopathy and competition from eight forage grass species. Results will be summarized in research and extension publications. Data from on-farm grazing demonstrations will be statistically analyzed at Cornell. Soil health parameters will be taken in Spring 2022 and compared to parameters prior to establishment of the trial pastures. A final Advisory Committee ZOOM conference will be organized for the four participating farms, researchers, and extension staff to network about results, conclusions, and future questions. We have already conducted additional in person IPM/ FAMACHA© Training workshops in September and October 2021 as well as presenting results on BFT and clover biomass establishment for different planting methods (traditional tillage, no till, spring harrow \"scratching\", frost seeding and mud seeding) during the Small Ruminant Livestock Section of the NYS Cornell Cooperative Extension Virtual In-Service Training in Nov 2021. More trainings are scheduled for 2022 and two of the farmers conducting the BFT establishment and grazing trials plan to present their experiences at producer meetings in 2022. We will also develop fact sheets on organic methods to establish legumes in pastures comparing pros and cons of complete pasture renovation versus less invasive methods of establishing different plant species. We are still waiting for some of the farmers participating in the on-farm grazing trials to complete their spreadsheets on labor costs for frost seeding and weed control in their BFT fields for 2021 before we can summarize economic performance. URI will complete the freeze-drying and sample preparation and will begin the analysis of in-vitro anti-parasitic activity for the 2020 BFT variety harvest in addition to tannin and isoflavone content. A third year of yield data on the variety plots will be collected No till seeding and other planting methods were disappointing in 2019 and 2020 because of very difficult planting conditions throughout New York. All farms plan to do some frost seeding in their established stands this spring to try to boost the percentage of BFT in the fields. No till seeding presented problems as far as accessibility of equipment to farmers and timing seeding when pasture stubble was low enough to allow for good seed contact. It was hard for new BFT stands to compete with established grasses in no till or lightly tilled pastures. We now have a list serv for our BFT demo farmers, researchers, student interns and educators. The list serv saw little traffic this summer. However, we will use it to organize a group ZOOM conference to discuss results for the 2020 and 2021 grazing seasons. **Impacts** What was accomplished under these goals? These experiments evaluate ways to improve establishment of birdsfoot trefoil pastures to prevent intestinal parasite infection in sheep. This may reduce economic losses for organic growers from reduced weight gains and loss of organic certification due to use of synthetic deworming agents. The methods may benefit conventional sheep growers by reducing need for deworming agents and slowing development of parasite resistance. Objective 1. Evaluate agronomic practices to improve BFT establishment in existing pastures. Trials established in 2019 at the WVU Organic Research Farm to evaluate the effect of soil fertility, cultivar, and tillage treatments on establishment of birdsfoot trefoil (BFT) in existing pastures were continued in 2021. Plots in a low-phosphorus pasture were unamended or amended with bone meal to supply 89 pounds P/acre. A high-P pasture was unamended, creating three phosphorus fertility levels. Four high-tannin forages (Delaney sanfoin, and Bull, Bruce and Norcen BFT) were planted using a Truax drill with either coulters or 3-inch sweeps to open sod in Sept. 2019. Additional plots were frost seeded in Feb. 2020, and again March 11, 2021. A new trial evaluated some of these treatments in a spring planting established on the low-phosphorus pasture. Five forages (Delaney and Shoshone sanfoin, and Bruce, Bull and Norcen BFT) were planted with the Truax drill using coulters versus 3-inch sweeps on April 28, 2021. Another trial evaluated the effect of seven forage grass species on BFT establishment. The grasses were established in 1.2 m2 plots in Fall 2020, and Norcen BFT was planted into the sod on April 28, 2021. Frost seeding was less successful in 2021 than in 2020, and the spring 2021 planting did not improve stands compared to the Fall 2019 planting. Sanfoin did not persist in any treatments in 2021. Norcen BFT planted with sweeps was the only treatment to persist through Fall 2021, but plant density did not reach the targeted 30 % of the pasture stand in any treatment. Significantly more BFT occurred in plots with bunch grasses (e.g., orchard grass, perennial ryegrass, timothy) than with rhizomatous grasses (e.g., smooth brome, tall fescue, reed canary grass, or Kentucky bluegrass). In summary, while BFT can be established into existing pastures if aided by sweeps, plant density was very low and did not reach the levels routinely achieved by planting into cultivated seed beds. Objective 2. Compare efficacy of

BFT pastures and supplemental feed in field-scale grazing trials \... Twelve pastures were established at the WVU Organic Research Farm in Fall 2019. Three 1.25-acre pastures each were planted with Norcen birdsfoot trefoil (BFT) or Bull BFT, and six with orchard grass and red clover (OG/RC). Grazing trials with four lambs per plot were conducted for 90 days, in June 2020 and again with six lambs per plot in 2021. Body weight, body condition index, FAMACHA scores and Fecal Egg Counts were conducted at 14-day intervals. Pasture forage was supplemented with corn and soybean in all plots at 0.75 % of body weight, but additional soybean was fed to lambs in half of the OG/RC plots to balance the protein content of BFT pastures (Total grain = 0.94 % body weight/day). Lambs with high FAMACHA scores (4 or greater) were treated with a synthetic dewormer (Cydectin) as needed. Wetter weather in 2021 produced much higher levels of infection than in 2020. Significantly more lambs on OG/RC required deworming than those on BFT (74 % versus 43 %). Lambs on BFT had higher weight gain per day (0.31 versus 0.22 lbs/day) and higher body condition index than lambs on OG/RC. BFT was 30-50 % of the pasture forage through the 2021 season, and due to abundant pasture growth, lambs grazed less than 15 % of the available forage. Objective 3. Determine the effect of BFT cultivars on gastrointestinal nematodes \.... In vitro testing of the anti-parasitic activity of 12 populations of BFT (Norcen, NB95-118, Langille, Witt, LTAN-17, Bruce, Pardee, MSP4068, BuUr-13, Leo, NB95-120, and Wellington) is underway at the University of Rhode Island. This randomized and replicated variety trial was established in the fall of 2019 with Leo as the check. Plots were harvested on June 29, 2020 and again on August 24, 2020. Two 0.5 kg subsamples were collected. One subsample from each plot was immediately frozen until freeze-drying. The other subsample was dried to constant moisture in a forced air cabinet at 40? to simulate haying. Frozen samples were shipped on dry ice to the Forage Testing Laboratory at the Univ. of Missouri for freeze drying. After samples were freeze-dried or air-dried, all samples were ground (Wiley Mill, 1mm screen). Extracts (organic solvent, inorganic and aqueous) are being prepared for testing for anthelmintic activity. Yield data were again collected in 2021 using the same methods as in 2020. June yields averaged 6500 kg/ha dry matter; August yields averaged 1800 kg/ha. Leo continues to perform in the middle of the pack; over the two years combined only NB95-118 significantly out-yielded Leo at P = 0.05. However, Norcen, Pardee and Wellington had numerically greater yields than Leo. Objective 4. Demonstrate research results in on-farm trials with select farmers. Four farms participating in field grazing trials with Cornell Univ. started grazing studies in July 2021, comparing a control pasture with a pasture planted with BFT. Forage samples at a farm in Oneida County, NY averaged 14, 4, 9% clover on 7/23, 8/06 and 8/21/21, respectively; and 94, 99, 98 and 88% BFT on 7/23, 8/06, 8/21, 9/04/21, respectively. The BFT pasture was replanted in 2020 but because of drought conditions, numerous bald spots were frost seeded with more BFT on 3/30/21 and had a lot of Canadian thistle so only areas where the BFT emerged were sampled. A farm in Cortland County no-till seeded half of their study field in Manitoba red clover and Rivendell white clover, and the other half was seeded in Bull BFT. On 5/29/21, the clover half averaged 9% clover, 4% BFT, 1511 lb. DM/acre and the BFT half averaged 13% BFT, 6.2% clover, 1834 lb. DM/acre. An area within the clover border appeared to be primarily BFT possibly due to either drift from the March 2021 frost seeding or preferential survival of volunteer BFT over clover during the 2020 drought along this ridge. Therefore, the clover paddocks sometimes contained more BFT than the BFT paddocks. A farm in Jefferson County frost seeded additional BFT on 3/21/21. During the grazing study, the BFT paddocks averaged 57, 42, 33, 33% BFT on 7/04, 7/18, 8/01 and 8/15/21 respectively. The quantity and quality of nutrition in the control pastures was lower than that of the BFT pastures throughout the study. Control kids were supplemented with ¼ lb. organic alfalfa pellets per day. Forage samples at a farm in Chenango County in the BFT paddocks ranged from 12-54 % during the grazing study. In the grazing studies, all lambs and kids were weighed at the beginning and end of each 8-week trial. Fecal samples and FAMACHA scores were taken every two weeks. Total strongyle and strongyloides worm eggs per gram (epg) for each fecal sample were determined. A lectin binding stain using a peanut agglutinin (PNA) was used to determine the percentage of Haemonchus contortus eggs among the Strongyle epg in the 1st (Day 0), 3rd (\~Day 28), and 5th (\~Day 56) fecal sample from each animal. Forage samples from the BFT and control pastures, taken every two weeks during the grazing studies, were divided into BFT, non-BFT legume, broadleaves and grasses/sedges prior to drying. Data analysis is on-going. Objective 5. Extend research results \...See description in Box \# 3. Objective 6. Evaluate success of outreach\...See description in Box \# 3. **Publications** - Type: Conference Papers and Presentations Status: Accepted Year Published: 2021 Citation: Blake, N. and J. Kotcon. 2021. Forage grass allelopathy in birdsfoot trefoil pasture establishment. Ecological Society of America Virtual Annual Meeting. Held on-line. Aug. 2-6, 2021. Available at: https://www.eventscribe.net/2021/ESA/fsPopup.asp?Mode=posterinfo&PosterID=407007 - Type: Conference Papers and Presentations Status: Accepted Year Published: 2021 Citation: Blake, N., and Kotcon, J. 2021. Forage Grasses and the Impact on Birdsfoot Trefoil. Ohio Ecological Food and Farming Association Annual Meeting. On-Line. Feb. 11-14, 2020. - Type: Websites Status: Published Year Published: 2019 Citation: \" Webpage listing the resources used in the IPM/FAMACHA workshops conducted through the project - http://blogs.cornell.edu/smallruminantparasites/resources-for-educators/

PROGRESS

2018/09 TO 2019/08 Target Audience: Students: Four graduate students received training from this project to conduct field and lab experiments to assessing the effects of different organic management practices on soil C and N dynamics and greenhouse gas emissions. Two PhD students will join the project in this coming August (2019) (one will be supported by this project). One postdoctoral scholar joined the project on April 1, 2019. Two visiting scientists were also involved and got trained through this project, although they did not receive financial supports from this project. The scientific community in general, particularly ecologists, soil scientists, and biologists and graduate and undergraduate students. Farmers and land managers. The field experiment was presented to farmers and land managers on Field Days. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? We have quickly assembled a strong team for the project and get our current technicians, postdoctoral or visiting scholars, and undergraduate and graduate students. Tom Stanton (a Research Technician and Scott Holden (an undergraduate) joined our team in September 2018 to set up the field gas emission experiment. Three undergraduate students, Once we received the official award notice, we started recruiting the postdoctoral scholar and the PhD student requested in our project. We recruited Dr. Arjun Pandey for the postdoc position and he has officially started his work here at NC State since April 1, 2019. We also recruited Mr. Christopher Gillespie (a student from an underrepresented group) from Oklahoma State University. How have the results been disseminated to communities of interest? Our field site at CEFS has hosted a Center for Environmental Farm Systems farm tour which included an information session on this innovative design (\~30ppl), the purpose of the tour was for interested faculty at NCSU not familiar with CEFS to come and potentially find collaborators for future research at CEFS. In addition, this site has hosted interested researchers from the Nature Conservancy and the Environmental Defense Fund. Lastly, this site was presented during a field trip by an undergraduate class focused on agroecology. Co-PL, Alex Woodley, will present our field N2O emission monitoring system in the ASA, CSSA and SSSA annual meeting in this coming fall. In addition, we had a very fruitful meeting with our collaborators at Rural Advancement Foundation International (RAFI)) on May 7, 2019 to identify 12 farms that span the three major soil types (sandy, loamy and clayey) in the region. We have decided basic criteria for farm selections and designed a concrete scheme for soil sampling on farm and planned to have our first on-farm meeting in October, 2019. What do you plan to do during the next reporting period to accomplish the goals? Research Program: We will continue to carry out the multiple experiments initiated recently to address our three long-term goals in understanding the effects of organic systems and tillage on soil C and N dynamics, N-cycling microbes, and greenhouse gas emissions. Objective 1. Characterize the impact of organic farming on N-transforming microbes. Our aim is to obtain the essential data of the N-cycling microbes so that the relationships between the functions (nitrification and denitrification potentials, N2O emissions) and the abundances and community composition of N-cycling microbes can be explored. Twelves (12) organic farms and their adjacent conventional farms will be selected through our collaboration with RAFI USA and sampled in April and/or July and October for two years (starting in July 2019). We will conduct analyses of soil physical, chemical, and microbial parameters, including Soil properties (The texture, pH and moisture; Total soil C and N; Microbial biomass C and N, Extractable N and C), Abundances of N-cycling microbes and their activities: Real-time PCR quantification of the amoA (AOA), amoA (AOB), nirS, nirK and nosZ genes, Nitrification potential and Denitrification potential. Objective 2. Identify the primary driving factors that modulate the effect of organic farming on N-cycling microbes. We will continue to conduct extensive analyses of soil physical, chemical and microbial parameters to examine the relationships between soil properties and N-cycling microbe for the samples collected in October 2020. We will start to establish Structural equation models to identify the primary driving factors that control the abundance of N-cycling microbes and ratios of different denitrifier groups. Objective 3. Validate the relationships between N transformations and abundances, composition and activities of N-cycling microbes both in field and controlled environments. Our major task will be Field assessment of relationships between communities of N-cycling microbes and N2O emissions Objective 4. Educate the next generation of organic agriculture researchers and farmers through student training and an active, multi-pronged outreach program. Students training: Training of individuals directly funded by this project will include a postdoc, a PhD student and one undergraduate student (one per year). Multiple other students will be also trained through this project, although they are supported by other funding sources. Outreach and provision of faculty-mentored research experiences for undergraduate students will also occur through the CEFS Sustainable Agriculture Internship Program. The PIs will direct 2-3 student mentored research projects in this coming year. Lastly, the PI (Shuijin Hu) will teach Soil Ecology in Spring, 2020. Research progress and results from this project will be presented to the students in the class. Outreach program: Reaching Farmers: Co-PIs, Chris Reberg-Horton and Alex Woodley will actively participate in numerous extension-oriented activities, including Field Days at our field site (CEFS, Goldsboro). We will introduce our project to a broad audience (\>500). We planned to have our first on-farm meeting in October or November, 2019. We will continue to our first-year activities, including Spring field day at CEFS and summer field day at cooperating organic farms on soil differences and sampling methods.

2019/09 TO 2020/08 Target Audience: Students: Two graduate students received training from this project to conduct field and lab experiments to assessing the effects of different organic management practices on soil C and N dynamics and greenhouse gas emissions. One PhD student (Mr. Christopher Gillespie) joined the project in this August 2019 and has been supported by this project. One postdoctoral scholar (Dr. Arjun Pandey) from Australia joined the project on April 1, 2019 but had to resign in September, 2020 because of a family emergency. A new postdoc (Dr. Sean Bloszies) was then hired in November, 2019. The scientific community in general, particularly ecologists, soil scientists, and biologists and graduate and undergraduate students. Farmers and land managers. The field experiment was presented to farmers and land managers on Field Days. Changes/Problems:We have to postpone some field sampling and lab analyses of soil samples because of COVID-19. We have some serious concerns about the negative impact of the pandemic on our project, but we will try our best to approach all of our goals listed in the grant proposal. What opportunities for training and professional development has the project provided?We have quickly assembled a strong team for the project and get our current technicians, postdoctoral or visiting scholars, and undergraduate and graduate students. Tom Stanton (a Research Technician and Scott Holden (an undergraduate) joined our team in September 2018 to set up the field gas emission experiment and has been working on this project since. Three undergraduate students, Once we received the official award notice, we started recruiting the postdoctoral scholar and the PhD student requested in our project. We recruited Dr. Arjun Pandey for the postdoc position and he has officially started his work here at NC State since April 1, 2019. PhD student (had to resign in October because of a family emergency). Mr. Christopher Gillespie (a student from an underrepresented group) joined the team in August, 2019. Dr. Sean Bloszies joined the team in November, 2019. We recruited two summer interns (undergraduate students) for 2020, but the COVID-19 forced us to cancel the program. How have the results been disseminated to communities of interest?Our field site at CEFS has hosted a Center for Environmental Farm Systems farm tour which included an information session on this innovative design (\~30ppl), the purpose of the tour was for interested faculty at NCSU not familiar with CEFS to come and potentially find collaborators for future research at CEFS. In addition, this site has hosted interested researchers from the Nature Conservancy and the Environmental Defense Fund. In addition, our field projects and results were introduced to students of a class entitled Ecology of Soil Ecosystems in Spring, 2020. Lastly, this site was presented during a field trip by an undergraduate class focused on agroecology. Co-PL, Alex Woodley, will present our field N2O emission monitoring system in the ASA, CSSA and SSSA annual meeting in this coming fall. We had another fruitful meeting with our collaborators at Rural Advancement Foundation International (RAFI)) in November, 2019 to reconfirm the sampling plan for the on-farm research and details for sample analyses. We initially identified 12 farms that span the three major soil types (sandy, loamy and clayey) in the region, but we had to reduce the field sampling to four farms because of COVID-19 travel and lab attendance limitations. What do you plan to do during the next reporting period to accomplish the goals? Research Program: We will continue to carry out the multiple experiments initiated recently to address our three long-term goals in understanding the effects of organic systems and tillage on soil C and N dynamics, N-cycling microbes, and greenhouse gas emissions. Objective 1. Characterize the impact of organic farming on N-transforming microbes. Our aim is to obtain the essential data of the N-cycling microbes so that the relationships between the functions (nitrification and denitrification potentials, N2O emissions) and the abundances and community composition of N-cycling microbes can be explored. Twelves (12) organic farms and their adjacent conventional farms have been selected through our collaboration with RAFI USA and we plan to sample from all farms once the pandemic limitations are over. Because of the COVID-19 cancellations in 2020, I plan to extend our final sampling in field to September 2021 and conduct analyses of soil physical, chemical, and microbial parameters, including soil properties (The texture, pH and moisture; Total soil C and N; Microbial biomass C and N, Extractable N and C), abundances of N-cycling microbes and their activities: Real-time PCR quantification of the amoA (AOA), amoA (AOB), nirS, nirK and nosZ genes, Nitrification potential and Denitrification potential. Objective 2. Identify the primary driving factors that modulate the effect of organic farming on N-cycling microbes. We will continue to conduct extensive analyses of soil physical, chemical and microbial parameters to examine the relationships between soil properties and N-cycling microbe for the samples collected through Spring, 2021. We will start to establish Structural equation models to identify the primary driving factors that control the abundance of N-cycling microbes and ratios of different denitrifier groups. Objective 3. Validate the relationships between N transformations and abundances, composition and activities of N-cycling microbes both in field and controlled environments. Our major task will be field assessment of relationships between communities of N-cycling microbes and N2O emissions. We plan to start our greenhouse (microcosm) experiments in April, 2021 to validate results obtained from the field experiments. We will use stable isotope-labelled fertilizers to trace the movement of chemical N through quantifying plant N utilization, N2O emissions and N remained in soil. Objective 4. Educate the next generation of organic agriculture researchers and farmers through student training and an active, multi-pronged outreach program. Students training: Training of individuals directly funded by this project includes a postdoc, a PhD student and one undergraduate student (one per year). Multiple other students will be also trained through this project, although they are supported by other funding sources. We are in the process recruiting two summer

interns (undergraduate students) for the summer 2021. Outreach and provision of faculty-mentored research experiences for undergraduate students will also occur through the CEFS Sustainable Agriculture Internship Program. The PIs will direct 2-3 student mentored research projects in this coming year. Outreach program: Reaching Farmers: Co-PIs, Chris Reberg-Horton and Alex Woodley will actively participate in numerous extension-oriented activities, including Field Days at our field site (CEFS, Goldsboro). We planned to introduce our project to a broad audience (\>500). We planned to have our on-farm meeting in this past spring, but it had to be canceled. We will continue to our field activities, including Spring field day at CEFS and summer field day at cooperating organic farms on soil differences and sampling methods.

2019/09 TO 2020/08 Target Audience:The Target Audiences are sheep and goat producers in the Northeast US. Grower trials are planned to demonstrated effectiveness of birdsfoot trefoil to suppress intestinal parasites, and parasite workshops and grower presentations will be held, including one focused on veterinarians. Field days and pasture walks focusing on organic management of sheep and goats and forage management to reduce parasite infection in sheep and goats will be held, as well as workshops on alternative forages with potential to assist in parasite management for participating researchers and farmers. Changes/Problems:At WVU, drought conditions slowed plant growth in birdsfoot trefoil pastures, resulting in a shift to lower-than-planned stocking rates. In addition, the COVID-19 epidemic prevented us from staffing the project at expected levels, thus the planned fall-2020 planting at WVU was not done, and while forage samples were collected for condensed tannin analyses, these have not yet been processed. An additional set of laboratory experiments was added to assess allelopathy of pasture grasses against birdsfoot trefoil. Trays (30-50-cm) were planted with eight common pasture grass species (tall fescue, meadow fescue, timothy, orchard grass, blue grass, reed canary grass, perennial ryegrass, and smooth brome grass) and maintained in the greenhouse. Shoot tissues were harvested after 60 days and dried, and ground. Extracts were prepared by mixing 0, 1.875, 3.75, 7.5 and 15 g in 25 ml distilled water. Three ml of each extract was placed on filter papers in petri dishes, and 20 birdsfoot trefoil seeds were added to assess effects of germination. Germination of birdsfoot trefoil as inhibited at the highest concentration of extracts of all grasses. Kentucky bluegrass, orchard grass, smooth brome and timothy were significantly less inhibitory than the other grasses at lower concentrations. Additional work is planned to evaluate allelopathy of root extracts as well as shoot extracts. Changes in land use and COVID-19 also precluded work at University of Rhode Island (URI). A change of objectives was approved by USDA in July to change Objective 3. In lieu of forage trials, URI will evaluate the Condensed Tannin profiles of birdsfoot trefoil forage preserved via freezing versus drying, and will characterize in vitro anti-parasitic activity of these samples. Persistence of anti-parasitic activity in storage will also be assessed using laboratory assays of the effects of birdsfoot trefoil extracts on egg hatch, larval motility, exsheathment, and growth. Severe drought at URI impacted BFT harvest yields. Poor planting conditions (extreme wet) delayed spring planting for New York growers in 2019 at two of the four farms and caused a failed planting at a third farm. At the fourth farm, the massive spring vegetative growth was still too thick in the fall despite grazing with lambs for the no till drill to penetrate the planned pasture. Instead, we had to do a fall no-till seeding into a different set of pastures that had been grazed more aggressively by the milking ewe flock. Because of weather, this fall seeding was also delayed a month past our planned seeding date and we will have to wait to see how successful it was as the BFT was seeded later than normally considered optimal. What opportunities for training and professional development has the project provided?Two graduate students (one each at WVU and URI) developed skills in sheep management, parasite monitoring, and research methods. Six undergraduate students were employed to assist with the sheep research and became skilled at a variety of field and laboratory techniques. In addition, the project provided training to five undergraduate interns at WVU and served as demonstrations/field trips for several undergraduate classes in Agriculture. How have the results been disseminated to communities of interest?A number of grower workshops were held to disseminate information about birdsfoot trefoil specifically, and sheep parasite management generally, were held during this reporting period. IPM/ FAMACHA© Training Workshop for Saratoga County CCE, Ballston Springs, NY. Sept 24, 2019. 5 hrs. Ten farmers and a CCE educator were certified in FAMACHA. Also met with a local veterinarian for 1 hr. to discuss parasite management techniques and selective deworming which this veterinarian had previously found to be a frustrating topic. Small Ruminant Parasites. Sponsored by Cornell Univ. Chapter of Amer. Assoc. of Sm. Ruminant Practitioner. Cornell Univ., Ithaca, NY, Oct 31, 2019. tatiana Stanton presented for 1 hr. to 37 Cornell veterinary students with a small ruminant interest. Sheep & Goat Health & Parasite Management Workshop. Waterloo, NY. Nov 20, 2019. Tatiana Stanton: co-instructor, 4 hr. twelve Amish goat & sheep farmers. Two hours of internal parasite instruction and 2 hours of field necropsies by state veterinarian. Innovations in Parasite Management Workshop at the 2019 Small Ruminant Management & Fiber Conference, Cornell Univ., Ithaca, NY, Nov 9, 2029. tatiana Stanton presented for 1 hr. 40 min. to 46 goat and sheep raisers and educators from throughout the US., ONT Province and Italy. \"Grazing Methods for Internal Parasite Control in Small Ruminants\" at NOFA-NY Winter Conference, Jan 18, 2020. 1.25 hr. 35 goat and sheep growers. Workshops and field days later in the year were canceled because of the COVID-19 pandemic. Instead, farmers and educators seeking IPM/ FAMACHA© Training were directed to the URI online training program at

https://web.uri.edu/sheepngoat/famacha/ . URI also assisted with the maintenance of the project webpage (https://web.uri.edu/sheepngoat/orgbft/) and events page. What do you plan to do during the next reporting period to accomplish the goals?WVU will determine stand density and overwintering survival in established pastures, and will repeat grazing trials in high-tannin forage pastures. Small plots will assess allelopathy and competition from eight forage grass species. URI will complete the freeze-drying and sample preparation and will begin the analysis of in-vitro anti-parasitic activity for the 2020 BFT variety harvest in addition to tannin and isoflavone content. During the summer of 2021 BFT varieties will again be harvested, prepared and analyzed for production characteristics as well as content of tannins and other isoflavones in addition to in vitro anti-parasitic activity. In New York, one farm will replant their birdsfoot trefoil (BFT) in spring 2020. Two other farms will also plant BFT in 2020. The farm that planted BFT in Fall 2019 plans to have their lamb grazing trial in summer/fall 2020. The other 3 grazing trials will be postponed until 2021. Several parasite and pasture management workshops are already scheduled for Winter 2020 including one presentation on \"Advanced grazing for parasite control\" at the NOFA-NY Winter Conference Jan 17-19. Cornell also has several parasite and pasture management workshops scheduled for fall 2019 and winter 2020. We hope to resume our in-person parasite and pasture management trainings as well as conduct a ZOOM conference for the BFT demo farmers, students and researchers. No till seeding and other planting methods were disappointing in 2019 and 2020 because of very difficult planting conditions throughout New York. All farms plan to do some frost seeding in their established stands this spring to try to boost the percentage of BFT in the fields. No till seeding presented problems as far as accessibility of equipment to farmers and timing seeding when pasture stubble was low enough to allow for good seed contact. It was hard for new BFT stands to compete with established grasses in no till or lightly tilled pastures. We now have a list serv for our BFT demo farmers, researchers, student interns and educators. The list serv saw little traffic this summer. However, we will use it to organize a group ZOOM conference to discuss results for the 2020 grazing season and plans for 2021.

IMPACT

2018/09 TO 2019/08 What was accomplished under these goals? In the first nine months of the project, we have assembled a strong team for the project, prepared our field plots, adjusted the crop rotation and have initiated multiple experiments, and conducted some outreach activities. In fall 2018, we made adjustments in winter crops with different types of cover crops/mixes depending on the treatment at the Center for Environmental Farming Systems (CEFS), Goldsboro, NC: all conventional treatments with wheat, both the organic clean-till and the organic reduce-till with clover and rye (We no longer use vetch in the organic cover crop mixture due to the difficulty to kill it in the organic reduce-till system). In the spring of 2019, we started sampling in these systems. Multiple field experiments are underway. Field trace gas emissions. At the Center for Environmental Farming Systems in Goldsboro, North Carolina, a field study was established in a long-term rotational study in March of 2019. Four rotational treatments were selected including: 3yr organic (corn, soybean and sunflower), a 6-yr organic production/forage (corn, soybean, sunflower, hay, hay, hay), a 3yr conventional no-till (corn, double crop wheat/soybean, sorghum) and a 3 yr conventional till (corn, double crop wheat/soybean, sorghum). In 2019, all phases of the rotation are in corn. In April of 2019, a system for semi-continuous measurement of nitrous oxide (N2O) was established in 3 locations within each cropping rotation treatment. This continuous measurement is achieved by using LI-Cor 8100-104 Long-Term Soil Gas Flux Automatic Chamber System (Figure 2a). These automated chambers are installed inter-row between the corn in representative areas in the field all equidistant (\~60m) to the trailer (Fig. 2b), they are placed on partially buried collars in the soil. At all times air is being simultaneously pulled (sample air) and pushed (return air) from pumps within a centrally located trailer at 2.8 LPM through two ports in the chamber lid. When a sample is taken the lid moves over the buried collar and each chamber is measured for 25 min, with a daily frequency of every 150 min, which allows for a high temporal resolution collection of seasonal N2O fluxes. A system of solenoids in the trailer divert the chosen chambers air to be run through a Teledyne 320U N2O analyzer for N2O analysis and a LiCor 840A for CO2 analysis. In addition, N2O is analyzed from the same sites using the static chamber method, which is more commonly used in N2O studies. Ancillary data being collected at a regular interval to supplement the N2O measurements include, repeated soil water nitrate collection at depths of 15.2, 30.4 and 60.9 cm by applying a vacuum to a porous cup soil solution access tube. This will allow for correlation between concentration of nitrate at varying depths with microbial community activity and nitrous oxide flux events. Soil temperature and soil moisture content is also being collected at each chamber for further interpretation of the soil microbial results (see more details below). Soil carbon and nitrogen dynamics: We collected soil samples right before planting in late April (and will continue the sampling quarterly) to monitor the soil C, N and microbial dynamics. An array of soil physical, chemical and microbial parameters are being analyzed. N-cycling microbes: Soil samples collected in April and May have been analyzed to determine the abundances of N-cycling microbes. Soil DNA have been extracted and partially

analyzed to determine the abundances of nitrifiers and denitrifiers through quantifying their functional genes. **PUBLICATIONS (not previously reported):** 2018/09 TO 2019/08 No publications reported this period.

2019/09 TO 2020/08 What was accomplished under these goals? Agricultural soils in the southeastern U.S., marked by high rainfall and warm temperatures, are often characterized with low organic C and soil pH (i.e., acidic soil). Organic farming relies on organic inputs and fundamentally alters the sources and forms of reactive carbon (C) and nitrogen (N) inputs. It also induces changes in soil physical and geochemical properties that may profoundly affect the abundances, composition and activities of soil microbes, including nitrifiers (i.e., ammonia-oxidizing bacteria and archaea) and diverse types of denitrifiers that critically control plant N use efficiency and the environmental consequences (such as N leaching to water systems and greenhouse gas (N2O) emissions) of chemical N fertilizers and organic manures. However, there is still a lack of a holistic picture of organic farming effects on N-cycling microbes and their activities. We have multiple ongoing experiment for this project and we have found some very exciting results. These results have been or will be disseminated to communities of interest mainly through publications and presentations to scientific meetings, as well as to farmers and land managers through field demonstrations and field days. The knowledge generated from our research may also help us design new N management regimes under future climate change scenarios. Multiple field experiments are underway. Field trace gas emissions. At the Center for Environmental Farming Systems in Goldsboro, North Carolina, a field study was established in a long-term rotational study in March of 2019. Four rotational treatments were selected including: 3yr organic (corn, soybean and sunflower), a 6-yr organic production/forage (corn, soybean, sunflower, hay, hay, hay), a 3yr conventional no-till (corn, double crop wheat/soybean, sorghum) and a 3 yr conventional till (corn, double crop wheat/soybean, sorghum). In 2019, all phases of the rotation are in corn. In April of 2019, a system for semi-continuous measurement of nitrous oxide (N2O) was established in 3 locations within each cropping rotation treatment. This continuous measurement is achieved by using LI-Cor 8100-104 Long-Term Soil Gas Flux Automatic Chamber System. Soil temperature and soil moisture content is also being collected at each chamber for further interpretation of the soil microbial results (see more details below). Field gas sampling were continuing until March 2020 when the COVID-19 field travel was halted. Soil carbon and nitrogen dynamics and N-cycling microbes in the long-term farming systems at CEFS and on farms of four organic farmers: Soil sampling: Soil samples were collected from 0-10 cm and 10-20 cm at five georeferenced sampling points at each sampling time. Soils were sampled at the end of a corn growing season on November 25, 2019, midseason in a soybean crop on July 14, 2020, and twice in the fall at or after soybean maturity on October 20, 2020, and November 10, 2020. In addition, soil sampling was conducted at four farms out of a planned twelve around the state on October 28, 2020 and November 18, 2020. Farms were located in Moore, Iredell, Chatham, and Rowan counties. A total of 8, 5, 4, and 4 fields were sampled at each of the four farms, with most recent crops including corn, soy, tobacco, sweet potato, and cattle pasture. Three of the four farms were 100% organic, while one farm was split conventional and organic. Soils were sampled from the 0-20 cm depth at five georeferenced points within each field on the farms. Soil and microbial analyses: An array of soil physical, chemical and microbial parameters have been or will be determined. Although some soil samples collected in 2020 have just been frozen without being analyzed because of limitations caused by COVID-19, data has been collected and statistically analyzed for potentially mineralizable C (PMC) and soil moisture for the first two sampling dates at the Goldsboro research station location. Nitrous oxide emissions (N2O) have been determined and analyzed for the second and third dates. In addition, microbial biomass carbon and nitrogen (MBC, MBN), dissolved organic carbon (DOC), potentially mineralizable N (PMN), mineral N (MIN), and soil pH have been analyzed for the first Goldsboro sampling date. Soil DNA have been or will be extracted and partially analyzed to determine the abundances of nitrifiers and denitrifiers through quantifying their functional genes. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 Type: Journal Articles Status: Other Year Published: 2020 Citation: Sean Bloszies, Alex Woodley, Chris Reberg-Horton, Shuijin Hu, 2020. Interactive impacts of nitrogen fertilizers and tillage practices on nitrous oxide emissions in conventional and organic systems. Intern review. To be submitted to Soil Biology & Biochemistry PROGRESS: 2020/09 TO 2021/08 Target Audience:Students: One PhD student received training from this project to conduct field and lab experiments to assessing the effects of different organic management practices on soil C and N dynamics and greenhouse gas emissions. One postdoctoral scholar has been working on the project. Four undergraduate summer students received training on greenhouse gas static chamber sampling and soil analyses. Also, a second postdoc received training in the automated chamber design and installation. The scientific community in general, particularly ecologists, soil scientists, and biologists and graduate and undergraduate students. We published papers in scientific journals that reach to the scientific community in general. Farmers and land managers. The field experiment was presented the nitrous oxide CEFS study at the North Carolina State University Organic Field Day August 2021 (75 people). Work from this project was also presented at a researcher and farmer focused Webinar titled \"Farming for Ecological Outcomes\" Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided?Two postdoctoral scholars, one technician, one PhD student, four undergraduate students received training through this project. Tom Stanton (a research technician)

and Scott Holden set up the field gas emission experiment and have been working on this project since. Three undergraduate students received trains The postdoctoral scholar who is financially supported by this project (Dr. Sean Bloszies) joined the team in November, 2019 and has been responsible for field sampling and analyses. How have the results been disseminated to communities of interest?Our field site at CEFS has hosted a Center for Environmental Farm Systems farm tour which included an information session on this innovative design (\~30ppl), the purpose of the tour was for interested faculty at NCSU not familiar with CEFS to come and potentially find collaborators for future research at CEFS. In addition, this site has hosted interested researchers from the Nature Conservancy and the Environmental Defense Fund. In addition, our field projects and results were introduced to students of a class entitled Ecology of Soil Ecosystems in Spring, 2020, again in the Spring, 2022. Lastly, the field experiment was presented the nitrous oxide CEFS study at the North Carolina State University Organic Field Day August 2021 (75 people). Work from this project was also presented at a researcher and farmer focused Webinar titled \"Farming for Ecological Outcomes\" What do you plan to do during the next reporting period to accomplish the goals?Research Program: We will continue to carry out the multiple experiments initiated recently to address our three long-term goals in understanding the effects of organic systems and tillage on soil C and N dynamics, N-cycling microbes, and greenhouse gas emissions. Objective 1. Characterize the impact of organic farming on N-transforming microbes. Our aim is to obtain the essential data of the N-cycling microbes so that the relationships between the functions (nitrification and denitrification potentials, N2O emissions) and the abundances and community composition of N-cycling microbes can be explored. Twelves (12) organic farms and their adjacent conventional farms have been sampled through our collaboration with RAFI USA and we plan to collect more samples from a few farms based on our results. Because of the COVID-19 cancellations in 2020, I had to extend our final sampling in field to September 2021 and conduct analyses of soil physical, chemical, and microbial parameters, including soil properties (The texture, pH and moisture; Total soil C and N; Microbial biomass C and N, Extractable N and C), abundances of N-cycling microbes and their activities: Real-time PCR quantification of the amoA (AOA), amoA (AOB), nirS, nirK and nosZ genes, Nitrification potential and Denitrification potential. Objective 2. Identify the primary driving factors that modulate the effect of organic farming on N-cycling microbes. We will continue to conduct extensive analyses of soil physical, chemical and microbial parameters to examine the relationships between soil properties and N-cycling microbe for the samples collected through November, 2021. We will start to establish Structural equation models to identify the primary driving factors that control the abundance of N-cycling microbes and ratios of different denitrifier groups. Objective 3. Validate the relationships between N transformations and abundances, composition and activities of N-cycling microbes both in field and controlled environments. Our major task will be field assessment of relationships between communities of N-cycling microbes and N2O emissions. Because of the COVID pandemic and our PhD student has some chronic respiratory disease history, we have to postpone our greenhouse (microcosm) experiments to March, 2022 to validate results obtained from the field experiments. We will use stable isotope-labelled fertilizers to trace the movement of chemical N through quantifying plant N utilization, N2O emissions and N remained in soil. Objective 4. Educate the next generation of organic agriculture researchers and farmers through student training and an active, multi-pronged outreach program. Students training: Training of individuals directly funded by this project includes one postdoc, a PhD student and two undergraduate students in 2021-2022. Multiple other students will be also trained through this project, although they are supported by other funding sources. We are in the process recruiting two summer interns (undergraduate students) for the summer 2022. Outreach and provision of faculty-mentored research experiences for undergraduate students will also occur through the CEFS Sustainable Agriculture Internship Program. The PIs will direct two student mentored research projects in this coming year. Outreach program: Reaching Farmers: Co-PIs, Chris Reberg-Horton and Alex Woodley will actively participate in numerous extension-oriented activities, including Field Days at our field site (CEFS, Goldsboro). We planned to introduce our project to a broad audience (\>500). We planned to have our on-farm meeting in this past spring, but it had to be canceled. We will continue to our field activities, including Spring field day at CEFS and summer field day at cooperating organic farms on soil differences and sampling methods. IMPACT: 2020/09 TO 2021/08 What was accomplished under these goals? Agricultural soils in the southeastern U.S., marked by high rainfall and warm temperatures, are often characterized with low organic C and soil pH (i.e., acidic soil). Organic farming relies on organic inputs and fundamentally alters the sources and forms of reactive carbon (C) and nitrogen (N) inputs. It also induces changes in soil physical and geochemical properties that may profoundly affect the abundances, composition and activities of soil microbes, including nitrifiers (i.e., ammonia-oxidizing bacteria and archaea) and diverse types of denitrifiers that critically control plant N use efficiency and the environmental consequences (such as N leaching to water systems and greenhouse gas (N2O) emissions) of chemical N fertilizers and organic manures. However, there is still a lack of a holistic picture of organic farming effects on N-cycling microbes and their activities. We have multiple ongoing experiment for this project and we have found some very exciting results. These results have been or will be disseminated to communities of interest mainly through publications and presentations to scientific meetings, as well as to farmers and land managers through field demonstrations and field days. The knowledge generated from our research may also help us design new N management

regimes under future climate change scenarios. Multiple field experiments are underway. Field trace gas emissions. At the Center for Environmental Farming Systems in Goldsboro, North Carolina, a field study was established in a long-term rotational study in March of 2019. Four rotational treatments were selected including: 3yr organic (corn, soybean and sunflower), a 6-yr organic production/forage (corn, soybean, sunflower, hay, hay, hay), a 3yr conventional no-till (corn, double crop wheat/soybean, sorghum) and a 3 yr conventional till (corn, double crop wheat/soybean, sorghum). In 2019, all phases of the rotation are in corn. In April of 2019, a system for semi-continuous measurement of nitrous oxide (N2O) was established in 3 locations within each cropping rotation treatment. This continuous measurement is achieved by using LI-Cor 8100-104 Long-Term Soil Gas Flux Automatic Chamber System. Soil temperature and soil moisture content is also being collected at each chamber for further interpretation of the soil microbial results (see more details below). Field gas sampling were continuing until March 2020 when the COVID-19 field travel was halted. In early 2020, we set up a long-term field experiment at CEFS that assesses the impact of biochar, chemical N fertilizer, and compost on N-cycling microbes and N2O emissions. The field continuous monitoring of N2O emissions have been ongoing since Spring 2020. We have recently collected soil samples for chemical and microbial analyses. Soil carbon and nitrogen dynamics and N-cycling microbes in the long-term farming systems at CEFS and on farms of four organic farmers: Soil sampling: Soil samples were collected from 0-10 cm and 10-20 cm at five georeferenced sampling points at each sampling time. Soils were sampled twice in the fall at or after soybean maturity on October 20, 2020, November 10, 2020, and June 7, 2021. Collaborators at RAFI provided a list of 12 organic farmers. All 12 were contacted by NCSU researchers and assisted with soil sampling on their farms, beginning with farms one and two on October 28, 2020 and ending with farm twelve on January 29, 2021. Soils were sampled from the 0-20 cm depth at five georeferenced points within each field on the farms. Soil chemical and microbial analyses: All samples from on-farm and the research station have been processed for pH, microbial biomass C and N, potentially mineralizable C and N, K2SO4-extractable C and NO3- and NH4+, and incubation N2O emissions potential. In addition, processing has begun for N cycling gene quantification, with four clades of nirK and two clades of nirS genes quantified for the June 7, 2021 soil using qPCR. Preliminary findings reveal a trend of higher N2O emissions potential among organic than conventional management soils in the research station samples, especially when complete denitrification to N2 is experimentally halted via acetylene. In addition, Fall 2020 emissions seem to have been elevated by the senescence of soybean root nodules, with roughly half the emissions of October 19 occurring on November 10, capturing when soybeans had passed from R7 to R8. Higher microbial biomass N was observed in organic soils than conventional at the June 2021 research station sampling, likely due to poultry litter applications to the 2021 sunflower crop. Under reduced tillage, potentially mineralizable C was enhanced in organic relative to conventional research station plots. Within the on-farm soil samples, dairies\' soils were notable for their elevated potentially mineralizable C and N, and microbial biomass C and N. Neither comparisons of organic versus conventional nor high versus low yield historical yield seem to have driven noticeable differences in N2O emissions among on-farm samples. PUBLICATIONS (not previously reported): 2020/09 TO 2021/08 1\. Type: Journal Articles Status: Published Year Published: 2021 Citation: Qiu, Y., Guo, L., Xu, X., Zhang, L., Zhang, K., Chen, M., Zhao, Y., Burkey, K.O., Shew, H.D., Zobel, R.W. and Zhang, Y., 2021. Warming and elevated ozone induce tradeoffs between fine roots and mycorrhizal fungi and stimulate organic carbon decomposition. Science Advances, 7(28), p.eabe9256. 2\. Type: Journal Articles Status: Under Review Year Published: 2021 Citation: Xuelin Zhang , Yunpeng Qiu, Frank S. Gilliam , Cong Tu , S. Chris Reberg-Horton, Shuijin Hu. 2021. Arbuscular mycorrhizae shift community composition of N-cycling microbes and suppress soil N2O emissions. Environmental Science and Technology 3\. Type: Journal Articles Status: Under Review Year Published: 2021 Citation: Sean A. Bloszies, S. Chris Reberg-Horton, Joshua L. Heitman, Alex L. Woodley, Julie M. Grossman, and Shuijin Hu. 2021. Tillage differentially affects soil N2O emissions in conventional and organic farming systems. Agriculture, Ecosystems & Environment **NO:** 1017168 **SUBFILE:** CRIS **PROJ NO:** WVA00914 **AGENCY:** NIFA WVA **PROJ TYPE:** OTHER GRANTS **PROJ STATUS:** NEW **CONTRACT/GRANT/AGREEMENT NO:** 2018-51106-28777 **PROPOSAL NO:** 2018-03547 **START:** 01 SEP 2018 **TERM:** 31 AUG 2021 **GRANT AMT:** \$499,817 **GRANT YR:** 2018 **AWARD TOTAL:** \$499,817 **INITIAL AWARD YEAR:** 2018 **INVESTIGATOR:** Kotcon, J.; Thonney, MI, L..; Brown, RE, N.; Petersson, KA, HA.; Griggs, TH, C.. **PERFORMING INSTITUTION:** WEST VIRGINIA UNIVERSITY 886 CHESTNUT RIDGE RD RM 202 MORGANTOWN, WEST VIRGINIA 26505-2742 ***OVERCOMING BARRIERS TO TRANSITIONING SMALL RUMINANTS TO ORGANIC PRODUCTION: EFFECTS OF FEEDING BIRDSFOOT TREFOIL ON PARASITE CONTROL, NUTRITIONAL STATUS AND PROFITABILITY***

2019/09 TO 2020/08 What was accomplished under these goals? These experiments evaluate ways to improve establishment of birdsfoot trefoil pastures to prevent intestinal parasite infection in sheep. This may reduce economic losses for organic growers from reduced weight gains and loss of organic certification due to use of synthetic deworming agents. The methods may benefit conventional sheep growers by reducing need for deworming agents and slowing development of parasite resistance. Objective 1. Evaluate agronomic practices to

improve BFT establishment in existing pastures. Two trials were established at the WVU Organic Research Farm to evaluate the effect of soil fertility, cultivar, and tillage treatments on establishment of birdsfoot trefoil in existing pastures. Soils were limed in June, 2019. Baseline plant community structure was assessed in Summer 2019. Half of the plots in the low-phosphorus pasture were amended with bone meal to supply 89 pounds P/acre. A high-P pasture was unamended, creating three phosphorus fertility levels. Four high-tannin forage cultivars (Delaney sanfoin, and Bull, Bruce and Norcen birdsfoot trefoil) were planted using a Truax drill with either coulters or 3-inch sweeps to open sod in Sept. Additional plots were frost seeded in Feb. 2020. Emergence data found higher seedling emergence using sweeps than coulters in fall 2019 plantings. Delaney sanfoin and all birdsfoot trefoil cultivars responded well to frost seeding in Feb. 2020 in low fertility plots, but stands declined by fall, 2020. Danish tines (sweeps) resulted in better stands than coulters. In a high fertility site, Norcen and Bull birdsfoot trefoil had significantly better stands than Delaney sanfoin by fall, 2002. Plant density did not reach the targeted 30 % of the pasture stand in any treatment. In summary, birdsfoot trefoil can be established into existing pastures if aided by sweeps, but plant density was low and did not reach the levels routinely achieved by planting into cultivated seed beds. Objective 2. Compare efficacy of BFT pastures and supplemental feed in field-scale grazing trials . Three 1.25-acre pastures were planted with Norcen birdsfoot trefoil, 3 with Bull birdsfoot trefoil, and 6 with orchard grass/red clover were established at the WVU Organic Research Farm in Fall 2019. Grazing trials with lambs were conducted for 90 days, beginning in June 2020. Four lambs per plot were rotationally grazed on paddocks for 7 days, followed by a 21-day rest. Body weight, body condition index, FAMACHA scores and Fecal Egg Counts were conducted at 14-day intervals. Lambs with high FAMACHA scores (4 or greater) were treated with a synthetic dewormer (Cydectin) as needed. Only 6 % of lambs grazed on birdsfoot trefoil required treatment, whereas 21 % of those on orchard grass/red clover were treated with deworming medication. The rate of parasite infection was lower than expected due to a prolonged drought which reduced parasite larval dispersal and infection. Total Weight Gain of lambs averaged 0.638 lb/day on BFT versus 0.60 lb/day on OG/RC (not statistically significant). The stocking rate of lambs (\~ 4 per acre) was relatively low, and clip samples of forage showed that only 10-20 % of available forage was consumed. Thus, pastures remained in very good condition all season, in spite of the prolonged drought. Objective 3. Determine the effect of feeding BFT processed as either hay, high- moisture hay or pellets on experimental and pasture gastrointestinal nematode infections in lambs. The scope of work for objective 3 was changed from animal feed trials using forage grown at URI to in vitro testing of the anti-parasitic activity of 12 populations of BFT established in the fall of 2019 on organic land located at the Agronomy Farm at the University of Rhode Island. The new objective will determine the effect of drying method, harvest date and length of storage on the in vitro anti-parasitic bioactivity of aqueous and organic fractions of 12 cultivars of BFT. Cultivars Norcen, NB95-118, Langille, Witt, LTAN-17, Bruce, Pardee, MSP4068, BuUr-13, Leo, NB95-120, Wellington and Leo were established in the fall of 2019. The trial entries were harvested on June 29, 2020 and again on August 24, 2020. At harvest each plot was clipped to a height of 12.5 cm to simulate mowing and the herbage was collected and weighed. One 0.5-kg subsample from each plot was immediately frozen and held at -20? until freeze-drying. Another 0.5-kg subsample was dried to constant moisture in a forced air cabinet at 40? to simulate haying. Frozen samples were freeze-dried at the Forage Testing Laboratory at the University of Missouri. After samples were freeze-dried or air-dried, samples were ground (Wiley Mill, 1mm screen). Objective 4. Demonstrate research results in on-farm trials with select farmers. On-farm demonstration were planned by Cornell., but no farms conducted grazing trials in 2020. Weather conditions for planting in both 2019 and 2020 were very challenging. One farm no till seeded a \~3-acre strip of pasture to red and white clover on 8/27/19 and a bordering 3-acre strip to cv. Bull birdsfoot trefoil on 9/01/19. Stands were less than the target of ≥25% biomass of clover and BFT respectively when forage was sampled on 5/27/2020. This farmer objected to weaning lambs from dairy ewes because of the effect of the COVID19 lockdown in NY on their cheese market. Additionally, Cornell University was closed to students through the summer. Therefore, a decision was made to postpone their lamb grazing trials until 2021. On 10/09/2020, the clover strip averaged 14.9% clover plus 5.6% BFT in the dried biomass. The BFT strip averaged 11.2% BFT plus 2.4% clovers in the dried biomass. The bulk of the biomass in both strips was unpalatable dead grass. Three other farms attempted to plant in 2020 despite a cold early spring followed immediately by persistent drought conditions. One farm was planted to Bull BFT on 8/12/2020. Some seed emerged 8/25/2020 but due to drought, the field was too short on 10/11/2020 to obtain any forage samples. Both other farms had planned to use no till seeding but were unable to. Objective 5. Extend research results to organic and conventional sheep and goat producers throughout the Northeast via Extension fact sheets, workshops, pasture walks, field days, and maintenance of the project website. Five workshops were held between Sept. 24, 20189 and Jan. 18, 2020, and reached 1o3 growers, and 38 veterinary students and practitioners. Workshops later in the year were canceled because of the COVID19 Pandemic. Instead, farmers and educators seeking IPM/ FAMACHA© Training were directed to the URI online training program at https://web.uri.edu/sheepngoat/famacha/ . URI also assisted with the maintenance of the project webpage (https://web.uri.edu/sheepngoat/orgbft/) and events page. Objective 6. Evaluate success of outreach activities through surveys of farmers and participants at workshops, as well as economic performance analyses of farmers participating in on-farm trials, or other adoption of management

recommendations. Farmers at one FAMACHA parasite workshop this year returned pre and post quizzes and answered questions on their plans. Scores were 62.5% for pre-quizzes and 90.6% for post quizzes, respectively. When asked about parasite management changes, 1, 2, 3, 1 and 1 respondents, respectively, indicated that they planned to start or make improvements in 1) pasture and/or barnyard management, 2) five-point checks for selective deworming, 3) fecal egg monitoring for selective deworming, 4) dewormer use, or 5) nutrition targeted at the immune system. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 1. Type: Conference Papers and Presentations Status: Accepted Year Published: 2020 Citation: Kotcon, J. High-Tannin Pasture Forages for Managing Intestinal Parasite in Sheep. Abstract \# 88003. Ecological Society of America Annual Meeting. Held on-line. Aug. 3-6, 2020. 2. Type: Conference Papers and Presentations Status: Accepted Year Published: 2020 Citation: Blake, N., and Kotcon, J. 2020. Allelopathy in birdsfoot trefoil pasture establishment.. Ohio Ecological Food and Farming Association Annual Meeting. Dayton, OH. Feb. 13-15, 2020. 3. Type: Websites Status: Published Year Published: 2019 Citation: https://web.uri.edu/sheepngoat/orgbft/ &#65533; Webpage listing the resources used in the IPM/FAMACHA workshops conducted through the project - http://blogs.cornell.edu/smallruminantparasites/resources-for-educators/

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Overcoming Barriers to Transitioning Small Ruminants to Organic Production: Effects of Feeding Birdsfoot Trefoil on Parasite Control, Nutritional Status and Profitability

Accession No.	1017168
Project No.	WVA00914
Contract / Grant No.	2018-51106-28777
Start Date	01 SEP 2018
Term Date	31 AUG 2021
Grant Amount	$499,817
Grant Year	2018
Investigator(s)	Kotcon, J.; Thonney, MI, L..; Brown, RE, N.; Petersson, KA, HA.; Griggs, TH, C..

NON-TECHNICAL SUMMARY

Small ruminants provide a profitable niche for organic growers. However, certain intestinal parasites, specifically gastrointestinal nematode (GIN) infections, are the major factor limiting organic production. [Consumption of condensed tannin (CT) containing forages aids in suppressing GIN infections, however, the bulk of the research on GIN control has been on forages adapted for warm climate]{.mark}s. Our previous work showed that a temperate forage, birdsfoot trefoil (BFT), has anthelmintic activity, however efficacy varies with the amount of CT-containing forage in the diet. [Because birdsfoot trefoil is slow to establish, improved methods to assure high levels in pastures or in ruminant rations are needed to realize the potential for GIN control]{.mark}. This proposal represents the next logical steps in research, by establishing a multi-state, multi-disciplinary project to enhance productivity and profitability of organic livestock production in the Northeast. Specifically, commercially-available cultivars of BFT will be evaluated by: 1) comparing agronomic pasture establishment practices, 2) determining efficacy of GIN suppression using in vitro and in vivo methods, including grazing trials and experimental infections, 3) demonstrating these improved pasture management practices in on-farm trials and 4) extending these practices to organic and transitioning growers. Outreach activities will include grower trials, IPM workshops, field days, and will also explore use of on-line services such as eOrganic and eXtension. Growers will realize increased animal productivity, reduced numbers of animals removed from organic status due to dewormer intervention, and will have increased confidence in coping with GIN during transition from conventional to organic farming. This project addresses OREI goals one, three, and four.

OBJECTIVES

This proposed project would enable us to continue studying the potential of birdsfoot trefoil (BFT) to combat two major barriers to organic small ruminant production: gastrointestinal parasite infections and the costs of organic concentrate feed that is often necessary to obtain sufficient growth rates in lambs and kids compromised by parasite infection. With this new project, our research objectives include evaluation of: agronomic practices to improve BFT establishment in existing pastures; the ability of protein supplementation to substantially increase parasite tolerance of organically managed lambs to gastrointestinal nematodes; and the effect of feeding BFT processed as either hay, high-moisture hay or pellets on experimental and natural gastrointestinal nematode infections in lambs. Outreach objectives include: teaching regional workshops on parasite management in small ruminants; conducting on-farm grazing trials with four goat or sheep farms (two certified organic and two still lacking confidence to transition) to demonstrate effective BFT management practices; organizing field days and pasture walks to demonstrate improved practices; preparing extension fact sheets to translate research results into practical management suggestions; and evaluating the impact of the field days, workshops and on-farm trials on grower adoption of best practices.Our overall goal is to develop organic grazing practices that suppress GIN parasites in sheep and goats while maintaining organic integrity. We propose new methods to validate harvesting methods that preserve the anthelmintic activity of the forage and develop best management practices for the

establishment of BFT. This project will integrate three research and three outreach objectives (described below).Research Objectives:1) Evaluate agronomic practices to improve BFT establishment in existing pastures.2) Compare efficacy of BFT pastures and supplemental feed in field-scale grazing trials to evaluate whether the use of BFT in the feeding program of sheep and goats can increase tolerance to gastrointestinal nematodes as well as replace or minimize grain supplementation needed for optimum growth.3) Determine the effect of feeding BFT processed as either hay, high- moisture hay or pellets on experimental and natural gastrointestinal nematode infections in lambs. Outreach and Extension Objectives:4) Demonstrate research results in on-farm trials with select growers.5) Extend research results to organic and conventional sheep and goat producers throughout the Northeast, via Extension fact sheets, workshops, pasture walks, field days, and continuation of the web page.6) Evaluate success of outreach activities through surveys of growers and participants at workshops, as well as economic performance analyses of growers participating in on-farm trials, or other adoption of management recommendations.

APPROACH

Obj. 1. Evaluate agronomic practices to improve BFT establishment in existing pastures. Plots (3 by 8 m) will be established at two pasture sites (both Westmoreland silt loams with 8-15 % slopes) differing in levels of soil phosphorus at the WVU Organic Research Farm. Half of the plots in the low-P site will be amended with cal-phos to match levels of available P in the high-P site. Cal-phos will also be applied to half of the plots at the high-P site. Three CT-containing forage legumes (Bruce and Pardee BFT, and sainfoin cv. Delaney) will be established in August 2019 via strip tillage with a split-hood rototiller or by using a no-till drill planting into strips from which the sod layer has been excised. Each plot will receive BFT or sainfoin alone, plus within-row mixtures with oats cv. Reins at 25% of total seed count or density. Additional untilled plots will be mowed at 5 cm in Fall 2019 whenever sward height exceeds 10 cm, then frost seeded with Pardee BFT (without oats) in February 2020. Three replicates of each treatment combination (2 phosphorus fertility levels times 3 seeding methods times 3 legume cultivars) will be arranged in randomized complete blocks in each site (108 plots total). The experiment will be repeated in 2020 and 2021. Data will be collected on legume emergence rate, and density of legume seedlings and established plants in Fall 2019, botanical composition and productivity of pastures at beginning and end of each growing season thereafter, pest damage to legume seedlings, degree of light interception or shading in seeded rows and adjacent canopies, and diet selection of grazing animals. Condensed Tannin concentration will be assessed via near-infrared reflectance spectroscopy.Obj. 2. Compare efficacy of BFT pastures and supplemental feed in field-scale grazing trials to evaluate whether the use of BFT in the feeding program of sheep and goats can increase tolerance to gastrointestinal nematodes as well as replace or minimize grain supplementation needed for optimum growth. A trial at WVU will compare performance and GIN infection of lambs on BFT pastures with lambs on conventional pasture with or without supplemental protein. One-acre plots will be established as a randomized complete block design with three replicates of BFT and six replicates of orchardgrass/red clover pastures. Forage samples will be clipped at 2-week intervals, sorted as legumes, grasses and weeds, and dried at 70oC to estimate pasture composition, and forage consumption. Lambs born in April will be weaned at \~ 60 days and placed on pastures in mini-flocks of six lambs each. Lambs will be rotationally grazed for 90 days using a \"challenge\" grazing regime of 7 days on a paddock, followed by 21 days rest for pasture regrowth, before lambs return to that paddock. Lamb weight, fecal egg counts, FAMACHA© and Body Condition Index scores will be determined at 2-week intervals. The experiment will be repeated in 2021 and 2022. Data will be analyzed using GLM procedures in SAS and unpaired Student t-test as appropriate.Obj. 3. Determine the effect of feeding BFT processed as either hay, high- moisture hay or pellets on experimental and pasture gastrointestinal nematode infections in lambs.Infective larvae will be obtained from donor lambs experimentally infected with H. contortus. BFT dry hay and high-moisture sweet hay will be grown and harvested from a 12-acre hay field, managed organically, at the University of Rhode Island\'s Peckham Farm. Pellets will be produced from the BFT:grass dry hay. Twenty-four 3-month-old lambs born and housed indoors, under conditions excluding nematode infection, will be administered 5,000 infective H. contortus larvae. After 4 weeks, lambs will be stratified into dietary treatment groups, balanced for FEC, sex and weight. Lambs will be fed for 8 weeks with: 1) BFT:grass dry hay, 2) BFT:grass high-moisture sweet hay, or 3) alfalfa:grass hay. Fecal egg counts, packed cell volume, FAMACHA score and weight will be measured weekly. In Year 2, 32 lambs will be administered 5,000 infective H. contortus larvae and the infection allowed to mature for four weeks. Lambs will be fed for 8 weeks with: 1) BFT:grass dry hay, 2) BFT:grass dry hay pellet, 3) alfalfa:grass hay and 4) pelleted alfalfa:grass hay. Fecal egg counts, packed cell volume, FAMACHA score and weight will be measured weekly. In Year 3, 32 lambs will graze GIN-infected pastures for a minimum of 4 weeks. Sixteen lambs will be removed from pasture and maintained indoor and fed either BFT-pellet or Control-pellet at 2.5% of body weight fo

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