

# OREI Project Details

## Award Year 2022

18 Research Projects

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# Scaling-up and Integrating Undergraduate Organic Agriculture Education Across Uc Flagship Campuses and Uc Agriculture & Natural Resources

<b>Accession No.</b>	1029024
<b>Project No.</b>	CALW-2022-04074
<b>Agency</b>	NIFA CALWA
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37965
<b>Proposal No.</b>	2022-04074
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2025
<b>Grant Amount</b>	\$749,821
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Philpott, S.; Bowles, TI, .; Parr, DA, .; Wilson, SA, HO.; Galt, RY, E.
<b>Performing Institution</b>	UNIVERSITY OF CALIFORNIA SANTA CRUZ, 1156 HIGH STREET, SANTA CRUZ, CALIFORNIA 95064

## NON-TECHNICAL SUMMARY

Successful continued growth of the organic agriculture sector requires meeting needs for a growing workforce that is trained in research, extension, and grower services, with professionals that fully understand the context of organic production and how to meaningfully engage with increasingly diverse participants in the organic industry. University degree programs focused on organic agriculture, coupled with training in cooperative extension and research will play a key role in producing these highly competent professionals. Our project harnesses rigorous experiential learning resources and training across three UC campuses (Santa Cruz, Davis, and Berkeley) and the Division of Agriculture & Natural Resources (ANR) to develop a series of new curricular programs in organic agriculture aimed at promoting underrepresented student success. Our project objectives are to: (i) Establish an intercampus exchange program in organic agriculture, (ii) Develop a research and extension internship program for students at UC ANR, and (iii) Plan and pilot a UC-wide Supercourse and Field Quarter to provide intensive training for students in organic agriculture. These curricular activities will support success of underrepresented students pursuing degree programs and careers by integrating curricular and career advising, mentored fieldwork and internships in production, extension, and research, and creating a Student Leadership Development Program. The project will foster increases in enrollments, graduation rates, and achievements of alumni, resulting in greater investments in organic education across the UC System. Ultimately, the project aims to create a broad-based cadre of professionals equipped to meet organic grower needs and move the field forward into the future.

## OBJECTIVES

The project addresses the following critical stakeholder needs: (1) the scarcity of organic agriculture education opportunities, (2) the absence of an advising and articulation structure that links educational and professional development opportunities across multiple University of California campuses, as well as between campuses and the UC Division of Agriculture & Natural Resources (UC ANR), and (3) inadequate educational opportunities and support for underrepresented students in organic agriculture who could meet the need for a diverse and inclusive workforce in organic research, extension, and industry. While undergraduate training in sustainable agriculture

methods is growing, specific education in organic agriculture lags behind and is piecemeal, and farmers have continued to identify needs for more service provider professionals to be trained in the principles and practices of organic production. Scaling up and integrating organic agriculture educational programs across the four UC entities involved in this project will enable a professional development pipeline where students acquire professional competencies in organic research and extension, and can meet the increasing demands of a growing organic industry in California and beyond. This project, led by a Hispanic Serving Institution, is a partnership among three of the academic institutions that comprise the 10-campus University of California System (UC Santa Cruz, UC Davis, UC Berkeley) plus the UC ANR campus. The long-term goal is to foster an exceptionally creative, competent, effective, diverse, and inclusive workforce for organic agriculture to grow into the future. Specifically, the goals of our Curriculum Development project are to (1) increase the quantity and quality of undergraduate educational opportunities across the U. of California (UC) in organic agriculture, (2) increase the numbers of people gaining UC bachelor degrees and entering careers in organic agriculture research, extension, and industry, and (3) increase the support and success of underrepresented students pursuing these UC degree programs and careers. This project represents work across a diversity of organic farming communities in California, from Coastal to Central Valley, cool weather vegetables and berries to orchards and field cropping systems. The OREI FY 2022 Priority served by this project is to develop curriculum for organic agriculture, with educational activities that include on-farm instruction, experiential learning, and student-farmer engagement for students enrolled in baccalaureate degree programs. Our objectives are to: (i) Establish a UC intercampus exchange program between flagship campuses teaching organic agriculture, (ii) Develop a UC ANR internship program linking cohorts of campus degree program students with UC ANR Cooperative Extension-led research projects, and (iii) Plan and pilot a UC Supercourse and Field Quarter program that provides intensive fieldwork training for students on regional organic farms, UC campuses, and UC ANR organic teaching and research farms. All three objectives occur in the context of supporting the success of underrepresented students pursuing these degree programs and career trajectories. The project integrates curricular and career advising, and mentored fieldwork in production- and research-based internships, as well as a Student Leadership Development Program at UC Santa Cruz and UC Davis student instructional and research farms.

## APPROACH

The methods for carrying out the project objectives relate to higher education curriculum development, course design, pedagogical innovations, as well as mentoring and related approaches to student retention and success. These methods are being employed by faculty and academic staff in the University setting, with support from additional key staff within our respective campuses. The instructional fieldwork involving farmer engagement and research activities are being informed by research faculty and cooperative extension staff, providing their expertise in applied, farmer-engaged research methods, in an educational and professional development context. Curricular Development activities: (i) Intercampus Exchange program. Students will gain access to enrolling in courses and internships in organic agriculture at multiple UC campuses facilitating a greater breadth and depth of instruction available to students. Key Personnel at each campus farm (Wong, Ullman, UC Berkeley) will provide internship coordination together with student peer advisors. Faculty, advising staff and student peer advisors will provide students materials and guide them in enrolling at multiple campuses, beginning in Y2 of the project, and continuing indefinitely. The Co-PDs and Key Personnel at each campus will contribute to the intercampus exchange advising conceptual development, with Co-PD Parr leading analysis, design, and final drafting of advising materials transfer credit agreements, and related protocols by end of Y1. In Y1, campus advising faculty, staff advisors, and student peer advisors will be provided guiding materials on the instructional opportunities at each campus as well as information on how to best host and support students enrolling at partner campuses. (ii) UC ANR Internship and Mentoring program. Co-PD Parr, Co-PD Wilson and Key Personnel at the UC ANR will research and develop advising protocols and materials. Students from UC Santa Cruz, UC Davis, and UC Berkeley will gain access to advising materials and support that connects them with available internships, paid student staff fieldwork opportunities, and professional development mentoring offered by UC ANR faculty and staff researchers conducting organic agriculture research and extension projects, state-wide. Key Person Muramoto will provide the California Organic Systems Researchers Map and introduce contacts with UC ANR organic researchers. Muramoto will also consult and advise on his previous OREI research projects experiences with mentoring student interns and staff in field research settings. In Y1, Co-PD Parr and Key Personnel at the UC ANR will research and produce critical mentoring professional development activities and resources for UC ANR mentors focused on underrepresented student support. These materials will be implemented in Y2. (iii) Supercourse and Field Quarter program. PD Philpott, Co-PD Parr and Key Person Wong will lead the development of the Supercourse using the UC Santa Cruz Center for Agroecology as the primary host site, with visits to organic farm operations, UC ANR research stations and UC Davis and UC Berkeley campuses (Y1), will gain UC Santa Cruz academic senate permission for offering the course (Y1), and will pilot the Supercourse in

Y2-3. Co-PD Galt and Key Person Ullmann will host the Supercourse at the UC Davis campus farm. Key Person Ichikawa and UC Berkeley Key Personnel will organize and lead an organic agriculture policy and economics training symposium for the Supercourse. In Y2, PD Philpott and Co-PD Parr will partner with Co-PD Wilson and UC ANR Key Personnel in researching, designing, and establishing a UC System-wide Field Quarter administrative home within UC ANR, in partnership with UC Santa Cruz Environmental Studies Department and faculty. The Field Quarter program will be capable of offering multiple Supercourses each year in Spring, Summer, and Fall quarters from 2026 forward.

Underrepresented Student Success activities: (i) Student Leadership Development Program. Key Person Wong and Co-PD Parr (UC Santa Cruz) and Key Person Ullman (UC Davis) will implement the Student Leadership Program at campus farms in Y1 with a specific focus on mentored fieldwork in research-based internships. Programming linkages with the UC Davis SCOPE project, co-directed by Ullmann will be made, and SCOPE project activities will be initiated at UC Santa Cruz with organic seed partners on the Central Coast beginning in Y2. Student Leadership Development Programming is expected to continue, indefinitely. (ii) Alumni career panels and research pathways workshops. We will deepen partnerships with UC Santa Cruz organizations and programs including the Educational Opportunities Program, Hispanic Serving Institution and Career Success, and the Graduating and Advancing New American Scholars: Promoting Postbaccalaureate Opportunities for Hispanic Americans (GANAS) grant in order to provide targeted curricular and career advising. Key Person Wong will host GANAS interns in campus farm research programming. Key Person Ichikawa and other UC Berkeley Key Personnel, Co-PD Parr, and UC Davis Key Personnel will co-organize alumni career panels and research pathway workshops featuring alumni and student researchers. (iii) Linkages with existing USDA Higher Education projects. PD Philpott, Co-PD Parr and Key Person Wong will manage program linkages at UC Santa Cruz between our OREI program activities and existing USDA funded fellowship (SUPERDAR) and scholarship (MSP) programs in Y1. Students in currently funded projects will be integrated into proposed activities including courses, UC ANR Internships, and Supercourse offerings. Best practices from these grant projects will be shared with partner campuses for potential adaptation. (iv) Professional development training workshops for UC ANR researchers. Co-PD Parr and Key Personnel at the UC OAI will lead the development of resources and critical mentoring competency workshops for working with underrepresented students (Y1). UC ANR Key Person Muramoto will introduce UC ANR organic researchers to these resources, and Parr and UC OAI personnel will provide resources and lead workshops with UC ANR intern mentors in Y2-3.

The evaluation of this "Curriculum Development" project will examine what was produced, what learning outcomes and impacts were achieved, and how the programming can better meet its goals. The evaluation will build off proven and vetted evaluation instruments, the UC Santa Cruz project team's previous work, and a theoretical evaluation framework responsive to the project's goals and intended audiences. Two vetted instruments provide both useful information about learning outcomes and impacts, as well as act as learning tools for individual professional development. To support mentorship development, we will use the Mentoring Competency Assessment (MCA). The goal of the instrument is to identify effective research mentor traits and skill level. The instrument assesses six mentor competencies, including maintaining effective communication, aligning expectations, assessing understanding, addressing diversity, fostering independence and promoting professional development. To identify learning outcomes for students participating in internships and Supercourses, we will use a modified Undergraduate Research Self-Assessment (URSSA) instrument. This instrument identifies how students are progressing in obtaining research skills, attitudes and affect. As it has been used by other programs nationally, it is possible to compare outcomes to larger groups to assess relative advancement. Outputs, developmental questions and summative outcomes will be tracked over the course of the project for each of the three primary curricular developments. Progress 09/01/23 to 08/31/24

Outputs Target Audience: The primary target audiences for this project's reporting period included underrepresented students studying Agroecology and Sustainable Agriculture & Food Systems. These students were enrolled in majors and courses that focus on the science and practices of organic agriculture. The experiential learning curricula included internships, work, and study in the campus organic farm-based Leadership Development Programming, and participation in an Agroecology Field Quarter. As of August 2024, UC Santa Cruz supported the enrollment of 96 Agroecology majors, a 20% increase in enrollment over the previous year of the grant. Hispanic and Latino identifying student enrollments in the major increased over 20% from year 2022-2023 to 2023-2024, growing from 14 students to 22 students, representing 31% of students in the Agroecology major. This 31% representation surpasses the UC Santa Cruz campus average of 26% for Hispanic/Latino students. Women-identifying student enrollments increased over 20% in 2023-2024, growing from 28 students to 41 students, representing 58% of students in the Agroecology major. This representation surpasses the UC Santa Cruz campus enrollment average of 48%. Relevant to gender equity efforts, non-binary students are also significantly overrepresented, two-to-one, in the Agroecology major, compared to campus averages. The Educational Opportunity Program (EOP) includes first-generation to college, low-income students with diverse social and personal identities from historically marginalized backgrounds. Enrollments of these students include California Dream Act recipients, independent students, formerly incarcerated and system-impacted students. We have seen EOP enrollments increase 100% in 2023-2024, growing from 9 to 18 students, now representing 25.4% of students in the major.

This 25.4% representation is trending quickly towards the campus enrollment average of 27.4%. In addition to the degree program gains in this project reporting period, UC Santa Cruz had 244 students served by OREI funded staff through the Leadership Development Program, Internships, and Field Quarter. The demographic backgrounds of student participants across all programs included African American representation of 8.6% compared to 4.5% campus-wide, Hispanic/Latinos at 27.5% compared to 26% campus-wide, Native American/Alaskan Native at 2.9%, compared to 0.7% campus-wide, Pacific Islander/Native Hawaiian at 0.4% compared to 0.3% campus-wide. Female identifying students made up 63.5% of participants compared with 48.3% campus-wide, and non-binary students represented 9% compared to 5.5% campus-wide. The UC Santa Cruz Center for Agroecology Leadership Development Program, specifically, had 80 undergraduate students participating in year 2 (Fall 2023-Summer 2024). Demographics included African American students at 11% compared to 4.5% campus-wide, Hispanic/Latinos at 36.3%, compared to 26% campus-wide, Native American/Alaskan Native 2.5% compared to 0.7% campus wide. Female students made up 56% of participants compared with 48.3% campus-wide, and non-binary students represented 10% of participants, compared to 5.5% campus-wide. The demographic background of the Field Quarter-Super Course participants included 13 undergraduate students, 11 of which identified as not white or male. Detailed demographics from UC Berkeley and UC Davis were not compiled during this reporting period. Changes/Problems: Y2 project deliverables were somewhat delayed in completion due to hiring and onboarding of Key Personnel. However, as we ended Y2 of the project, all key personnel have been hired or replaced, and the project is now fully staffed. The key personnel replacements occurred across the three lead partner campuses (UC Santa Cruz, UC Berkeley, UC Davis) as well as at the UC Agriculture and National Resources Organic Agriculture Institute. At UC Berkeley, Berkeley Food Institute Executive Director (an OREI Key Person) left their position and was replaced in January 2024. UC Berkeley Education Coordinator staff also departed and a new hire finalized in June 2024. At UC Davis, the Student Farm Director (an OREI Key Person) left their position and was replaced with a new Director who was settling into the position in 2024. The UC Agriculture and National Resources Organic Agriculture Institute position was newly hired in July 2024. What opportunities for training and professional development has the project provided? UC undergraduate students from three UC campuses participated in the first pilot of a 7-week UC Agroecology Field Quarter - or "Supercourse", hosted by UC Santa Cruz. There were 13 students enrolled in 17 units, a program consisting of three classes and a lab. Courses included; ENVS 133C - Agroecology Practicum, ENVS 130A - Agroecology and Sustainable Agriculture, ENVS 130L - Laboratory, and ENVS 130C - Field Experiences in Agroecology and Sustainable Food. Students lived, worked, and traveled as one cohort for the full 7 weeks. Lectures and demonstrations were combined with field applications to give students direct experience and knowledge of organic agriculture and horticulture practices and principles. The UC Santa Cruz Center for Agroecology's Farm and Garden served as the residential location for the first 3 weeks of hands-on instruction. Partner hosts include UC Davis Student Farm, UC Berkeley Oxford Tract, UC Agriculture and Natural Resources Kearney Agricultural Research and Extension Center. Field work took place on 15 regional organic farming operations. Together, these farms serve as living laboratories for integrating theory and practice. Students built a professional network with prospective mentors and employers across university, farm operations, state and federal agencies, and non-profit and community-based organizations. Student mentoring, recruitment, advising and retention through the Student Leadership Development Programming. New activities within this project reporting period include informal professional development for UC Santa Cruz Leadership Development Program core team staff. This happens through inclusion of academic research based in theoretical and philosophical frameworks to better contextualize, understand, and implement effective educational programming, and the collection of professional and continuing education topics of interest for undergraduate staff. In the Y2 reporting period, the project partners continued to supervise and facilitate 140 Student Leadership staff across the 3 partner campuses to act as peer mentors and role models for advising early career undergraduate students pursuing hands-on organic agriculture, research and education. These students typically worked 8-20 hours per week and participated in organic agriculture skills and leadership development activities led by staff supported by this grant. Of the 140 students, UC Santa Cruz employed 85 undergraduate student staff, UC Davis employed 2 Graduate students and 36 Undergraduate student staff, and UC Berkeley employed 5 Undergraduate student staff. Of all students participating in these programs, four were paid directly from this grant (two at UC Santa Cruz and two at UC Davis). All the remaining student staff were supported by multiple different funding sources. However, program development has had an impact far beyond the students funded by this grant - and all student staff have been critical to supporting the main goals and objectives of this project. Furthermore, the Leadership Development Program student staff are supervised by professional staff that are paid for by this OREI project. These grant-paid staff are responsible for designing and managing these student employment and career development programs. Internship and Course Enrollments. In the Y2 reporting period, the project partners continued to supervise and facilitate student interns (UC Santa Cruz - 156, UC Davis - 261, UC Berkeley - 80), resulting in nearly 500 students enrolled in for-credit internships related to Agroecology and Organic Agriculture. The internship activities took place at the three campus farms, gardens, produce stands, and cafés. Students were engaged in learning experientially for 6 to 12 hours per week alongside our seasoned researchers,

production managers and student staff. At UC Davis, 148 students participated in internships across 9 projects related to organic agriculture (African Food Basket, Ecological Garden, Flower Project, Fresh Focus, Kids in the Garden, Market Garden, Student Collaborative Organic Plant Breeding Education or SCOPE, Farm Shop, Vineyard). Of this total, 69 students returned for at least one additional quarter, in which they deepened their knowledge in organic agriculture by staying within a project or expanded their knowledge of organic agriculture by shifting to a different project. Summer interns (45) participated in weekly seminars with sustainable agriculture professionals from UC Davis, UC Agriculture and Natural Resources, and the regional agriculture community. Five new internships were created related to food sovereignty (African Food Basket & Fresh Focus Seed Saving) that directly supported underrepresented BIPOC students. Relatedly, 15 graduate and undergraduate students enrolled in AAS190 - Race & Agriculture in Black California, physically hosted and partially staffed by the UC Davis Student Farm. The UC Davis PLS49 - Organic Vegetable Production class hands-on activities and staff were supported by this grant. Over 200 UC Davis students conducted field research in classes related to organic agriculture and agroecology, with student and professional staff supported by this grant. UC Berkeley, supervised interns worked at the campus Oxford Tract farm, focused on student-led workshops and programming, including regular work days, communications, and travel arrangements. UC Berkeley held Alumni Career Panels and Research Pathways, taking 8 students on a field trip to UC Santa Cruz to participate in a panel with local organic farmers discussing crop rotation, soil conservation, no till methods, and cover crop techniques. We co-presented research on organic no-till methods as modes for sustainable agriculture then toured the farm and gardens at UC Santa Cruz. UC Berkeley provided three workshops at Oxford Tract Farm, including instruction on: no-till, land sovereignty, processing food for added value, herbal for 30 students from UC Berkeley and UC Santa Cruz for 3 hours as part of a student run DeCal course at Berkeley Student Farms. The workshop trained 12 UC Berkeley students and 30 community members on how to use BCS tractors for productive urban farming. The group also visited UC Davis to learn about tractor training in a full day train-the-trainer workshop, to prepare for managing training for UC Berkeley students. How have the results been disseminated to communities of interest? UC Santa Cruz has reached our target audience of underrepresented undergraduate students by creating and utilizing a lower division, large capacity course (ENVS 80F - introduction to Agroecology and Sustainable Food Systems) that also meets a General Education requirement. This course includes 3 hours per week of fieldwork in a discussion section that brings students to the campus farm and cafe over a 10-week quarter period. These direct experiences in organic farming and the broader food system often sparks interest and inspiration to get further involved. The course has between 100-170 students enrolled who are curious about agroecology and organic food and farming. We guest present and publicize additional educational opportunities within this course, encouraging interested students to take the next step and intern with us, consider pursuing the Agroecology major and related coursework. We make students aware of student staff employment opportunities, and the Leadership Development Program as a whole. We also publicize any fellowship and scholarship opportunities that we and others may offer. This course fieldwork activities at the UC Santa Cruz farm are supervised and near-peer mentored by Leadership Development Program student staff who are underrepresented and were once in the shoes of the new-to-campus undergraduate students. We create an intergenerational mentoring and advising structure where underrepresented students can role model and recruit from within relatable communities of students. Outreach for all of the programming opportunities mentioned above is also emailed to advisors, specifically focused on 1st year students and transfers. Opportunities are also shared through Student Affairs professional staff and their list serves, such as the Hispanic Serving Institution (HSI) Initiative, the Office of African, Black, and Caribbean (ABC) Student Success, and Educational Opportunities Program (EOP). Our staff are present at partner events with these units and seek out underrepresented students who may be interested in Agroecology and Organic Agriculture. We make presentations in classes with similar interested student groups. We manage student facing resource-focused email lists for Agroecology major students and Food, Agriculture, Natural Resources and Human Sciences engaged scholars. Internships, Leadership Development Program employment positions, and Field Quarter enrollments are all recruited using the activities above. What do you plan to do during the next reporting period to accomplish the goals? Major goals for the next year (Y3 of the project) are to: Begin to establish a UC System-wide Field Quarter administrative home within UC Agriculture and Natural Resources Organic Agriculture Institute, in partnership with UC Santa Cruz Environmental Studies Department faculty and academic staff. Redesign the UC Agroecology Field Quarter and offer it in Summer 2025, based on experiences and feedback from students, instructors, hosts, and partners. Partners will create a shared logic model based on feedback and previous course experience. Evaluation tools will be revised, and implemented, for further course improvement. Complete the intercampus exchange advising conceptual development, with UC Santa Cruz leading analysis, design, and final drafting of advising materials for transfer credit agreements, and related protocols. Project partner faculty and staff will work to provide advising to faculty, staff advisors, and student peer advisors guiding materials on the instructional opportunities at each campus as well as information on how to best host and support students enrolling at partner campuses UC Santa Cruz and UC Agriculture and Natural Resources Organic Agriculture Institute staff will research and produce critical mentoring professional development activities and resources for UC Ag and Natural Resources, Organic Ag

Institute mentors focused on underrepresented student support. Project staff will develop critical mentoring competency workshops for working with underrepresented students. Provide professional development training workshops for UC Agriculture and Natural Resources researchers. The Co-PDs and Key Personnel will research and develop advising protocols and materials for the UC Agriculture and Natural Resources Internship and Mentoring program. UC Santa Cruz will finalize the Leadership Development Program logic model and outcome development based on the recent feedback from stakeholders, and develop an outcome survey for UC Santa Cruz's Leadership Development Program team. We will work closely with other UC campuses to explore and support their evaluation development in their specific contexts. UC Santa Cruz will implement professional development workshops for their Leadership Development Program based on staff and student needs and interests.

**Impacts** What was accomplished under these goals? The two major accomplishments in the Y2 reporting period included piloting the UC System-wide Agroecology Field Quarter or "Supercourse", and building the programmatic foundation for the student Leadership Development Program. Additionally, project partners increased and improved educational opportunities in organic agriculture through internships and employment on campus organic farms, as well as increased the numbers of people gaining University of California bachelor degrees focused on organic agriculture research, extension and industry. All of these curricular advancements occurred in the context of increasing support and success of underrepresented students pursuing UC degree programs and careers. Successfully piloting the UC Agroecology Field Quarter accomplished significant progress towards our project's primary three goals. Our 1st goal of creating quality organic education was accomplished by gaining UC Santa Cruz Academic Senate course approval and Chancellor course fee exception approval to launch the UC Agroecology Field Quarter in Summer 2024. Students from three different UC campuses (UC Berkeley, UC Santa Barbara and UC Santa Cruz) enrolled, with 50% of students reporting they visited farm projects in the communities they grew up in. Enrolling students from different UC campuses officially initiated the UC intercampus exchange program in teaching organic agriculture practices and research across campuses. Thirteen students enrolled in the first UC Agroecology Field Quarter, 11 of which identified as underrepresented by race and gender. More than 50% of students enrolled reported that the course offering reduced attendance costs and shortened their time-to-degree. All enrolled students completed the course. Given the UC Agroecology Field Quarter enrollment demographics, we accomplished the project's 3rd goal of increasing support for underrepresented students. All students were invited to take an online post-course survey three weeks after the course conclusion. Seven students responded (54% response rate). The findings showed clear progress towards our 2nd goal, to increase degree completion and enter organic careers. All survey respondents reported that the course helped increase their understanding of organic agriculture (57% 'a great deal' and 43% 'a lot'). Similarly, all respondents were either 'extremely likely' (86%) or 'likely' (14%) to contact someone they met through this course for both academic opportunities (internships, coursework, etc.) and for work opportunities (jobs before or after graduation). All survey respondents (100%) 'strongly agreed' that the course helped them find a pathway forward in academia that resonates with them, and all agreed (86% 'strongly agreed' and 14% 'agreed'), that the course helped them find a pathway forward in work/career that resonates with them. Nearly 90% of students reported they would "Definitely recommend the Field Quarter to other students." Direct student feedback through the survey and end of session focus group exemplifies and expands on all these findings. For example, students stated the course was "Life changing and fun! Exhausting but worth it! I feel more confident in my career path after college now", and "The course gave me tangible ways to take next steps. Seeing young people in these farming operations makes career paths less daunting" and "When I started college, I found it hard to connect. Traveling in this course helped me break out of my mold." Another student reported "Visiting different sites across California is a really valuable experience and spending time with like-minded students, faculty, and people certainly gives you a chance to grow and refine." These findings further show progress to our 3rd goal, to increase the success of underrepresented students, in that the majority (71%) of evaluation respondents were from racially underrepresented backgrounds. We continued developing outcomes for the Leadership Development Program. Building on the efforts of the UC partners in Y1, we worked with the UC Santa Cruz Leadership Development Program core staff team to further develop these outcomes, created a logic model, and integrated a philosophical and theoretical framework to connect academic and co-curricular goals with a broader theory of change. This contributed to goal 1, increasing the quality of programming. Outcomes continued to be developed through a participatory process, by obtaining input from professional staff supervising undergraduates and undergraduate student staff participants. At UC Berkeley, 32 students participated in Spring capstone internships with community-based organizations, such as school district gardens and kitchen classrooms, farmers' markets, botanical gardens, coalitions, seed libraries, and anti-hunger organizations. Students gained practical experience working with the community, and valuable hands-on career readiness in an organic food system. One student reported that the internship, "Expanded my passion for Food Systems and gardening and made me realize that volunteering at a similar org is something I want to continue in my career". "I think one of the skills I was lacking before this experience was hands-on field work so this opportunity filled that gap in my skill set." These impacts for students point to the project accomplishing an increase in the quality of undergraduate educational opportunities in organic agriculture, and increase in the numbers of students pursuing careers in

organic agriculture. Publications Progress 09/01/22 to 08/31/23 Outputs Target Audience: The primary target audiences for this project's reporting period included underrepresented Agroecology, Sustainable Agriculture and Food Systems undergraduate major students, undergraduate student interns, and undergraduate students enrolled in the campus farm-based Leadership Development Programming at UC Santa Cruz, UC Davis, and UC Berkeley.

1. Internship enrollments. Across the three UC campuses, there were 463 student interns (UCSC - 156, UCD - 227, UCB - 80) enrolled in for-credit internships related to Agroecology and Organic Agriculture. The internship activities took place at the three campus farms, gardens, produce stands, and cafés. Students were engaged in learning experientially for 6 to 12 hours per week alongside our seasoned researchers, production managers and student staff.

2. Student mentoring, recruitment, advising and retention through the Student Leadership Development Programming. The project coordinated 144 Student Leadership staff across the 3 partner campuses to act as peer mentors and role models for advising early career undergraduate students pursuing hands-on organic agriculture and agroecology education. Of the 144 students, UCSC employed 86 undergraduate student staff, UCD employed 2 Graduate students and 47 Undergraduate student staff, and UCB employed 9 Undergraduate student staff. Some of these students were paid by the funds from this grant; others were paid for by multiple different sources, but still supported the main goals and objectives of the project. There were a total of 62 undergraduates enrolled in the UCSC agroecology major's degree program, 100 were enrolled in the UCD Sustainable Agriculture and Food Systems major degree program, and 40 were enrolled in the Food Systems minor program.

Changes/Problems: Year 1 project deliverables were significantly limited in scope and delayed in completion due to the unanticipated loss of nearly all the project's Key Personnel to departures and leave periods. This occurred across the three lead partner campuses. At UCSC, Key Personnel, Center Farm and Research Lands Manager, Darryl Wong left their position, and was not replaced. Key Personnel, Jan Perez took personal leave for a significant period of the year. At UC Berkeley, Key Personnel, Berkeley Food Institute Executive Director, Nina Ichikawa, left their position and was not replaced. At UC Davis, Key Personnel and Student Farm Director Katharina Ullman left their position and was replaced with a new Director from outside UC Davis. Hiring is underway for replacing these Key Personnel across the partner campuses and accelerated progress is expected in Year 2 of the project.

What opportunities for training and professional development has the project provided? This OREI project is entirely dedicated to professional development through the expansion of undergraduate degree programs and curricular offerings. Please see above for more details.

How have the results been disseminated to communities of interest? We have not yet disseminated results to communities of interest.

What do you plan to do during the next reporting period to accomplish the goals? We plan to accomplish the remaining Year 1 project deliverables within the next reporting period. Year 1 project deliverables were significantly limited in scope and delayed in completion due to the loss of nearly all the project's Key Personnel to departures and leave periods. The following are the deliverables not yet addressed in Year 1 that are planned for completion in Year 2. The Co-PDs and Key Personnel at each campus will complete the intercampus exchange advising conceptual development, with UCSC leading analysis, design, and final drafting of advising materials for transfer credit agreements, and related protocols. Project staff will work to provide advising faculty, staff advisors, and student peer advisors guiding materials on the instructional opportunities at each campus as well as information on how to best host and support students enrolling at partner campuses UCSC and ANR Organic Agriculture Institute staff will research and produce critical mentoring professional development activities and resources for UC ANR mentors focused on underrepresented student support. The Co-PDs and Key Personnel will research and develop advising protocols and materials for UC ANR Internship and Mentoring program. The Co-PDs and Key Personnel will research and produce critical mentoring professional development activities and resources for UC ANR mentors focused on underrepresented student support. Project staff will develop critical mentoring competency workshops for working with underrepresented students. Provide professional development training workshops for UC ANR researchers. Project participants will continue researching, designing, and beginning to establish a UC System-wide Field Quarter administrative home within UC ANR, in partnership with UC Santa Cruz Environmental Studies Department faculty and academic staff. The project evaluator will work on the following activities: Finish development of participatory evaluation plan, particularly including students in the development process. Conduct formative evaluation for supercourse and exchange activities, to better recruit students. Develop instruments and implement evaluation for Leadership Development Program and the Supercourse.

Impacts What was accomplished under these goals? The accomplishments occurred mainly under the project's first goal (1) increase the quantity and quality of undergraduate educational opportunities across the U. of California (UC) in organic agriculture, and (3) increase the support and success of underrepresented students pursuing these UC degree programs and careers. In the first year, the project began to integrate curricular and career advising, and mentored fieldwork in production-based and research-based internships. This happened primarily through the expansion of the Student Leadership Development Program at the campus instructional and research farms. The professional staff employed by this grant at each campus farm provided significant internship coordination and hosted a total of 463 undergraduate student interns. UC ANR faculty and staff researchers conducted organic agriculture research and extension projects, state-wide. A Bay Area "No-Till" research field trip to UC Berkeley was co-organized by campus and

ANR partners and attended by 25 undergraduate students. Key Person Muramoto developed and updated the California Organic Systems Researchers Map, which indicates the most relevant ANR researchers with whom we will develop relationships to set up mentorship with undergraduate student interns. UCSC Co-PI Parr initiated development of the Supercourse and Field Quarter program using the UC Santa Cruz Center for Agroecology farm as the primary host site, with visits to organic farm operations, UC ANR research stations and UC Davis and UC Berkeley campuses. The first pilot of the Supercourse is scheduled for summer 2024. Project staff researched and began designing a UC System-wide Field Quarter administrative home within UC ANR, in partnership with UC Santa Cruz Environmental Studies Department faculty and academic staff. Undergraduate Student Leadership Development Program staff acted as peer mentors and role models for advising earlier career undergraduate students pursuing organic agriculture and agroecology education and careers. UCSC employed 86 undergraduate student lead staff, UCD employed 2 graduate students and 47 undergraduate lead student staff, UCB employed 9 undergraduate lead student staff. Project staff developed programming linkages between the UC Davis SCOPE project and UC Santa Cruz with organic seed partners on the Central Coast. As part of these linkages, UCSC started a common bean field research trial. In addition, project staff from UCD and UCSC developed a new, now funded OREI SCOPE 3.0 project that will continue building the student-centered organic seed breeding program across our campuses. UCSC project staff held an alumni career panel and research pathways workshop, with Parr presenting. UCSC project staff created an internship as part of a partnership between UC Santa Cruz organizations and programs including the Educational Opportunities Program, Hispanic Serving Institution (HSI) Initiatives and Career Success, and the Graduating and Advancing New American Scholars: Promoting Postbaccalaureate Opportunities for Hispanic Americans (GANAS) grant. This internship and partnership will provide targeted curricular and career advising. A GANAS intern was hosted by the UCSC campus farm research and education programming. UCB created and hosted a career panel and research pathway workshop featuring UC alumni and student researchers. The event was attended by 35 undergraduate students. UCSC Project staff worked to create program linkages between our OREI program activities and two other USDA-funded grants on our campus: (1) Research and Extension Experiences for Undergraduates Grant (a.k.a. SUPERDAR) and (2) the Multicultural Scholars Program Grant). As part of these program linkages, students joined a Bay Area "No-Till" Field Trip to regional organic farms and campus farms at UCSC and UCD. Relatedly, UCSC hosted a jointly organized "Soil as Teacher" workshop, attended by 25 undergraduates, including UCSC USDA-funded fellowship students. Program staff began development of a participatory evaluation framework. They identified desired short, medium and long-term outcomes for the effort to include in evaluation instruments. Publications

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# Solutions for Controlling Mastitis and Improving Milk Quality in Organic Dairy Farms: an Integrated Approach

<b>Accession No.</b>	1028981
<b>Project No.</b>	COL0-2022-04034
<b>Agency</b>	NIFA COL\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37880
<b>Proposal No.</b>	2022-04034
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2026
<b>Grant Amount</b>	\$2,999,946
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Pinedo, P.; Cabrera, VI, E.; Schuenemann, GU, MA.; Heins, BR, .; De Vries, AL, .; Miller-Cushon, EM, KA.; Lynch, RO, .; Silva, ER, .

## NON-TECHNICAL SUMMARY

Mastitis is the most common and costly disease affecting organic dairy cows, ranking within the top two reasons for early removal of cows within US organic herds. Effective mastitis control is of utmost importance, but options for prevention and treatment are limited in organic herds. The long-term goal of this integrated proposal is to identify and develop cost-effective management solutions that will control mastitis, improve milk quality, promote welfare, and enhance the sustainability of the organic dairy community. The overall goal is to develop and assess an integrated systems approach for mastitis control and welfare. The central hypothesis is that combining environmental and animal-level solutions will be effective to control mastitis and improve milk quality and cow welfare. We propose the following specific objectives: 1) Developing and delivering a comprehensive mastitis and milk quality Extension program with emphasis on prevention and control practices. 2) Assessing the effect of management practices during the dry period on early lactation mastitis and testing the efficacy of novel interventions at dry-off to develop mastitis control solutions; 3) Developing a mastitis index as financial and welfare measures of the mastitis and milk quality burden to identify top areas for improvement; 4) Evaluating the impact of cow comfort on mastitis and milk quality by using precision technologies; and 5) Identifying leading risk factors associated with mastitis and milk quality in organic dairies. Developing and delivering an integrated approach with best solutions will reduce mastitis, improve milk quality, and enhance the long-term sustainability of organic dairy herds.

## OBJECTIVES

The long-term goal of this integrated proposal is to identify and develop cost-effective management solutions that will control mastitis, improve milk quality, promote welfare, and enhance the sustainability of the organic dairy community. The overall goal is to develop and assess an integrated systems approach for mastitis control and welfare. The central hypothesis is that combining environmental and animal-level solutions will be effective to control mastitis, improve milk quality, and cow welfare. We propose the following specific objectives: 1) Developing and delivering a comprehensive mastitis and milk quality Extension program with emphasis on prevention and control practices. 2) Assessing the effect of management practices during the dry period on early lactation mastitis and testing the efficacy of novel interventions at dry-off to develop mastitis control solutions; 3) Developing a mastitis index as financial and welfare measures of the mastitis and milk quality burden to identify top areas for improvement; 4) Evaluating the impact of cow comfort on mastitis and milk quality by

using precision technologies; and 5) Identifying leading risk factors associated with mastitis and milk quality in organic dairies. Developing and delivering an integrated approach with best solutions will reduce mastitis, improve milk quality, and enhance the long-term sustainability of organic dairy herds.

## APPROACH

**Objective 1 (Extension)** Sub-objective 1A and 1B: With the continuous assistance of the advisory panel and considering the diverse organic dairy farm community, educational needs will be identified for the workshops and webinars starting in year 1 through year 4. Findings from the research Objectives will also be integrated and used during the educational training. Workshops will consist of 1-day training modules. All training workshops and webinars will be offered at no cost and open to all stakeholders. Each identified educational topic or area will be developed around common themes with several interconnected topics delivered using short webinar to support the overall theme. The webinar series will be recorded and available online. The content of the modules will be captured digitally and loaded to electronically assessable formats. We will promote participants' interaction through short oral presentations followed by discussion and hands-on demonstrations. For Sub-objective 1C, we will focus on changes in attendees' satisfaction, knowledge gain, and willingness to adopt best management practices evaluated by 1) pre- and post-tests of knowledge and questionnaire surveys that will be developed to set a baseline on performance of herds and to measure willingness to adopt best management practices. Participants will have the opportunity to evaluate the program and instructors and provide feedback using predesigned evaluation instruments and the Evaluation of Effective Extension Teaching (The Ohio State University).

**Objective 2 (Dry-off methods)** Sub-objective 2.A.: We will perform a controlled experiment to evaluate the effect of two separated strategies at dry-off: i) Gradual cessation of milking before dry-off; and ii) Intramammary application of a natural product based on carvacrol administered at dry-off. Based on records indicating the future dates for dry-off, 1,764 cows will be randomly assigned to be submitted to either abrupt or gradual dry-off (1x/day final week of lactation. Fifty percent of the cows in each group will be administered a natural product based on essential oils containing carvacrol after the final milking at dry-off. Milk samples will be collected at the last milking and within the first 3 days of lactation for culture and for somatic cell count (SCC). A third sample will be collected at 15 days in milk for SCC. Culture data and SCC will be compared among the four groups. Occurrence of clinical mastitis within 30 days in milk is another relevant outcome of interest. Milk analyses will be completed at the Quality Milk Production Services, Animal Health Diagnostic Center at Cornell University.

**Objective 3 (Mastitis index)** Sub-objective 3.A.: We will rely on the Co-PDs contacts and the assistance of the Advisory Committee members (especially Dr. Guy Jodarski, Organic Valley Veterinarian) to reach our target farms for surveying. Co-PDs and graduate students will perform field visits using a survey instrument that will be pre-tested in a focus group. The surveys will collect data about specific risk factors associated with on-farm management aspects related to mastitis and milk quality, including housing, milking, and dry period management. Study personnel will score cows for udder hygiene and teat condition. Information regarding pasture and crop management and nutritional quality will be made available to us (Organic Valley databases).

Sub-objective 3.B.: We will calculate failure costs of mastitis in organic dairy cows using methodology in the literature, including milk loss, reduced reproductive performance, increased culling, loss of organic status, and lower milk quality. We will use the dynamic programming approach of De Vries (2004, 2006) and new extensions for cow specific modeling currently in development (USDA NIFA FACT 2019-67021-28823) and from Cha et al. (2011) and other published mastitis models. This modeling approach is needed because failure cost depends on the best decision made and the opportunity and their costs that directly or indirectly help prevent mastitis on organic dairy farms using data from our sub-objective 3A surveys and the approach of Van Soest et al. (2016). The financial mastitis index will be expressed in dollars per cow per year). Our methodology of the financial mastitis index allows to be approximated by organic dairy farms by entering their data in a user-friendly tool.

Sub-objective 3.C.: We will identify on-farm practices that lead to low mastitis index values using analysis of variance. These practices are top management opportunities for reducing the mastitis burden.

**Objective 4 (Cow behavior)** Sub-objective 4.A. and 4B: Methods to collect precision dairy behavior data and other cow event data include backups from on-farm software and access to cloud-storage of dairy data. We will use SAS to organize the data due to our long-term experience with that data management and statistical software. We will analyze the collected database with classical regression as well as random forest machine learning methods. For the association analyses, data from affected cows will be matched with healthy controls and analyzed to determine relationships between behavioral traits indicative of cow comfort and the risk of developing mastitis.

**Objective 5 (Risk factors)** Sub-objective 5.A. Farm data provided by Dairy Herd Improvement Association (DHIA) and Organic Valley will be edited and organized in lactation records, differentiating organic and conventional farms. Data from individual cows will be the base for herd level calculations. Files will be prepared in a format that is adequate for the subsequent analyses to be completed with SAS (SAS institute Inc., Cary, NC).

Sub-objective 5.B. Statistical analyses will depend on the nature of the variables under analysis (continuous/categorical). Briefly, ANOVA, logistic regression, and time to event analysis will be considered for the analyses. The focus will be placed on

characterizing mastitis and milk quality at the individual and at the herd level, comparing variables related to the cow and the herd, as well as considering the organic and conventional status of farms. Sub-objective 5.C. Association analysis will help identify important risk factors for mastitis and suboptimal milk quality. Of special interest for organic dairies are breed, longevity, milk yield, dry-off and dry period characteristics, among others. Herd level variables include herd size, type of housing, location, seasonality, etc. Univariate calculations of incidence risk for mastitis will be used to describe overall disease frequency. Additionally, univariate survival analysis will be used to determine the Kaplan-Meier median DIM to the first occurrence of mastitis event per lactation. Associations between risk factors and udder health outcomes will be assessed through multivariable logistic regression. Sub-objective 5.D.: Datasets from 8 large organic herds in CO and TX, including 95,000 lactations will be organized into records with the data collection starting at the dry-off date from the previous lactation in multiparous cows or at calving in primiparous cows. Milk yield and SCC are also available. Incidence risk for mastitis will be calculated considering stratification by multiple variables, such as parity number, 305 d ME milk yield during the previous lactation, last milk yield recorded before dry-off, season of dry-off, access to grazing at dry-off, length of the dry period, length of the close-up period, gestation length, season of calving, access to grazing at calving, and concurrent health conditions. Progress 09/01/23 to 08/31/24 Outputs Target Audience: The target audience reached during the reported period included dairy veterinarians and consultants, the scientific community (including academia (faculty and students)), and dairy farmers, in organic and conventional systems. This audience was reached through abstract presentations (2024 American Dairy Science Association Annual Meeting, University of Florida, Animal Sciences Graduate Student Symposium), short extension articles, and a series of webinars and workshops. Specifically, the extension component led by Dr. Gustavo M. Schuenemann has delivered a total of 5 webinars, 3 in-person workshops, 11 extension presentations, and developed 3 short extension articles reaching multiple audiences that include dairy producers, personnel, students, veterinarians, consultants, allied companies, extension professionals, and academia. Changes/Problems: The Highly Pathogenic Avian Influenza (HPAI) outbreak in dairy cattle that was confirmed by USDA in March 2024 significantly impacted the beginning of the farm visits (survey on mastitis risk factors and milking parlor and facilities assessment) planned for 100 dairies in multiple states (CO, MN, WI, OH, NY, among others). Our team developed biosecurity protocols to be followed when the visits could be started. As the potential role of milk in the transmission of the virus has gained support, more restrictions for farm visits developed, as a milking parlor assessment is a key component of the assessment of the farm. After these actions, we have been able to start with the farm visits (including milking parlor equipment assessment) and a total of dairies have been surveyed in OH, NY, and CO). What opportunities for training and professional development has the project provided? Graduate students have been involved in the development of the survey instrument (Colorado State University, University of Wisconsin, The Ohio State University, University of Minnesota). These students have participated in farm visits to complete the surveys and milking parlor/farm facilities assessment (~38 organic dairies in OH, CO, TX, MN, and NY). Graduate students have also been involved in developing and coordinating field studies conducted in CO (coordinating farm visits, IACUC updates, compliance with organic regulations, enrolling cows, administering treatments, completing follow-up of mastitis cases, etc.). Finally, these students have received intense training in managing large databases and completing data analysis using statistical software (R). How have the results been disseminated to communities of interest? Study results have been mostly disseminated to the scientific community through presentations at scientific conferences (2024 American Dairy Science Annual Meeting, Palm Beach FL). The extension materials have been disseminated by a series of webinars (n=5), in-person workshops (n=3), extension presentations (11), and short extension articles (3). What do you plan to do during the next reporting period to accomplish the goals? Our goals during the next reporting period are to complete the survey by visiting 100 dairies and finalize the data organization originating from these assessments (Objective 3). We are expanding our efforts to multiple states, including OH, NY, MN, GA, CA, and OR. Second, the field study on dry-off preventive therapy should be completed and the data analyzed to be presented in scientific conferences and subsequently published (Objective 2). Related to Objective 4, we will continue the analyses on the associations between behaviors indicative of individual cow comfort and welfare and the likelihood of developing mastitis by using precision technologies. We will expand the extension activities (Objective 1) presenting our results from this project. Finally, we will continue organizing and analyzing the following databases: Dairy Herd Improvement Association (DHIA): Herd and cow information, including lactation records from both organic-certified herds and conventional herds in multiple regions from across the US to explore leading risk factors associated with mastitis and suboptimal milk quality in organic dairies (Objective 5). Organic-certified grass feed herd in TX: Cow comfort and mastitis risk (manuscript in progress) Robotic dairy farm in Colorado: Cow behavior and mastitis by mastitis-causing pathogen (manuscript in progress) Organic-certified herds in TX-CO: Retrospective analysis to investigate associations between dry-off and dry period and risk of mastitis. Impacts What was accomplished under these goals? We have developed a survey that we are applying through visits to dairies (Objective 3). Visits are occurring in both cool and warm seasons for each dairy, until completing a total of 100 farms. The instrument is available both on paper and as an electronic instrument (Qualtrics) that can connect online with the general database. The survey consists of three main sections: i) a

questionnaire to be responded by the farmer in a in personal interview; ii) an assessment of the milking parlor and udder health during the milking; and iii) an assessment of facilities for the analysis of risk factors for mastitis and suboptimal milk quality. The information that has been collected at the farms is crucial for the completion of Objective 3, which is focused on developing a mastitis index as a comprehensive financial and non-financial measure of the mastitis and low milk quality burden. The goal is to quantify mastitis levels in individual organic dairies and determine the best opportunities for prevention and control through the use of field data. During this period, we have developed a working relationship with the technical team at Organic Valley to identify and reach organic dairy farms that could be included in the survey activity. Supporting Objective 2, our field study testing a natural product for dry-off therapy in an organic dairy in CO is close to completion. This experiment aims to test the efficacy of a natural product based on essential oils containing carvacrol. The product is applied after the last milking at dry-off (UterFlush), which is the most efficient time for treating and controlling mastitis. This is a commercial natural product that can be used in organic-certified cows. We assessed the efficacy of the treatment by performing milk bacteriological cultures before product application (baseline at the time of dry-off) and in the subsequent lactation (3 and 15 days post-calving). We have also completed a careful examination of the udders, where signs of inflammation were assessed. A total of 203 cows were enrolled in the experiment and about 80% of the animals have completed the follow-up sampling and clinical examination. The extension component (Objective 1, led by Dr. Gustavo M. Schuenemann) has completed the following activities: Programs and Workshops: 2024 Transition cow management program. The 5-day program was held in Columbus, OH on April 1-5, 2024 with a total of 52 participants from 7 US states. Audience: veterinarians, nutritionists, Extension personnel, dairy producers, and students). 2024 dairy scouting tour: 1-day visit to different dairy farms in Ohio, Michigan and Wisconsin to see housing facilities (for cows and calves) and precision technology. A total of 43 participants attended the tour from 4 US states. Audience: veterinarians, nutritionists, Extension personnel, and students). On-farm education: At least 1-day visit to 28 certified organic dairy farms located in OH and NY to assess their milking equipment/routine, housing facilities for animals and management/protocols. The overall emphasis was on improving milk quality and animal health. A total of 60 participants from 2 US states. Audience: Dairy producers, veterinarians, Extension personnel, and students). Webinars: A total of 5 webinars with 7692 participants who attended or watched the webinars afterward. A total of 10 CE certificates were provided to veterinarians. Cultivating sustainable solutions to nourish generations by Dr. Ron Shuller (400 registered) Molds and mycotoxins in dairy cattle: Effects, diagnosis, and control by Gustavo Schuenemann (3070 registered) Udderly Important: Solutions to mastitis challenges by Dr. Luciana da Costa (460 registered) The DairyPrint model: Helping dairy farmers towards higher sustainability by Dr. Victor Cabrera (471 registered) Maternity and newborn dairy calf care management Gustavo Schuenemann (1300 registered) Presentations: Gustavo Schuenemann. Parlor management and milking routine. Celina, OH. January 31, 2024. Gustavo Schuenemann. Calm animal handling for best performance. Celina, OH. February 01, 2024. Gustavo Schuenemann. Managing health alerts with precision algorithm. Celina, OH. February 01, 2024. Gustavo Schuenemann. Solutions to optimize dry matter intake. 2024 Dairy Herd Manager Retreat. Wisconsin Wells, WI. February 10, 2024. Gustavo Schuenemann. Solutions to optimize dry matter intake. 2024 Dairy Herd Manager Retreat. Frankenmuth, MI. February 17, 2024. Gustavo Schuenemann. Transition cow management: Solutions to optimize dry matter intake. 2024 High Plains Dairy Conference. Amarillo, Texas. March 5-6, 2024. Gustavo Schuenemann. Back to basics in transition cow management. 2024 Dairy Herd Manager Retreat. Arnolds Park, IA. March 9, 2024. Gustavo Schuenemann. Interaction of mycotoxins and gestation length with health and survival of dairy cows and calves. Global Ruminant Days 2024. June 6, 2024. Vienna, Austria. Gustavo Schuenemann. Maternity, colostrum and newborn dairy calf care. Appleton, Wisconsin. June 26, 2024. Gustavo Schuenemann. Leadership and communication: Five principles of teamwork. July 13, 2024. Paulding, OH. Gustavo Schuenemann. Maternity, colostrum and newborn care. Custar, OH. September 24, 2024. Short articles: Gustavo Schuenemann. First colostrum meal is vital for calf survival and health. Farm and Dairy. February 22, 2024. Gustavo Schuenemann. Avoiding dairy calf mortality. Country Flocks. February 22, 2024. Gustavo Schuenemann, Shaun Wellert, Justin Kieffer, Armando Hoet, Greg Habing, and Owen Mickley. Avian Influenza Detected in Dairy Cattle. Buckeye Dairy News. July 11, 2024. We have received a massive database from Dairy Records Management Systems (DRMS, North Carolina State University) that includes detailed information from organic dairies across the US. This information will be used to complete Objective 5: Develop a mastitis database with data originating from the Dairy Herd Improvement Association (DHIA) including lactation records from both organic-certified herds and conventional herds in multiple regions from across the US. Associated with Objective 4, we obtained and edited a large database that compiles information from organic-certified cows (Holstein, Jersey, and Holstein x Jersey crosses) maintained in an organic-certified grass-fed herd in Central TX, USA. These cows are milked in a robotic system, which provides massive detailed information on milking behavior and performance. Health data (mastitis and other diseases) is also available for this research. In specific, studies in progress are centered on three areas: i) milking behavior and performance of primiparous and multiparous cows, ii) milking behavior dynamics before clinical mastitis diagnosis by pathogen category, and iii) effect of pre-milking waiting time on milking behavior and performance. As we continue with this analysis we expect to gain a better understanding of

the impact of cow comfort and subsequent behavior on the occurrence of mastitis. Finally, we built a second database from a large robotic dairy in CO where similar behavioral parameters are recorded in every milking, providing an opportunity for the analysis of behavioral patterns associated with clinical mastitis caused by different categories of pathogens. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Use of the VaDia vacuum recorder for detection of factors related to mastitis and somatic cell count in organic dairy herds. 2024 B. J. Heins, K. T. Sharpe, P. J. Pinedo, A. DeVries, E. Miller-Cushon, V. E. Cabrera, E. M. Silva, R. A. Lynch, and G. M. Schuenemann. J. Dairy Sci. 107 (Suppl.1) ADSA 2024 Annual Meeting June 2024, West Pam Beach FL. Type: Other Status: Other Year Published: 2024 Citation: Description of milking procedures in organic dairy herds UF Animal Sciences Graduate Student Symposium C. NinodeGuzman, L. Prada, S. Bellinzoni, P. Muñoz, C. Hernandez, C. Ibarguren, R. Weng, G. M. Schuenemann, P. Pinedo, R. Lynch, V. E. Cabrera, B. J. Heins, A. De Vries. Type: Other Status: Published Year Published: 2024 Citation: Short extension article: Gustavo Schuenemann. First colostrum meal is vital for calf survival and health. Farm and Dairy. February 22, 2024. Type: Other Status: Published Year Published: 2024 Citation: Short extension article: Gustavo Schuenemann. Avoiding dairy calf mortality. Country Fairs. February 22, 2024. Type: Other Status: Published Year Published: 2024 Citation: Short extension article: c. Gustavo Schuenemann, Shaun Wellert, Justin Kieffer, Armando Hoet, Greg Habing, and Owen Mickley. Avian Influenza Detected in Dairy Cattle. Buckeye Dairy News. July 11, 2024. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Pathogen-specific clinical mastitis and its association with daily body condition score in dairy cows. 2024. P. Munoz-Boettcher, C. Hernandez-Gotelli, and P. Pinedo. J. Dairy Sci. 107 (Suppl.1). ADSA 2024 Annual Meeting June 2024, West Pam Beach FL Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Associations of pre-milking waiting time with milking behavior and performance of cows in an automated batch milking system. 2024. R. Weng Zheng, J. Velez, N. Rodriguez, and P. Pinedo. J. Dairy Sci. 107 (Suppl.1) ADSA 2024 Annual Meeting June 2024, West Pam Beach FL. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Milking behavior dynamics within the first 4 weeks of lactation in primiparous and multiparous cows in an automated batch milking system. 2024. P. Munoz-Boettcher, J. Velez, N. Rodriguez, and P. Pinedo. J. Dairy Sci. 107 (Suppl.1) ADSA 2024 Annual Meeting June 2024, West Pam Beach FL. Progress 09/01/22 to 08/31/23 Outputs Target Audience: The target audience reached during the reported period included dairy veterinarians and consultants, the scientific community (including academia faculty and students), and dairy farmers, in organic and conventional systems. This audience was reached through abstract presentations (2023 American Dairy Science Association Annual Meeting) and a series of webinars. In specific, the extension component led by Dr. Gustavo M. Schuenemann has delivered a total of 10 webinars (6 in 2022 and 4 in 2023) centered on multiple areas of best dairying practices, reaching an estimated of 3,083 participants (dairy producers, personnel, students, veterinarians, consultants, allied companies, Extension professionals, and academia) from 20 US states with ~3895 page views so far after presentations. Three additional webinars are scheduled for December 2023. In addition, a total of 28 in-person workshops were delivered from September 2022 to November 2023 reaching an estimated of 917 participants (Amish youth, dairy producers, personnel, veterinarians, consultants, students, allied companies, and Extension professionals) serving an estimated of 550,000 dairy cows from 10 US states (OH, IN, MI, MD, PA, TX, CO, SD, MN, VA, and WI). Several workshops are planned for 2024. Although the presented topics are not restricted to mastitis in organic dairies, all of them have an impact on health, welfare, and performance of the dairy cow. Changes/Problems: We have not faced specific challenges in developing the proposed objectives. However, checking compliance with the organic dairy certification in the therapeutics included in the field studies has taken some extra time and effort. What opportunities for training and professional development has the project provided? Graduated students have been involved in the development of the survey instrument (Colorado State University, University of Wisconsin, The Ohio State University). Grad students have also been involved in developing and coordinating the field studies to be completed in CO (coordinating farm visits, IACUC updates, compliance with organic regulations, etc.). How have the results been disseminated to communities of interest? Study results have been mostly disseminated to the scientific community through presentations at scientific conferences (2023 American Dairy Science Annual Meeting). The extension materials have been disseminated by a series of webinars (n=10) and in-person workshops (n=28). What do you plan to do during the next reporting period to accomplish the goals? Our first goal during the next reporting period is to complete the survey (100 dairies) and finalize the data organization originating from these visits (Objective 3). Second, the first field study on clinical mastitis therapy should be completed. Concurrently, a second study on dry-off preventive therapy should be initiated (Objective 2). Related to Objective 4, we will initiate the analyses on the associations between behavior indicative of individual cow comfort and the likelihood of developing mastitis by using precision technologies. We will expand the extension activities (Objective 1) focusing more specifically on mastitis and presenting our results from this project. In addition, as part of the efforts to disseminate best practices for milk production the following workshops are planned for 2024: 2024 Transition cow management. This 2-day event will cover nutrition-related information to optimize dry matter intake, health, and productivity of dairy cattle. The target audience are dairy producers, veterinarians, consultants, Extension personnel and

students. Registration will open in December 2023. The workshop will be held in Columbus OH in Spring 2024 (Date TBD). 2024 Dairy heifer replacement program. This 2-day event will cover housing, nutrition, and management information to optimize health, growth, and productivity of replacement heifers. The target audience are dairy producers, veterinarians, consultants, Extension personnel and students. Registration will open in December 2023. The workshop will be held in Columbus OH in Fall 2024 (Date TBD). 2024 Scouting dairy tour. This is an educational multi-state tour with various educational workshops delivered at selected locations (TBD) across US addressing dairy cattle health, nutrition, reproduction, replacement heifers, sustainability, and precision technologies of dairy farming. The target audience are dairy producers, veterinarians, consultants, Extension personnel and students. Registration will open in 2024 (Date TBD). Finally, a database with data originating from the Dairy Herd Improvement Association (DHIA) including lactation records from both organic-certified herds and conventional herds in multiple regions from across the US will be constructed to allow for the identification of leading risk factors associated with mastitis and suboptimal milk quality in organic dairies (Objective 5). Impacts What was accomplished under these goals? Significant research efforts during the reported period have been focused on developing the survey instrument that will be applied through visits to 100 dairies in two seasons (cool and warm seasons). The information that will be collected at the farms is crucial for the completion of objective 3, which is focused on developing a mastitis index as a comprehensive financial and non-financial measure of the mastitis and low milk quality burden. The goal is to quantify mastitis levels in individual organic dairies and determine the best opportunities for prevention and control through the use of field data. During this period, we have developed a working relationship with the technical team at Organic Valley to identify and reach the organic dairy farms that will be included in the survey activity. Supporting objective 2, our first field study is organized in an organic dairy in CO to test a natural therapy for clinical and toxic mastitis. The objective is to test the efficacy of a treatment for clinical mastitis in organic-certified dairy cows, consisting of intra-oral bolus application of a natural product based on electrolytes and sugars (treatment). This is a commercial natural product that can be used in organic-certified cows. We will assess the efficacy of the treatment by performing milk bacteriological cultures post-therapy and completing a careful examination of the udder, where signs of inflammation will be assessed. Treated cows will be compared with affected control cows receiving the standard therapy, which consists of an increased number of milkings to maintain the mammary gland empty of milk. Depending on the results of this trial, we will test the efficacy of this treatment as a dry-off therapy. The extension component (Objective 1, led by Dr. Gustavo M. Schuenemann) has established a series of webinars (6 in 2022 and 4 in 2023) centered on multiple areas of best dairying practices, reaching an estimated of 3,083 participants from 20 US states with ~3895 page views so far after presentations. A total of 28 in-person workshops were delivered from September 2022 to November 2023 reaching an estimated of 917 participants (Amish youth, dairy producers, personnel, veterinarians, consultants, students, allied companies, and Extension professionals) serving an estimated of 550,000 dairy cows from 10 US states (OH, IN, MI, MD, PA, TX, CO, SD, MN, VA, and WI). Although the presented topics are not restricted to mastitis in organic dairies, all of the contents have an impact on the health, welfare, and performance of the dairy cow. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Association between clinical mastitis and body condition score pattern and pregnancy at first artificial insemination. P. Munoz Boettcher<sup>1</sup>, A. De Vries<sup>2</sup>, D. Manriquez<sup>1,3</sup>, and P. Pinedo<sup>1</sup>, <sup>1</sup>Colorado State University, Fort Collins, CO, <sup>2</sup>University of Florida, Gainesville, FL, <sup>3</sup>School of Veterinary Medicine of Toulouse, Toulouse, France. 2023 American Dairy Science Association Annual Meeting, Ottawa, Ontario, Canada. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Benchmarking first-lactation organic Holstein, Jersey, and crossbred cows for production, somatic cell score, and days open in the United States. B. J. Heins<sup>1</sup>, K. T. Sharpe<sup>1</sup>, P. J. Pinedo<sup>2</sup>, A. De Vries<sup>3</sup>, E. K. Miller-Cushon<sup>3</sup>, V. E. Cabrera<sup>4</sup>, E. M. Silva<sup>4</sup>, R. A. Lynch<sup>5</sup>, and G. M. Schuenemann<sup>6</sup>, <sup>1</sup>University of Minnesota, Morris, MN, <sup>2</sup>Colorado State University, Fort Collins, CO, <sup>3</sup>University of Florida, Gainesville, FL, <sup>4</sup>University of Wisconsin, Madison, WI, <sup>5</sup>Cornell University, Ithaca, NY, <sup>6</sup>The Ohio State University, Columbus, OH. 2023 American Dairy Science Association Annual Meeting, Ottawa, Ontario, Canada. Type: Journal Articles Status: Published Year Published: 2023 Citation: D Kness, T Grandin, J Velez, J Godoy, D Manriquez, F Garry, P Pinedo. 2023. Patterns of milking unit kick-off as a proxy for habituation to milking in primiparous cows. JDS Communications 4(5):385-389. doi: 10.3168/jdsc.2023-0384. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Risk factors associated with clinical mastitis in certified organic dairy herds. P. Munoz Boettcher<sup>1</sup>, A. De Vries<sup>2</sup>, E. Miller-Cushon<sup>2</sup>, B. J. Heins<sup>3</sup>, V. Cabrera<sup>4</sup>, E. Silva<sup>4</sup>, R. A. Lynch<sup>5</sup>, G. M. Schuenemann<sup>6</sup>, D. Manriquez<sup>1,7</sup>, J. Velez<sup>8</sup>, and P. Pinedo<sup>1</sup>, <sup>1</sup>Colorado State University, Fort Collins, CO, <sup>2</sup>University of Florida, Gainesville, FL, <sup>3</sup>University of Minnesota, St. Paul, MN, <sup>4</sup>University of Wisconsin, Madison, WI, <sup>5</sup>Cornell University, Ithaca NY, <sup>6</sup>The Ohio State University, Columbus OH, <sup>7</sup>National School of Veterinary Medicine of Toulouse, Toulouse, France, <sup>8</sup>Aurora Organic Dairy, Platteville, CO. 2023 American Dairy Science Association Annual Meeting, Ottawa, Ontario, Canada.

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# Building Resilient Pest Management Strategies for Organic Hemp Systems

Accession No.	1029067
Project No.	COL0-2022-04053
Agency	NIFA COL\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2022-51300-38010
Proposal No.	2022-04053
Start Date	01 SEP 2022
Term Date	31 AUG 2025
Grant Amount	\$749,999
Grant Year	2022
Investigator(s)	Nachappa, P.; Uchanski, MA, .; Prenni, JE, .; Szczepaniec, AD, .; Trivedi, CH, .

## NON-TECHNICAL SUMMARY

The recent passage of the Hemp Access and Consumer Safety Act has cleared the way for organic farmers to increase hemp acreage and organic brands to develop and diversify new organic hemp products. However, there are many challenges to producing an organic hemp crop, particularly with regards to pest management strategies. For instance, lack of knowledge on the impact of organic management on crop resistance to hemp pests, and lack of information on efficacy of OMRI-approved insecticides. Hence, the long-term goal of this multi-disciplinary project is to create a robust and resilient organic hemp production system against arthropod pests which will provide growers with management tools to improve crop health and sustainability. The long-term goals will be accomplished by the four specific objectives: Objective 1. Quantify the effects of organic production systems on plant resistance to hemp pests through changes in rhizosphere microbiome. Objective 2. Evaluate hemp cultivars for resistance to hemp pests in organic production systems. Objective 3. Determine the effectiveness of OMRI-approved insecticides in combination with biological control for suppression of hemp pests. Objective 4: Develop effective methods to provide research-based information to hemp growers and other stakeholders. These objectives will be addressed through field experiments in certified organic hemp fields that has been under organic production for over 19 years at the Agricultural Research, Development and Education Center at Colorado State University. The research we propose addresses USDA-OREI program priorities: (1) and (6) and legislated goals (1), (2) and (3).

## OBJECTIVES

Long-term and short-term goals Our long-term goal is to create a robust and resilient organic hemp production system against arthropod pests which will provide organic hemp growers with management tools to improve crop health and sustainability. To achieve this, our proposal will focus on the short-term goals of understanding the role of rhizosphere microbiome associated with plant resistance to pests, impact of organic inputs (resistant cultivars, OMRI-approved insecticides, and natural enemies) in reducing pest populations, and dissemination of these results (Fig. 2). The short-term goals of the project will be accomplished by the following four objectives: Objective 1. Quantify the effects of organic production systems on plant resistance to hemp pests through changes in rhizosphere microbiome. Hypothesis: Organic production improves plant resistance to arthropod pests through changes in rhizosphere microbial communities. Objective 2. Evaluate hemp cultivars for resistance to hemp pests in organic production systems. Hypothesis: There will be variability among hemp

cultivars in host plant resistance to hemp pests in organic systems. Objective 3. Determine the effectiveness of OMRI-approved insecticides in combination with biological control for suppression of hemp pests. Hypothesis: Organic insecticides augmented by biological control can suppress key pests of hemp below damaging levels. Objective 4: Develop effective methods to provide research-based information to hemp growers and other stakeholders. Hypothesis: Access to information and recommendations for pest suppression in organic hemp production will increase adoption of sustainable pest management practices within the context of IPM

## APPROACH

**Objective 1. Quantify the effects of organic production systems on plant resistance to hemp pests through changes in the rhizosphere microbiome. Methods and feasibility.** The experimental design will be a completely randomized block with three replications/plots of each treatment (organic and conventional). For organic and conventional production, rooted clones of a certified CBD cultivar (Unicorn) will be excised from mother plants grown in the greenhouse at CSU and planted in the field at the end of May/first week of June. In each replicate or plot, seedlings will be transplanted into rows separated by 76 cm, and 91 cm spacing between each plant with 12 rows and 12 plants in each row. Pest, natural enemy sampling, and phytohormone analysis. At each field, 20 arbitrarily selected plants will be sampled for the two key hemp pest populations (cannabis aphids and hemp russet mites), natural enemies, leaf tissue and flowers, and rhizosphere sampling. Arthropod populations will be sampled three weeks after transplanting (early vegetative), 4-6 weeks (late vegetative), 6-8 weeks (flowering or anthesis) and 10-12 weeks (at harvest). Arthropods will be sampled by manually examining 10 leaves per plant and samples will be bagged and frozen until arthropods are sorted and counted (Fig. 8). During arthropod sampling, approximately 100 mg of developmentally similar true leaves from three hemp plants per field will be removed for phytohormone analysis. There will be a total of 72 plants sampled (2 treatments (organic vs conventional) x 3 plants x 4 sampling times x 3 replicates). The samples will be stored in the -80°C until phytohormone analysis. Absolute quantitation of 18 phytohormones will be determined using an established targeted LC-MS assay developed in Co-PI Prenni's lab (Sheflin et al. 2019). Plant yield and cannabinoid analysis. At maturity, plant biomass will be measured as the mass of the aboveground portion of all the plants in each row. Plants will be cut at the soil surface and air-dried for a minimum of 30 days. The plants (including flowers and leaves without stem and branches) will then be weighed to determine yield. To analyze cannabinoid levels, hemp flower samples will be collected from six plants per replicate. Hence, a total of 2 treatments x 6 plants x 3 replicates = 36 plants). Dried flower samples will be homogenized using a bead beater and phytochemicals will be extracted using established protocols in Co-PI Prenni lab. Samples will be analyzed using a targeted liquid chromatography mass spectrometry (LC-MS) quantitative assay for 20 cannabinoids (Bowen et al. 2021) (Fig. 8). Individual plants within each replication/plot will be pooled for yield and quality determination. Rhizosphere sampling. In each replication/plot, the same 20 plants sampled for pest and natural enemies will be targeted for rhizosphere sampling. The loosely attached soil on the roots will be carefully removed. The rhizosphere soil will be collected by gently brushing the remaining soil adhering to the roots using brush pencils. There will be a total of 120 samples (2 treatments (organic vs conventional) x 20 plants x 3 replicates). DNA will be extracted using Qiagen PowerSoil® DNA isolation kit following the manufacturer's instructions. Extracted DNA will be quantified using Qubit and stored at -80°C until further use. PCR will be performed targeting the V4 region of the bacterial 16S rRNA gene using primer-pair 515F/806R (Caporaso et al. 2012) and sequencing will be done at the Colorado State University Next Generation Sequencing facility (Fort Collins, USA) through Illumina MiSeq 2x 300 bp paired end sequencing.

**Objective 2. Evaluate hemp cultivars for resistance to hemp pests in organic production systems. Methods and feasibility.** The experiment will be comprised of 30 different hemp lines, planted in a randomized complete block design with three replications/plots. Each plot will consist of 30 plants planted in six rows that are 6.1 m in length with approximately 0.25 m spacing between rows. Plants will be naturally infested with pests throughout the season. We will monitor populations of hemp russet mite and cannabis aphids at the same time-points described in Objective 1: early vegetative, late-vegetative, flowering or anthesis and at harvest (Fig. 8). At maturity, plant biomass will be measured as the mass of the aboveground portion of all the plants in a plot. The dried hemp flower samples from three plants per replicate/plot will be combined and submitted for cannabinoid analysis, hence a total of 90 plants (30 lines x 3 replications) (Bowen et al. 2021).

**Objective 3. Determine the effectiveness of organic insecticides in combination with biological control for suppression of hemp pests. Methods and feasibility.** The experiments will be conducted on certified organic hemp fields at the CSU ARDEC Research Center over two growing seasons in 2024 and 2025. The experimental design will be a split plot design with three OMRI-approved insecticides (rosemary oil - TetraCURB Max, sulfur - MicroThiol Dispress, mineral oil - Suff-Oil) and untreated control as whole plot factors, and presence or absence of natural enemies (commercially purchased minute pirate bugs, predatory mites, and lacewing larvae will be released - 10 of each predator species per plant - two days after pest infestations) as the split plot factors. Each treatment combination will be replicated three times with 20 plants per plot (n=480). Insecticide treatments will be applied one month after transplanting (mid-July) and pest and natural enemy densities will be counted weekly thereafter for seven

weeks. Arthropod sampling and counts will be conducted as described in Obj. 1 (Fig. 8). At maturity, plant biomass will be measured as the mass of the aboveground portion of all the plants in a plot. Plants will be cut at the soil surface and air-dried for a minimum of 30 days to determine yield.

**Objective 4: Develop outreach and educational materials that can be disseminated to hemp growers, industry agronomists, crop consultants, diagnostic labs, researchers and other stakeholders.** Hemp Resource Center/ Hemp Insect Website and social media. The Hemp Resource Center/ Hemp Insect Website will serve as a centralized repository for all extension and outreach materials (posters, factsheets, videos, press releases, etc.) and to receive feedback from stakeholders. The website will be hosted at the CSU Hemp Resource Center website (<http://hemp.agsci.colostate.edu/>). Meetings. A second means of distributing information is through in-person meetings. The principal investigators will meet with Advisory Committee comprising of grower participants and industry representative twice a year to discuss research progress and outreach needs. The CSU Extension service workshops, annual field days organized as in-person and virtual format, the High Plains Organic Conference, pest management professionals meeting, CSU Agricultural Extension Service (AES) meetings will also be used as means to disseminate information. Researchers will present project findings at professional meetings such as Entomological Society of America (ESA) Pacific and North Central branch meetings and annual meetings. Publications. Lastly, information will be communicated via extension and scientific publications. Outreach materials will include state-specific extension factsheets, local newsletter articles, popular press articles, posters and presentations at regional and national meetings/field days will be deposited in the Hemp Resource Center website. At least one Extension factsheet will be developed for each of the research objective, and quarterly updates will be disseminated through press releases. Further, research findings will be published in peer-reviewed journals. Progress 09/01/22 to 08/31/23 Outputs Target Audience: The target audience were hemp growers, industry agronomists, crop consultants, diagnostic labs, researchers, and other stakeholders. Outreach and education were fully integrated into our project at each step of development and execution of the objectives. Diverse and numerous multimedia and presentation strategies were used to reach our most diverse stakeholders. We targeted hemp producers, as well as crop advisors, crop consultants, extension personnel and hemp industry personnel (seed, genetics) with a variety of digital and traditional media. We used three main channels by which this information was disseminated: 1) CSU hemp entomology website (<https://www.csuhempentomology.com/>), 2) in-person meetings, and 3) publications. The CSU hemp entomology website serves as a centralized repository for all extension and outreach materials (posters, factsheets publications etc) and to receive feedback from stakeholders. We gave the following presentations: Schmidtbauer, M., Jessica, P., Szczepaniec, A., Uchanski, M., Nachappa P. Don't panic, it's organic: Building resilient pest management strategies for organic hemp systems. Entomological Society of America Conference, 2024, Phoenix, AZ. Schmidtbauer, M., Jessica, P., Szczepaniec, A., Uchanski, M., Nachappa P. Don't panic, it's organic: Building resilient pest management strategies for organic hemp systems. North Central Branch-Entomological Society of America Conference, 2024, Fort Collins, CO. Changes/Problems: We were awarded the grant on September 1, 2022, but we missed the 2022 field season. Hence, we requested a no-cost extension until August 31, 2026, so we can have three field seasons and time to analyze the data and write publications. There is no change in the scope of work, and we will complete all the goals and objectives delineated in the grant. What opportunities for training and professional development has the project provided? We trained one Postdoctoral Fellow, one PhD student, and one undergraduate student. The postdoctoral fellow has been trained in metabolomics, data analysis, grant and manuscript writing. The MS student has been trained in experimental design, insect identification, metabolomics, statistical analysis, and manuscript writing. The undergraduate student has been trained in insect identification and data collection. How have the results been disseminated to communities of interest? The target audience were hemp growers, industry agronomists, crop consultants, diagnostic labs, researchers, and other stakeholders. Outreach and education were fully integrated into our project at each step of development and execution of the objectives. Diverse and numerous multimedia and presentation strategies were used to reach our most diverse stakeholders. We targeted hemp producers, as well as crop advisors, crop consultants, extension personnel and hemp industry personnel (seed, genetics) with a variety of digital and traditional media. We used three main channels by which this information was disseminated: 1) CSU hemp entomology website (<https://www.csuhempentomology.com/>), 2) in-person meetings, and 3) publications. The CSU hemp entomology website serves as a centralized repository for all extension and outreach materials (posters, factsheets publications etc) and to receive feedback from stakeholders. We gave the following presentations: Schmidtbauer, M., Jessica, P., Szczepaniec, A., Uchanski, M., Nachappa P. Don't panic, it's organic: Building resilient pest management strategies for organic hemp systems. Entomological Society of America Conference, 2024, Phoenix, AZ. Schmidtbauer, M., Jessica, P., Szczepaniec, A., Uchanski, M., Nachappa P. Don't panic, it's organic: Building resilient pest management strategies for organic hemp systems. North Central Branch-Entomological Society of America Conference, 2024, Fort Collins, CO. What do you plan to do during the next reporting period to accomplish the goals? We are in the midst of our third field season, during which we plan to repeat experiments related to Objective 3. We will also disseminate results through various avenues and publish results we have obtained so far. Impacts What was accomplished under these goals? Objective 1. In 2023 and

2024, pest and beneficial insect populations were significantly higher in the organic field. A total of 4,098 pests were recorded in the organic plots, compared to 2,583 in the conventional plots. Similarly, natural enemy abundance was elevated in the organic field, with 729 individuals observed versus 184 in the conventional field. Key pest taxa enriched in the organic system included plant bugs (Miridae) ( $42 \pm 9$ ,  $p = 0.002$ ), leaf beetles (Chrysomelidae) ( $8 \pm 2$ ,  $p = 0.004$ ), and owl moths (Noctuidae) ( $4 \pm 1$ ,  $p = 0.003$ ). Grasshoppers (Acrididae) ( $5 \pm 1$ ,  $p = 0.003$ ) were the only pest taxon found in greater abundance in the conventional field. Prominent natural enemies in the organic field included minute pirate bugs (Anthocoridae) ( $27 \pm 5$ ,  $p < 0.0001$ ), damsel bugs (Nabidae) ( $22 \pm 5$ ,  $p = 0.002$ ), and lady beetles (Coccinellidae) ( $19 \pm 3$ ,  $p < 0.001$ ). A similar pattern was observed in 2024, with 4,014 pest captures and 914 natural enemies recorded in the organic field. In contrast, both pest and beneficial insect counts declined in the conventional field, with only 370 of each observed. Cannabis aphids (Aphididae) ( $475 \pm 170$ ,  $p = 0.019$ ), thrips (Thripidae) ( $33 \pm 9$ ,  $p = 0.007$ ), and leaf beetles (Chrysomelidae) ( $21 \pm 3$ ,  $p < 0.001$ ) were significantly more abundant in the organic field. Meanwhile, chalcid wasps (Chalcidoidea) ( $80 \pm 14$ ,  $p < 0.001$ ), lady beetles (Coccinellidae) ( $4 \pm 1$ ,  $p < 0.001$ ), green lacewings (Chrysopidae) ( $3 \pm 1$ ,  $p = 0.003$ ), and syrphid flies (Syrphidae) ( $2 \pm 1$ ,  $p = 0.010$ ) were also enriched in the organic field. Damsel bugs (Nabidae) ( $3 \pm 1$ ,  $p = 0.019$ ) were the only beneficials found in greater numbers in the conventional field. Despite increased pest pressure, the organic field consistently produced greater yields across both years. Specifically, yield was assessed using three metrics: total biomass, flower, and fiber. In 2023, fiber yield was significantly greater in the organic field compared to the conventional field ( $54.6 \pm 25.1$ ,  $p = 0.034$ ), while flower and total biomass were also higher, though not statistically significant. In 2024, all three yield metrics, total biomass ( $304.6 \pm 116.7$ ,  $p = 0.005$ ), flower ( $210.5 \pm 74.2$ ,  $p = 0.003$ ), and fiber ( $94.1 \pm 46.5$ ,  $p = 0.024$ ), were significantly increased in the organic field relative to the conventional field. Phytohormone levels also differed between management systems. Phytohormone analysis was conducted at three developmental stages: vegetative, flowering, and harvest. Salicylic acid levels were elevated in the organic field during the vegetative timepoint ( $2347 \pm 1273$ ,  $p = 0.049$ ). Abscisic acid was significantly increased in the organic field during both the flowering ( $25,467 \pm 13,217$ ,  $p = 0.043$ ) and harvest timepoints ( $39,967 \pm 13,763$ ,  $p = 0.009$ ). Dihydrophaseic acid was consistently higher in the organic field across all three timepoints: vegetative ( $140,733 \pm 47,613$ ,  $p = 0.008$ ), flowering ( $25,383 \pm 12,661$ ,  $p = 0.038$ ), and harvest ( $26,523 \pm 9,092$ ,  $p = 0.009$ ), as was phosphatidic acid: vegetative ( $13,757 \pm 5,638$ ,  $p = 0.019$ ), flowering ( $8,083 \pm 2,560$ ,  $p = 0.006$ ), and harvest ( $6,960 \pm 2,211$ ,  $p = 0.006$ ). In contrast, methyl jasmonic acid ( $121.7 \pm 59.4$ ,  $p = 0.035$ ) was elevated in the conventional field during the vegetative stage. Additionally, 12-oxo-phytodienoic acid ( $20,982 \pm 9,530$ ,  $p = 0.028$ ) and jasmonic acid ( $4,913 \pm 2,009$ ,  $p = 0.019$ ) were significantly higher in the conventional field during flowering. Finally, 1-aminocyclopropane-1-carboxylic acid ( $673 \pm 348$ ,  $p = 0.042$ ) was elevated in the conventional field at harvest. Phytohormone samples from the 2024 season have been submitted, and results are pending. In 2023, cannabinoid profiles showed limited variation between the two field treatments. Of the 18 cannabinoids analyzed, CBCO, CBCV, CBDV, CBL, CBN, CBT, and THCV were not detected. No significant differences were observed between fields in the levels of CBDA, CBD,  $\Delta^9$ -THCA,  $\Delta^9$ -THC, CBCA/CBLA, CBC, CBDVA, CBGA, CBNA, or THCV. The only significant difference detected was for CBG ( $0.0033 \pm 0.0016$ ,  $p = 0.033$ ), which was more abundant in the conventional field during the flowering timepoint. Cannabinoid samples from the 2024 season have been submitted, and results are pending. Preliminary results from the 2023 microbiome analysis suggest differences in microbial community composition between the organic and conventional fields. The conventional field exhibited significantly higher Shannon entropy ( $0.303 \pm 0.110$ ,  $p = 0.011$ ) and observed amplicon sequence variants (ASV) ( $87 \pm 36$ ,  $p = 0.021$ ), while Pielou's evenness did not differ between treatments. Despite higher alpha diversity in the conventional field, ANCOM (analysis of composition of microbiomes) indicated a greater abundance of nitrogen-fixing microbial taxa in the organic field, which may help explain the observed yield advantages. Microbiome samples from the 2024 season are scheduled for preparation and submission by the end of June 2025. Objective 2. In 2023, all experimental plots experienced comparable levels of cannabis aphid infestation during the flowering timepoint. While some genotypes showed clear resistance, others were severely impacted, with near-total mortality. Due to this uniform infestation pressure, aphid abundance alone was not a reliable indicator of plant quality. Instead, yield performance under infestation was used to identify genotypes with potential aphid resistance. For example, genotype 7550 consistently exhibited high biomass yield compared to genotypes 8259 ( $169.1 \pm 58.9$ ,  $p = 0.045$ ), 7619 ( $203.1 \pm 57$ ,  $p = 0.024$ ), 4473 ( $182.9 \pm 61$ ,  $p = 0.040$ ), and 3583 ( $185.8 \pm 56.8$ ,  $p = 0.031$ ) to name a few. Although aphid counts varied by genotype, 7550 consistently showed lower infestation levels, consistently high biomass yield, and is a strong candidate for transcriptomic analysis to uncover mechanisms of insect resistance. Objective 3. In 2024, insecticide applications affected aphid and beneficial insect populations in the field. Field level aphid counts were recorded across nine sampling timepoints, with corresponding insect samples currently in cold storage. These samples are being processed and will provide greater resolution to support the field observations. Preliminary findings suggest that certain insecticides reduce cannabis aphid infestations while remaining compatible with beneficial insect releases. Although azadirachtin treatments (AzaGuard) appeared to increase overall yield, the increase was not statistically significant compared to the untreated control. Hemp flower samples have been submitted for cannabinoid profiling to evaluate whether

pest pressure influences cannabinoid content, or if cannabinoid levels contribute to pest resistance. These trials are scheduled to be repeated in the summer of 2025. ?Objective 4. To foster engagement and promote hemp research, findings from these experiments have been shared at both national and regional Entomological Society of America meetings, as well as the Cannabis Research Conference. To broaden outreach to stakeholders, the Colorado State University Hemp Entomology website (<https://www.csuhempentomology.com>) was developed to serve as a central repository for research updates and extension materials. Publications

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# Exploring Grower Interest in Microbial Predation and the Impact on Nutrient and Microbial Turnover in Organic Production

<b>Accession No.</b>	1028985
<b>Project No.</b>	FLA-PLP-006232
<b>Agency</b>	NIFA FLA\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37888
<b>Proposal No.</b>	2022-04078
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2023
<b>Grant Amount</b>	\$46,664
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Martins, S. J.; Bull, CA, TH.; Martin, KE, JA.; Triplett, LI, .; Kovac, JA, .; Emerson, JO, B.; Zasada, IN, .; Taerum, ST, EX.
<b>Performing Institution</b>	UNIVERSITY OF FLORIDA, G022 MCCARTY HALL, GAINESVILLE, FLORIDA 32611

## NON-TECHNICAL SUMMARY

Our research team composed of experts of different fields (plant and human bacteriology, bacteriophage, nematology, protists, microbial ecology) from six states and led by the southern region (Florida) is exploring the impact of soil microbial predation on a suite of soil, plant, and human health outcomes in agricultural production. We recently wrote a published review article describing the state of the art for applying high throughput and metagenomic sequencing and other tools to quantifying these impacts. As organic agriculturalists are among the most interested in soil microbial processes for soil health, we propose to provide educational resources about the potential roles of soil microbial predation on farms, while simultaneously hosting organic grower round tables to co-create hypotheses that will become the basis for future research. Webinars, presentations and focus groups at regional and national organic grower conferences, a webpage, and an extension article posted on eOrganic will help us to identify organic growers interested in the science of soil microbial processes so that we can use our training and expertise to serve their needs and answer their questions. Our planning proposal aligns with six of eight goals of OREI priorities legislatively-defined by the Farm Bill: 1, 2, 3, 4, 5, 8.

## OBJECTIVES

To extend the insight and applied value of current advances in microbiome sciences and soil microbial predation to organic growers. We plan to leverage the planning grant funds to work with growers and co-develop hypotheses that will be a cornerstone of a fully integrated OREI proposal.

## APPROACH

Provide educational resources about the potential roles of soil microbial predation on farms  
Host organic grower round tables to co-create hypotheses that will become the basis for future research  
Create webinars, presentations and focus groups at regional and national organic grower conferences  
Develop a webpage and an extension article posted on eOrganic to help in identifying organic growers interested in the science of soil

microbial processes Progress 09/01/22 to 02/29/24 Outputs Target Audience: Growers, grower organizations, the scientific community. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? Some of the growers provided letters of collaborations for our full proposal and agreed to compose our advisory board. How have the results been disseminated to communities of interest? They were disseminated via presentations at EcoFarm in CA, Pennsylvania Sustainable Agriculture Association, and Northeast Organic Farming Association of Connecticut. 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 presented at 3 conferences (EcoFarm in CA, Pennsylvania Sustainable Agriculture Association, and Northeast Organic Farming Association of Connecticut) and hosted 3 organic grower round tables to co-create hypotheses that became the basis for future research in our full OREI proposal. Moreover, a webinar on "Microbial Predators in Soil: Hunting for Healthy Soil Ecosystems" was presented and is publicly available at: <https://www.youtube.com/watch?v=vX701ePu2xo> Additionally, we met once a month (virtually) to plan and discuss the results of the surveys we applied at the meetings. We met in Portland (in person) to write the hypothesis based on the feedback we received and attended the 2023 SACNAS meeting. This meeting was important for our team, as some of us were attending for the first time. Based on our conversation with growers we wrote our objectives around the impact of organic matter on the micropredator dynamic in the soil. The most used organic matter source that growers were interested in us doing research on were: compost, manure, feather meal, and others (bone, fish, etc). Moreover, some of the growers became members of our panel for the full proposal. Publications Progress 09/01/22 to 08/31/23 Outputs Target Audience: Growers, grower organizations, the scientific community 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? Write the full proposal. Impacts What was accomplished under these goals? We presented at 3 conferences (EcoFarm in CA, Pennsylvania Sustainable Agriculture Association, and Northeast Organic Farming Association of Connecticut) and hosted 3 organic grower round tables to co-create hypotheses for our full OREI proposal. Publications

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# Systems Approach to Organic Management of the Asian Citrus Psyllid

<b>Accession No.</b>	1029015
<b>Project No.</b>	FLA-SWF-006234
<b>Agency</b>	NIFA FLA\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37889
<b>Proposal No.</b>	2022-04080
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2026
<b>Grant Amount</b>	\$2,032,887
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Qureshi, J. A.; Stelinski, LU, LE.; Setamou, MA, .; Wade, TA, .; Martini, XA, PH.; Sciligo, AM, .
<b>Performing Institution</b>	UNIVERSITY OF FLORIDA, G022 MCCARTY HALL, GAINESVILLE, FLORIDA 32611

## NON-TECHNICAL SUMMARY

The Asian citrus psyllid (ACP), vector of the devastating disease huanglongbing (HLB) or citrus greening, is currently the most severe threat to citrus production and requires management in all production systems, including organic citrus. Most efforts to control ACP focus on application of chemical insecticides prohibited from use by organic standards. The need to find solutions to ACP management for the organic citrus industry (worth \$90 million) was also highlighted by the stakeholders in our USDA-NIFA-OREI planning grant. This proposed project will provide organic citrus growers nationwide with critical tools to reduce and manage ACP in their groves. The focus is on a season-long systems approach to managing psyllids utilizing research and extension expertise and preliminary findings, including three research and education centers of the University of Florida, Texas A&M University-Kingsville, and The Organic Center. Field studies supported with laboratory and greenhouse experimentation will employ a range of tools and tactics to test and implement: 1) conservation and augmentation biological control including naturally occurring and commercial predators, 2) reflective mulches integrated with applications of organic insecticides to disrupt ACP colonization and infestation of citrus trees, 3) multiple treatments of biopesticides and programs to control ACP, and 4) integrated pest management programs backed by scientific data. An economic analysis will be conducted to determine the cost and benefits of the proposed tactics. Stakeholders will be educated with the latest knowledge of ACP and HLB management through outreach and extension initiatives. This research and associated outcomes will address four goals of OREI.

## OBJECTIVES

1: Development of a season-long management strategy for Asian citrus psyllid (ACP). 2: Economic analysis of the production practices and proposed strategies for a profitable organic citrus production. 3: Develop and disseminate outreach and extension materials on organic management of ACP and HLB.

## APPROACH

Objective 1: Development of a season-long management strategy for Asian citrus psyllid (ACP) Objective 1a-i. Screening of arthropod predators against ACP eggs and early nymphal instars. Selected natural enemies will be evaluated for nymph predation, egg predation and attraction to citrus volatiles and methyl salicylate (MeSA), the main component of PredaLure that will be used in the field trial to attract natural enemies. Predation tests will be conducted under laboratory conditions to identify suitable predators. As our goal is to lure and retain predators into citrus groves for increased ACP predation, we will integrate mass release of the selected predator(s) with the addition of PredaLure, a known and commercially available kairomone (active ingredient is Methyl salicylate) that attracts beneficial insects. We will test predator response to volatiles. The olfactometer setup will be adapted for testing the behavioral needs of each predator species. Objective 1a-ii. Conservation and augmentation biological control. We will choose four commercial organic citrus groves (sweet orange, *Citrus sinensis*), two each in FL, and TX. Treatments will be applied to an organically treated block: 1) organic insecticide only (positive control), 2) MeSA lures + organic insecticides, 3) Wheat stations + organic insecticides, and 4) MeSA lures + Wheat stations + organic insecticides. Organic insecticides will consist of OMRI listed biopesticides. Psyllid adults will be estimated by tap samples. Psyllid nymphal and egg populations will be estimated by examining 5 flushes collected in each row (25 per plot) in the randomly selected tree. The abundance of the important coccinellid predators will be sampled by an insect vacuum. Yellow sticky traps will be placed in the same trees used for tap sampling to assess the populations of arthropods. Parasitization by *T. radiata* will be assessed by examining the flush samples collected for nymph infestation levels. The total number of nymphs will be counted and then the number of 3rd, 4th and 5th instar nymphs will be counted which are maximally parasitized by *T. radiata*. The field trials will be conducted in the same location as for years 1 and 2. There will be four treatments: 1) organic insecticide only (positive control), 2) Best treatment from yr 1 and 2 experiments + commercial predator 1, 3) Best treatment from yr 1 and 2 experiments + commercial predator 2. Predators 1 and 2 will be selected based on the lab trial from objective 1a-i and will correspond to those that will combine high predation and high attraction to MeSA, citrus volatiles, and Wheat. Sub-objective 1b. Evaluation of citrus trees protection from ACP using UV-reflective mulch and organic insecticides. Advance technologies to deter ACP from colonizing citrus trees have been demonstrated to reduce ACP populations and improve tree health, such as UV-metalized reflective mulches (Croxtton and Stansly 2014). We propose to conduct replicated field experiments in FL and TX with organic growers. These replicated randomized complete block design experiments will be arranged in new 2-5-acre blocks with 4 replicates including three treatments: 1) trees planted on the UV reflective mulch; 2) trees planted on UV reflective mulch and treated with organic insecticides; and 3) bare ground trees treated with organic insecticides. ACP populations (adults and nymphs) will be monitored every 2 weeks using visual sampling for adults supplemented by tap sampling starting the second year (Qureshi et al. 2009). Immature stages will be sampled on flush taken to the laboratory and assessed under a stereoscopic microscope. Procedures described for tap sampling and flush examination under objective 1 will be followed. PCR will be used to assay for HLB every 6 months. The trunk diameter of rootstock and scion will be measured every six months to determine effects on tree growth. Sub-objective 1c. ACP control using biopesticides. The following treatments of OMRI approved products will be evaluated in the dormant season field experiments in FL and TX: Organic insecticide 1 with appropriate adjuvant; Organic insecticide 2 with appropriate adjuvant; Grower standard treatment. Following the dormant season evaluations the following programs will be evaluated in the growing season: Oil-based program; Oil and soap-based program; Oil, soap, and Kaolin based program; Grower standard. Promising treatments from the Objectives 1a, b, and c will be integrated into programs for protecting young and mature trees. Objective 2: Economic analysis of the production practices and proposed strategies for profitable organic citrus production. To evaluate the economic feasibility, this project will examine the cost of implementing the new systems and quantify changes in management practices such as changes in fertilizer use, water and fuel use, pesticide use, and labor. Partial budgeting analysis will be used to assess how changes in management practices and yields affect net returns. Further, 20-year net present value (NPV) will be developed to project future net returns and returns on investments from proposed management practices. In addition to production analysis, the project team will develop a survey instrument to determine cultural changes in production practices, costs, and expected yields of organic groves because of HLB infection. Florida and Texas survey data will highlight how practices, costs, and yields have changed with the advent of HLB. California surveys will reflect a pre-HLB and proactive management production environment. This information will be critical to understanding the barriers to adoption and extension and outreach objectives. The survey questionnaire will be developed with significant input from organic citrus growers and research and extension experts on the proposal. Objective 3. Develop and disseminate outreach and extension materials on organic management of ACP and HLB. The Organic Center, Florida Organic Growers (FOG), and California Certified Organic Farms (CCOF) will collaborate with the University of Florida, and Texas AandM Kingsville to provide outreach and extension for the proposed project. Our outreach goal is to deliver practical, research-based solutions to the national management of ACP and HLB that meet organic standards. The proposed outreach plan is multi-tiered to ensure constant stakeholder engagement and effective results dissemination from local to national audiences. The outreach material presented by The Organic Center will also target consumers, who will learn the value of organic citrus and the

challenges the industry faces. This is expected to increase consumer awareness, demand for organic citrus, and support for ongoing research for organic production. We will also launch a social media campaign surrounding the research, via The Organic Center, FOG, CCOF, the Organic Trade Association, and Uncle Matt's Organic Facebook and Twitter accounts. This campaign will be continued for the duration of the project and will be used to announce farmer listening sessions, research phase announcements, requests for stakeholder input, research findings, stakeholder meeting updates, field days, conference and workshop information, links to publications produced by our research, media coverage, webinars, and educational videos. We will publish research updates in The Organic Center newsletter (The Organic Scoop), the Organic Trade Association's weekly News Flash publication, and the Organic Trade Association's quarterly news magazine, The Organic Report, FOG's Newsletter, and CCOF's Newsletter. These newsletters have a combined reach of over 24,000 individuals. The Organic Center will also provide write-ups for external newsletters, targeting farmer-focused publications such as Acres USA, Florida Citrus Exchange, Florida Grower Magazine, Citrus Grower Magazine, Florida Citrus Reporter, and the Citrus and Vegetable Magazine. Progress 09/01/23 to 08/31/24 Outputs Target Audience: Our target audience for this project included citrus growers, industry groups, food brands, consumers, undergraduate and graduate students from universities, colleges, and schools, research and extension specialists from multiple institutions and disciplines, and experts from state and federal departments such as FDACS and USDA. The research and extension specialists on this project have given presentations at statewide and national events such as the Citrus and Specialty Crops Expo, Science fair and exhibits, Florida Entomological Society Meeting, and the nation's largest natural food expositions Natural Products Expo West, in Anaheim where over 65,000 people attended in 2024. Additionally, virtual seminars, in-person open-house presentations, demonstrations, and field visits (>300 people) were organized for citrus producers. Other educational events included training attended by undergraduate and graduate students, growers, researchers, and extension specialists. Graduate students and postdoctoral research associates were also trained on specific research proposed for this project. The population groups that attended our events were from different racial and ethnic backgrounds including minorities. Changes/Problems: Low ACP populations and shoot production at certain times limited evaluation of the proposed spray programs and biological control. Field studies were also impacted by weather events, such as heavy rains, which influenced the effectiveness of spray programs. However, we were able to evaluate the populations of beneficial organisms and ACP and conduct experiments to evaluate the natural mortality contributed by biological control agents. Some delays or changes in spray applications resulted in accommodating grower preference, such as one participant declining the use of oil in his grove. Hence the program was modified in that location to test the effectiveness of insecticidal soap, kaolin, and diatomaceous earth versus the grower control. One challenge observed was that the psyllid-only focus as a pest control strategy was not a viable approach for growers producing citrus for the fresh fruit market. There is a suite of arthropod pests that growers need to target simultaneously, thus requiring the use of a multi-target management strategy. What opportunities for training and professional development has the project provided? PD and Co-PDs trained their staff, postdocs, and students to provide a foundation for understanding the significance of the proposed research and methods of conducting the proposed experiments. They were also trained in disseminating information to stakeholders. Online seminars, open house demonstration through posters, movies, and hands-on training; presentations at state and national exhibits and workshops provided several opportunities for the staff, postdocs, and students to learn and improve and expand their skills in preparing the materials for such events, writing and presenting to the stakeholders. The stakeholder groups that attended the events including growers, research and extension personnel, school and college students, and graduate students from multiple disciplines, were also trained in identifying harmful and beneficial organisms in citrus orchards and their management. Three PhD students, one PhD from Haiti (Romain Exilien) under the supervision of Dr. Martini and two PhD students one each from Ghana (Seth Tsatsu) and Egypt (Mohamed Ali) were trained under the supervision of Dr. Qureshi, Romain presented his research on repellents and ACP management from at the Entomological Society of America and Florida Entomological Society meetings. Mohamed also presented at the professional meetings and Southwest Florida Research and Education Center (SWFREC) open house attended by citrus producers and others. Seth shared knowledge from his research at the same open house. How have the results been disseminated to communities of interest? Three workshops to disseminate the results of the findings to citrus homeowners, one each in Tallahassee, FL (Leon County), Milton, FL (Santa Rosa County), and Lake City, FL (Columbia County), were organized. These workshops aim to give homeowners tools to control ACP that do not rely exclusively on conventional insecticides. Romain Exilien delivered the information from his research to homeowners. Mohamed Ali work was presented at the International Congress of HLB. Seth Tsatsu and Mohamed Ali shared knowledge from their research at the open house organized at the SWFREC. In addition, a summary of the research findings of Romain and Mohamed has been published in the 2023 and 2024 research book "Keeping Florida Growers Informed" published by the Citrus Research and Education Center. A grower presentation was made on September 21, 2023, during the annual meeting of the Texas Citrus Mutual, attended by 103 growers. A summary of organic psyllid control strategies was discussed. Furthermore, informal meetings with each of the three collaborating growers in the project were held in January 2024 to refine the program and

discuss its challenges. Faculty, postdocs, and students discussed research on ongoing tools for citrus pest management during their visits to the citrus orchards. Information on citrus pest management was also disseminated through UF's Electronic Delivery Source (EDIS) system, including a production guide and citrus industry magazine. Presentations and demonstrations at the open house, expos, workshops, and national and international meetings were attended by several segments of society. These include citrus growers, industry groups, food brands, consumers, undergraduate and graduate students from universities, colleges, and schools, research and extension specialists from multiple institutions and disciplines, and experts from state and federal departments such as FDACS and USDA. A website, Science for Citrus Health (<http://ucanr.edu/sites/scienceforcitrushealth/>), was co-maintained to educate growers on the research and methods being conducted and developed to protect citrus from the ACP-HLB complex. What do you plan to do during the next reporting period to accomplish the goals? Objective 1: Development of a season-long management strategy for Asian citrus psyllid (ACP). We will conduct detailed investigations to evaluate the effects of beneficial organisms on ACP in the controlled and field conditions including their interactions with kaolin and thyme oil and insecticides. We also plan to investigate the effects of entomopathogenic fungi on ACP populations under controlled and field conditions. Large-scale field studies will evaluate the integrated effects of insecticides and biological control in season-long management programs implemented in Florida and Texas. We will continue to monitor the effectiveness of different organic programs in suppressing ACP populations, including evaluations of different OMRI-approved biopesticides for suppressing ACP during winter months. We will monitor the populations of natural enemies in citrus orchards under different organic programs and employ exclusion techniques to determine natural mortality in the developing colonies of ACP immatures. We will evaluate the parasitism by *Tamarixia radiata* in the organic management programs for ACP by examining and rearing the field-collected nymphs of ACP. We plan to replicate the experiments in Valencia sweet orange using MeJA for its effects on ACP including understanding the mechanisms of action by which MeJA treatment affects ACP populations. Objective 2: Economic analysis of the production practices and proposed strategies for profitable organic citrus production. The economics team will meet with the other team members to incorporate new practices and lessons learned from experiments into the survey instrument. This is an interactive process that will be completed in 2025. The team already identified recent Florida and California production budgets that will be used in partial budget analysis. They will also levy work done on citrus trunk injection costs from another project to assess the cost of production if trunk injection is included as a new cultural practice. They will also meet with Co-PIs to collect and process information on management practices and related costs for budget analysis. Objective 3: Develop and disseminate outreach and extension materials on organic management of ACP and HLB. PI, Co-PIs, postdocs, and students will present research findings to the stakeholders through online seminars and in-person meetings with growers, research and extension specialists, students, extension agents, and industry brands. We will work with Florida Organic Growers (FOG) and California Certified Organic Farmers (CCOF) to host informational events such as workshops and survey organic growers. Findings from different studies will be presented at the 'Citrus Health Forum' workshop Dr. Martini organized annually, attracting over 120 growers from FL, AL, and GA. Finally, we plan to present the results from this project at the meeting of the Entomological Society of America, Florida Entomological Society, and the International IPM seminar, as well as in the open house and citrus seminars offered online or in person. We plan to publish articles on the natural enemies and kaolin suppression of ACP and their interactions. We will continue communication efforts including maintaining the website, updating the website with progress reports provided by the project team, and publicizing farmer listening sessions, research phase announcements, and requests for stakeholder input. The Organic Center is currently redesigning its entire website, and in 2025, the entire website will be available in multiple languages, while all information products, like reports, will be slowly translated and offered in Spanish. This will expand the reach of the project website which will be updated with information from new research findings in Florida and Texas. As a part of this overhaul, the TOC communications team and Director have been working with its web designing company to increase the user-friendliness of all research project microsites, which will also help increase engagement with the outreach material from this project. While TOC added a Resources page to the project website, it is not very engaging and interactive. The team is currently brainstorming ways to use better graphic design to increase interaction with material from this page: <https://www.organic-center.org/resources-2>. Impacts What was accomplished under these goals? Objective 1: Experiments were conducted to evaluate commercially available ladybeetle *Cryptolaemus montrouzieri*, lacewing *Chrysoperla rufilabris*, minute pirate bug *Orius laevigatus*, and predatory mites *Amblyseius swirskii* for predation on ACP. The natural enemies were isolated on single shoots within a 2-inch vial at one individual per vial with 20 to 40 eggs of ACP. Eggs were counted before the experiment and at 48h and 96 h after the release of the natural enemies. Considering less efficacy of *A. swirskii* against it was excluded from the next experiment. A follow-up experiment with additional candidates was conducted on small citrus plants under laboratory conditions. Natural enemies were allowed to forage on the citrus plant infested with ACP eggs before 48h and 96h count. This experiment revealed that *D. catalinae* is more efficient than the other tested candidates in predating ACP eggs. A field experiment arranged as a randomized block design in a newly planted block of Star Ruby grapefruit was conducted to test the

hypothesis that Methyl jasmonate (MeJA) phytochemical could reduce ACP and citrus greening or Huanglongbing (HLB). MeJA and its derivatives, collectively known as jasmonates, are fatty acid-derived cyclopentanones that regulate growth and development, responses to various environmental stresses, and gene expression, playing a key role in plant defense responses against pathogens and pests. Trees received either of two treatments of weekly applications: 1) control of Tween 80 aqueous solution, or 2) MeJA + Tween 80. ACP adults, nymphs, and eggs found on plants were counted weekly. Flush growth on six trees per replicate plot was monitored weekly by counting all flush within a 50 cm<sup>2</sup> cube randomly positioned within the canopy of the tree. PCR was performed on a subset of plants in each treatment replicate at the beginning and two months after initiation of the experiment (06/26/24) (6 samples per treatment replicate) to test for CLas pathogen of HLB. Significantly ( $P < 0.01$ ) fewer ACP were observed in plots treated with MeJA (mean  $\pm$  SD:  $0.32 \pm 1.07$ ) than in control plots (mean  $\pm$  SD:  $2.53 \pm 5.13$ ). CLas remained undetectable in plots treated with MeJA by PCR at 2 months after applications. However, we found a 67% infection rate in control plots. Findings support the hypothesis that methyl jasmonate can potentially reduce ACP-HLB. In an ongoing study evaluating season-long control of ACP in Florida, OMRI-approved organic insecticides were evaluated in winter and growing seasons. In February 2024, Aza-Direct at 16 oz/ acre, Entrust at 2.0 oz/ acre, PyGanic at 10 oz/acre were applied in a block of citrus in a randomized complete block design. ACP populations were suppressed from all treatments, with the significant effects observed from Entrust, averaging 41% reduction in April and 56% in early May. JMS stilet oil, M-pede (soap), and Surround (Kaolin), respectively, were evaluated during the growing season in three spray programs i) oil-based, ii) oil plus soap-based, and iii) oil plus soap and kaolin-based, respectively. Although rains impacted the effectiveness of the sprays conducted in July a significant effect of all treatments was observed at 7 days after treatment application. An average ACP reduction of 79%, 63%, and 36% was observed from the treatments of M-pede (soap), JMS stilet oil, and Surround (Kaolin), respectively. Exclusion experiments conducted in May and July used cohorts of developing colonies of ACP nymphs protected or exposed to natural enemies. ACP suppression averaged 23-75%, suggesting the contribution of biotic mortality factors in reducing pest populations in all programs. Samples of ACP nymphs were collected and examined in the laboratory for evidence of parasitism by the parasitoid *Tamarixia radiata* released in the study grove. Programs 1, 2, and 3 contained 66%, 14%, and 61% colonies, respectively, with parasitized nymphs. The parasitism rate in the parasitized colonies averaged 64%, 83%, and 72%, respectively. Overall parasitism, including colonies without parasitism, averaged 42%, 12%, and 43%, respectively, in programs 1, 2, and 3, respectively. In Texas, four project goals were targeted: 1) compare the effectiveness of the season-long psyllid control program relative to the grower standard; 2) compare the efficacy of threshold-based spray vs monthly spray program; 3) determine the effectiveness of the implemented organic ACP control program on the incidence of HLB in the grove and 4) conduct outreach activities to disseminate best management practices for ACP control. The effectiveness of the season-long psyllid control program was tested as grower participatory trials in two groves. In this study, three ACP control programs, including i) oil-based, ii) oil plus soap-based, and iii) oil plus soap and kaolin-based, were compared to the standard grower control. In both locations, programs based on oil plus other organic pesticides were the most effective at reducing ACP compared to the grower standard or the oil-only program. A threshold-based spray program was compared to a calendar spray program in two groves. This study used the organic program combined with grower standard and diatomaceous earth based on the preference of a collaborating grower. During this reporting period, nine calendar and 8 threshold-based sprays were performed at each site. Those two spray programs were comparable except in one grove in which the monthly calendar spray program resulted in lower psyllid densities. HLB incidence was evaluated in one of the groves in which the season-long efficacy of four treatments was compared. Since the study was being conducted for the second year in the same grove, the percentage of HLB-affected trees was evaluated on the total number of experimental trees per treatment ( $n = 35$ ). No significant differences, and HLB incidence was 23% to 34% across treatments. Objective 2: The economics team met with the PI and Co-PIs in Florida and Texas and reached growers to collect information on the costs associated with implementing tools and tactics for managing ACP. The team also identified recent Florida and California production budgets to be used in partial budget analysis. Objective 3: The concept of the system approach to organic management ACP, including the role of key predators, parasitoids, and entomopathogenic fungus in its suppression, was demonstrated through posters and live demonstrations in the 1) 2023 Fall open house at the Southwest Florida Research and Education Center (> 300 participants), and a collaborative 2024 Junior Achievement Inspire event held at the Florida Gulf Coast University (>5,000 participants). A presentation on effective psyllid control strategies in organic groves was given at the Texas Citrus Mutual annual meeting in September 2023. The Organic Center updated the project website disseminating the research team's progress in 2024 to TOC's vast consumer and industry networks (<https://www.organic-center.org/news-and-updates>). The combined reach of the organization's social media pages is over 156,000 individuals on Facebook, and there are over 41,000 followers on X (formerly known as Twitter). In 2024 TOC increased its social media network by amplifying updates on LinkedIn, a new platform for the organization. The project website was maintained and updated with project updates. Outreach materials were developed to educate a primarily industry audience at The Organic Center's annual benefit on March 13, 2024. This event showcased a science fair before the main

"Organic Oscars" event, and an exhibit was dedicated to the ACP citrus greening research project. The poster described what it means to take whole systems approach in organic pest management and presented some preliminary results from the research team. A QR code was presented on the poster, which leads to the project website to generate more exposure to this work. ? ? Publications Progress 09/01/22 to 08/31/23 Outputs Target Audience: Our target audience for this project were from several segments of society. These include citrus growers, industry groups, food brands, consumers, undergraduate and graduate students from universities, colleges and schools, research and extension specialists from multiple institutions and disciplines, and experts from state and federal departments such as FDACS and USDA. The research and extension specialists on this project have given presentations at statewide and national events such as Citrus and Specialty Crops Expo, Science fair and exhibits, Florida Entomological Society Meeting, Entomological Society of America Meeting and the nation's two largest natural food expositions Expo West and Expo East. Similarly, virtual citrus seminars were given which were attended by a variety of audiences particularly growers, students, research, and extension faculty. Additional opportunities for reaching several of the above-listed target audiences were created through open house and workshops which were attended by lower-level students (freshman and sophomores), undergraduate and graduate students, growers, researchers, and extension specialists. Graduate students and postdoctoral research associates were also trained on specific research proposed for this project. The population groups that attended our events were from different racial and ethnic backgrounds including minorities.

Changes/Problems: ACP populations were low at the study sites during the early part of the year, which limited evaluation of the proposed spray programs at start. However, we were able to evaluate the populations of beneficial organisms and ACP as well as conduct experiments to evaluate the natural mortality contributed by biological control agents. During the active phase of the program, one participant declined the use of oil in his grove; the program was modified in that location to test the effectiveness of insecticidal soap, kaolin, and diatomaceous earth versus the grower control. By working directly with growers, we will ensure rapid adoption of the most effective method of psyllid control as the data generated by the project came directly from their groves and work. What opportunities for training and professional development has the project provided? PD and Co-PDs trained their staff, postdocs, and students to provide a foundation for their understanding of the significance of the proposed research and methods of conducting the proposed experiments. Online seminars, open house demonstration through posters, movies, and hands-on training; presentations at state and national exhibits and workshops provided several opportunities for the staff, postdocs, and students to learn and improve and expand their skills in preparing the materials for such events, writing and presenting to the stakeholders. The stakeholder groups that attended the events, including research and extension personnel, school and college students, and graduate students from multiple disciplines, were also trained in the identification of harmful and beneficial organisms in citrus orchards and their management. Postdocs and students availed the opportunity to present their work at professional meetings such as national and branch meetings of the Entomological Society of America, Florida Entomological Society etc. How have the results been disseminated to communities of interest? Faculty, postdocs, and students presented the work at several professional and grower meetings with extensive participation. Presentations and demonstrations at the open house, expos, workshops, and national and international meetings were attended by several segments of society. These include citrus growers, industry groups, food brands, consumers, students from universities, colleges, and schools, and research and extension specialists from multiple institutions and disciplines.

Qureshi, J.A. 2023. Beneficials for management of Asian citrus psyllid (predators, parasitoids, entomopathogens). Citrus Insect, mite, and nematode workshop. 19 January. UF/IFAS Citrus Research and Education Center, Lake Alfred, FL. Stelinski, L.L. et al. 2023. Asian citrus psyllid research and management. Citrus Insect, mite, and nematode workshop. 19 January. UF/IFAS Citrus Research and Education Center, Lake Alfred, FL. This was a workshop included hands-on demonstrations in addition to a series of presentations during which time there was a longer period of stakeholder engagement. An 80-page educational binder was produced for all attendees that included copies of all presentations given.

Stelinski, L.L. 2023. Managing psyllids and other citrus pests. Citrus Expo. 16-17 August. (Several hundred Attendees). Florida State Fairgrounds, Tampa, FL. A factsheet was developed as an education tool regarding our research and this project. This described our progress, challenges, and future applied opportunities. This was distributed as a hard-copy booklet at the meeting. Stelinski, L.L. et al. 2022. Getting the most bang out of Asian citrus psyllid management under endemic greening. Citrus Expo. 17-18 August. (Several hundred Attendees). Lee Civic Center, Fort Myers, FL. The seminar updated growers on our objective, discussed our progress to date, and challenges/opportunities for the future. Qureshi, J.A. 2023. Evolving management of Asian citrus psyllid and interactions with citrus pest complex. Citrus Seminar, 31 October, Southwest Florida Research and Education Center, Immokalee, FL. This seminar was attended by citrus growers and extension specialists and included information on the organic and conventional methods of ACP management. Qureshi, J.A. 2023. Citrus pests: Asian citrus psyllid and beneficial organisms (poster presentation). Open house, 8 November, Southwest Florida Research and Education Center, Immokalee, FL. Qureshi, J.A. et. al. 2023. Live demonstration of Asian citrus psyllid and beneficial organisms used for its biological control with audience from multiple segments of the society. Open house, 8 November, Southwest Florida Research and Education Center, Immokalee, FL. Ali, M., B.

Kostyk, M. Triana, and J. A. Qureshi. 2023. Demonstration of the effectiveness of UV-metalized reflective mulch in reducing Asian citrus psyllid populations (poster presentation and demonstration). Open house, 8 November, Southwest Florida Research and Education Center, Immokalee, FL. These open house presentations and demonstration focusing on cultural and biological methods of ACP control were attended by more than 300 people including growers, extension and research staff, and several other segments of society including homeowners producing citrus in their backyards and included field tours. Qureshi, J.A. 2023. Reduce Pests with Natural Enemies. Citrus Industry Magazine, 104 (4), 16-18. This article discussed the role of beneficial organisms in managing ACP and other pests and was distributed electronically and in hard copies to stakeholders in multiple states. Setamou, M. 2023. Approaches of psyllid management. Meeting with organic growers and master gardeners. TX. This presentation described the approaches for managing psyllids and focused on organic pest management. Several other channels of communication, including websites, newsletters, science fairs, exhibits etc. were used to reach and share knowledge and findings with several stakeholders, including citrus producers and consumers. The Organic Center made a press release and grower group announcements to a wide variety of venues publicizing the planned research, <https://www.organic-center.org/usda-awards-major-organic-research-grant-fight-citrus-greening>. An interview was organized with The Packer in advance of the press release, which was published the same day as the press release, <https://www.thepacker.com/news/organic/organic-research-combat-citrus-greening-gets-2m-usda-funds>. The press release was also picked up and reported on by AndNowUKnow Produce Industry News, <https://www.thepacker.com/news/organic/organic-research-combat-citrus-greening-gets-2m-usda-funds>. Additionally, a social media campaign surrounding the proposed research was launched by pushing the project launch through social media channels and through the Organic Trade Association's and The Organic Center's newsletters. The combined reach of the organization's social media pages is over 156,000 individuals on Facebook, and there are over 41,000 followers on Twitter. The project website was maintained and updated with project updates. Outreach materials were developed to educate a primarily industry audience at The Organic Center's annual benefit on March 8, 2023. This event was later transformed into the format of a science fair and an exhibit was dedicated to the citrus greening research project. The timeline of the funding acquisition process and the importance of the research topic was highlighted. The timeline was printed on a trifold poster board accompanied by a digital sign that showcases the research goals. A QR code will be presented on the digital sign, which leads to the project website to generate more exposure of this work. Two specialty cocktails were developed for the event to highlight citrus and the importance of finding organic solutions to citrus greening (imagine if you didn't have your lemons/limes/grapefruits for these drinks!). Some updates from the project research were publicized via social media and The Organic Center's June newsletter that reaches over 25,000 subscribers. We also used the Science for Citrus Health website (<https://ucanr.edu/sites/scienceforcitrushealth/>) as a web-based medium for disseminating new information related to HLB. The SCH team continues to meet bi-monthly to discuss the next tasks relevant to the goals of the group. The Science for Citrus Health (SCH) team (current lead members: P. Lemaux and L.L. Stelinski) is comprised of outreach specialists, postdocs, and graduate students. The group produces outreach/extension documents and organizes/delivers events. An information flier about the Science for Citrus health website was published in the California Research Board E-News mailer and the Florida Citrus Industry magazine newsletter to increase grower traffic to the site. We also published several new snapshots on the website site. What do you plan to do during the next reporting period to accomplish the goals? Objective 1: Development of a season-long management strategy for Asian citrus psyllid (ACP). During the next period we plan to conduct experiments to study the oviposition and development of ACP on citrus treated with kaolin and thyme oil. This would help us to determine if the residual number of ACP that laid eggs on citrus treated with kaolin and thymol is impacted by the treatment. We will also start experimenting with natural enemies to determine the best predator(s) against ACP eggs and nymphs. We plan to have the new olfactometer working and test the effect of volatile pulse on the response of ACP to volatiles. Samples of natural enemies from experiments that evaluated ant baits and Predalure dispensers will be examined to collect data on the predators and ACP abundance under different treatments. Field experiments with Antixx Ant Bait and Predalure dispensers will be replicated at least one more time temporally during the next season. We will continue to monitor the effectiveness of different organic programs in suppressing ACP populations including evaluations of different OMRI approved biopesticides for suppressing ACP during winter months. We will monitor the populations of natural enemies in citrus orchards under different organic programs and employ exclusion techniques to determine natural mortality in the developing colonies of ACP immatures. Parasitoid *Tamarixia radiata* will be released in the organic management programs for ACP and rates of parasitism will be determined through examination of the nymphs. We will evaluate the effects of UV-metalized reflective mulch on ACP and citrus trees. Objective 2: Economic analysis of the production practices and proposed strategies for profitable organic citrus production. In early 2024, economics team will meet with the other team members to incorporate new practices and lessons learned from experiments into the survey instrument. This is an interactive process that will be completed in 2025. The team already identified recent Florida and California production budgets that will be used in partial budget analysis. They will also levy work done on citrus trunk injection costs from another project to assess cost of production if

trunk injection is included as new cultural practice. In January 2024, they will meet with Co-PIs to begin work on collecting information on management practices and related costs for partial budgets analysis. Objective 3: Develop and disseminate outreach and extension materials on organic management of ACP and HLB. PI, Co-PIs, postdocs, and students will present findings of research to the stakeholders through online seminars, and in-person meetings with growers, research and extension specialists, and industry brands. Findings will continue to be shared with target audiences through the project website hosted by The Organic Center (<https://www.organic-center.org/news-and-updates>) and websites of the FL and TX participating research and education centers. Event and exhibit reporting will continue through The Organic Center and Organic Trade Association newsletters (The Organic Scoop; OTA Newsflash) and social media channels. We will continue communication efforts including maintaining the website, updating the website with progress reports provided by the project team, and publicizing farmer listening sessions, research phase announcements, and requests for stakeholder input.

Impacts What was accomplished under these goals? Objective 1: We were able to initiate several experiments between laboratory and field in Florida and Texas toward developing tools and tactics useful for suppressing the populations of ACP. Experiments were conducted to use kaolin clay as a nanocarrier for essential oils to repel ACP so that their colonization of citrus trees is reduced. Four different oils (fir, coriander, lavender, thyme) were tested in combination with kaolin clay. We found that kaolin increased the repellent activity of the four oils. However, out of the four treatments, the combination of thymol and kaolin offered the best protection to citrus. In a subsequent experiment mixing kaolin with blue and red colorant, later combination significantly increased the repellent effect of kaolin clay and reduced the number of ACP eggs laid on citrus. Finally, we tested a treatment of kaolin + red colorant + thymol and found that in a choice test red kaolin + thymol reduced the number of ACP by 90% as compared to the control plant. Oviposition of ACP was also significantly reduced with this treatment. These findings suggest that red kaolin is an effective visual repellent against ACP useful for reducing its populations in citrus trees, however, red kaolin had no greater effect than kaolin when combined with thymol. In Florida, a large-scale field study was initiated with the state's largest organic citrus producer. This study is intended to evaluate the effects of three organic programs and a grower standard program as well as of biological control on the populations of ACP. Populations of ACP and natural enemies were monitored in the study block using tap and suction sampling methods during 2023. The three organic programs to be evaluated against the grower standard are oil based and with soap or soap plus kaolin combinations. Initially, ACP populations were low which did not warrant need for spray applications. Additionally, grower needed information on the products to be used in the proposed programs including labels and time to get approval for his organic farm plan. TriTek (80% mineral oil) at 1% of the application volume was sprayed across all programs in the first week of August. ACP populations averaged at 0.45 adults per tap sample at the end of August which was 37% less than the numbers observed in early August. We also employed exclusion experiments to evaluate the natural mortality in the nymphal populations of ACP in this block. The developing colonies of ACP immatures on citrus shoots were protected with sleeve cages or left exposed to the natural enemies for a period of about two to three weeks to assess the impact of natural mortality factor on ACP populations. An average of 36% reduction in the nymphal populations of ACP in the colonies developing on citrus shoots exposed to natural enemies compared to the ones protected was observed. Releases of the parasitoid *Tamarixia radiata* which attack ACP nymphs preferably instars 4-5 were also made and parasitism rates were evaluated in the block. We observed evidence of parasitism in 43% of nymphal colonies with parasitism averaging 29% from 13-50% per colony. To improve the role of biological control in the citrus groves by excluding ants, two experiments were conducted in an organic grove in Lake Wales, FL. We tested commonly used ant baits and OMRI listed ant bait formulation approved for organic pest management. We also evaluated MeSA dispensers (Predalure) attractive to natural enemies and useful to enhance biological control in citrus groves. In a proof-of-concept study we showed that an ant bait treatment of groves could substitute the need for application of the labor intensive Tanglefoot sticky barrier used at the base of trees to reduce ant populations in citrus orchards. We therefore evaluated the OMRI listed ant bait treatment Antixx Ant Bait (Spinosad). We also evaluated Predalure dispensers which release a kairomone that attracts beneficial insects as a method of augmenting and/or conserving biological control agents of ACP. Our results indicate that a similar, albeit somewhat less effective, reduction of fire ants can be achieved by application of the OMRI listed Antixx Ant Bait. This treatment may be a useful tool for citrus growers in FL to increase the effectiveness of biological control of ACP via fire ant removal. Our initial evaluation of Predalure dispensers as a method for enhancing biological control of ACP suggests that these dispensers may also be a viable method for enhancing biological control of ACP by attracting natural enemies to treated plots. In Texas, field trials to meet the main project goal consisting in the development of a year-long psyllid control program were conducted as grower participatory trials with three growers. Three programs (Program 1 = oil based only; Program 2 = Oil based plus insecticidal soap in rotation, and Program 3 = oils, insecticidal soaps, and kaolin in rotation) were tested in a replicated design along with the traditional grower program as control. Similar trends were observed in ACP population in the two locations where all four programs were tested. ACP populations were very low at the onset of the trial in both locations in May 2023 and remain low for the early part of summer. Program 3 that combined the three types of biopesticides (oil, insecticidal soap and kaolin in rotation) was the most effective in

reducing psyllid densities. The present study shed light on the benefits of using different types of biopesticides for managing ACP. Objective 2: The economics team drafted the grower adoption survey. This is an interactive process that will be completed in 2025. The team identified recent Florida and California production budgets that will be used in partial budget analysis. Objective 3: The Organic Center made a press release and grower group announcements to a wide variety of venues publicizing the planned research, <https://www.organic-center.org/usda-awards-major-organic-research-grant-fight-citrus-greening>. An interview was organized with The Packer in advance of the press release, which was also published, <https://www.thepacker.com/news/organic/organic-research-combat-citrus-greening-gets-2m-usda-funds>. The press release was also reported on by AndNowUKnow Produce Industry News, <https://www.thepacker.com/news/organic/organic-research-combat-citrus-greening-gets-2m-usda-funds>. Additionally, a social media campaign surrounding the proposed research was launched by pushing the project launch through social media channels and through the Organic Trade Association's and The Organic Center's newsletters. The combined reach of the organization's social media pages is over 156,000 individuals on Facebook, and there are over 41,000 followers on Twitter. The project website was maintained and updated with project updates. Outreach materials were developed to educate a primarily industry audience at The Organic Center's annual benefit on March 8, 2023. This event was later transformed into the format of a science fair and an exhibit was dedicated to the citrus greening research project. Some updates from the project research were publicized via social media and The Organic Center's June newsletter that reaches over 25,000 subscribers. We also used the Science for Citrus Health website (<https://ucanr.edu/sites/scienceforcitrushealth/>) as a web-based medium for disseminating new information related to HLB. An information flier about the Science for Citrus health website was published in the California Research Board E-News mailer and the Florida Citrus Industry magazine newsletter. Two grower meetings were organized in Texas, one in December 2022 and the second one in September 2023 to explain the goal of the program and provide the preliminary results of this project. Another educational meeting was organized in September 2023 for master gardeners, during which different organic psyllid control approaches were presented.

Publications Type: Other Status: Published Year Published: 2023 Citation: Qureshi, J.A. 2023. Evolving management of Asian citrus psyllid and interactions with citrus pest complex. Citrus Seminar, 31 October, Southwest Florida Research and Education Center, Immokalee, FL. Type: Other Status: Other Year Published: 2023 Citation: Qureshi, J.A. 2023. Citrus pests: Asian citrus psyllid and beneficial organisms (poster presentation). Open house, 8 November, Southwest Florida Research and Education Center, Immokalee, FL. Type: Other Status: Other Year Published: 2023 Citation: Qureshi, J.A. et. al. 2023. Live demonstration of Asian citrus psyllid and beneficial organisms used for its biological control with audience from multiple segments of the society. Open house, 8 November, Southwest Florida Research and Education Center, Immokalee, FL. Type: Other Status: Other Year Published: 2023 Citation: Ali, M., B. Kostyk, M. Triana, and J. A. Qureshi. 2023. Demonstration of the effectiveness of UV-metalized reflective mulch in reducing Asian citrus psyllid populations (poster presentation and demonstration). Open house, 8 November, Southwest Florida Research and Education Center, Immokalee, FL. Type: Journal Articles Status: Published Year Published: 2023 Citation: Qureshi, J.A. 2023. Reduce Pests with Natural Enemies. Citrus Industry Magazine, 104 (4), 16-18. Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Qureshi, J. A. 2022. Biological control of invasive Asian citrus psyllid in commercial citrus orchards. Symposium Biological Control of Major Invasive Pests: Achievements and Current Development. 12-17 November. Joint Annual Meeting of the Entomological Society of America and Entomological Society of Canada, Vancouver, BC, Canada. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Stelinski, L.L. 2023. Managing psyllids and other citrus pests. Citrus Expo. 16-17 August. (Several hundred Attendees). Florida State Fairgrounds, Tampa, FL. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Stelinski, L.L. et al. 2023. Asian citrus psyllid research and management. Citrus Insect, mite, and nematode workshop. 19 January. UF/IFAS Citrus Research and Education Center, Lake Alfred, FL. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Qureshi, J.A. 2023. Beneficials for management of Asian citrus psyllid (predators, parasitoids, entomopathogens). Citrus Insect, mite, and nematode workshop. 19 January. UF/IFAS Citrus Research and Education Center, Lake Alfred, FL. Type: Websites Status: Published Year Published: 2023 Citation: Qureshi, J.A. 2023. Biologically-based management of citrus pests. August 1. Tip of the week for citrus industry. Citrus Industry. AgNet Media. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Qureshi, J.A. 2023. A decade of psyllid suppression and strategies with newly developed and conventional chemicals. 4 April. Citrus Growers Institute. Avon Park, FL Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Stelinski, L.L. et al. 2022. Getting the most bang out of Asian citrus psyllid management under endemic greening. Citrus Expo. 17-18 August. Lee Civic Center, Fort Myers, FL. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Exilien R and Martini X. 2023. Symposium. Plant-based essential oils and kaolin to control Asian citrus psyllid. November 5-8. 2023. Entomological Society of America Annual Meeting. National Harbor MD. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Martini X. 2023. Keynote address. Use of nanoclay and essential oil for whitefly

and Asian citrus psyllid control. November 10 November 12. 12th Sustainable Nanotechnology Organization Meeting. Marina Del Rey CA. Type: Other Status: Other Year Published: 2023 Citation: Setamou, M. 2023. Approaches of psyllid management. Meeting with organic growers and master gardeners. TX. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Cardozo, I., A. Sciligo, J. Qureshi, and X. Martini. 2023. Organic Management of Citrus Greening Disease (HLB) requires the control of its vector, the Asian Citrus Psyllid. Natural Products Exposition East, Philadelphia, PA. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Cardozo, I., A. Sciligo, and X. Martini. 2023. Organic Management of Citrus Greening Disease: Example of how diligent fundraising and non-stop research collaboration has finally resulted in a win for organic research. Irene Cardozo, Amber Sciligo, Violet Batcha. 2023 Natural Products Exposition West, Anaheim, CA. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Qureshi, J.A. 2023. Approaches to Asian citrus psyllid (*Diaphorina citri*) management in young citrus plantings. Florida Entomology Society Annual Meeting, 30 October to August 2, Jupiter Beach Resort and Spa, Jupiter FL.

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## Rooting for Ecosystem Services

<b>Accession No.</b>	1029002
<b>Project No.</b>	ILLU-875-648
<b>Agency</b>	NIFA ILLU\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37882
<b>Proposal No.</b>	2022-04045
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2026
<b>Grant Amount</b>	\$1,499,090
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Ugarte, C. M.; Wander, MI, M.; Bohn, MA, .
<b>Performing Institution</b>	UNIVERSITY OF ILLINOIS, 2001 S. Lincoln Ave., URBANA, ILLINOIS 61801

### NON-TECHNICAL SUMMARY

This project uses multi-disciplinary expertise and farmer feedback to identify and evaluate traits that can document organic systems' contribution to supporting and provisioning ecosystem services. This addresses the need for continuous innovation necessary for the development and sustainability of organic agriculture. Our objectives are to: 1) Develop a region-specific ideotype for maize roots suitable for organic production; 2) Evaluate traits associated with ecosystem services and increase our understanding on how to manage the rhizosphere to optimize carbon storage, resource use efficiency and productivity; and 3) Develop a clear understanding of how farmers can incorporate knowledge on crop and soil traits in management and optimization of cropping system functions. By conducting a bioinformatics survey of maize genes associated with phenotypes that we propose as ecosystem service proxies, we can efficiently generate root ideotypes that may perform well in variable soil environments. By conducting experimental work in an organic breeding nursery and organic systems trial that has already established treatment differences, we can quickly gain an understanding of the range of root responses possible and reasonably attempt to relate these traits to ecosystem services. To increase awareness and appreciation for the value of root traits among farmers, we will develop mental models of farmer's goals and constraints to prioritize trait development by conducting on-farm strip trials. Results will provide the basis for rotation planning and management and orient systems-based breeding efforts in the Midwest.

### OBJECTIVES

The overarching goal of this project is to identify maize ideotypes that are good candidates for systems-based breeding and quantify ecosystem services associated with soil and root traits by improving our understanding of how crop rotation and organic fertility (green and brown manures) interact and influence traits and their relationships to nutrient and water use efficiency, C sequestration, and overall productivity. We will also assess best approaches to incorporate these traits into systems management design. Specifically, efforts will contribute to: 1) Develop a region-specific ideotype for maize roots suitable for organic production. We argue that cultivars with a high degree of plasticity will be better fit for organic systems; 2) Evaluate traits associated with ecosystem services and increase our understanding of how to manage the rhizosphere to optimize C storage, resource use efficiency and productivity. We argue that integrative soil traits can predict root response and be related to ecosystem provision; and 3) Understand how farmers can incorporate knowledge on crop and soil traits through

planning to optimize cropping system functions. We assume only farm relevant solutions should be prioritized by systems-based breeding efforts.

## APPROACH

Objective 1: To develop a region-specific ideotype for maize roots suitable for organic production. We will use bioinformatics to identify "root haplotypes" in our current and diverse maize germplasm and evaluate the root function in selected groups. Objective 2: To evaluate traits associated with ecosystem services and increase our understanding on how to manage the rhizosphere to optimize C storage, resource use efficiency and productivity. For this, we will evaluate clusters of maize hybrids identified in Objective 1 and quantify their root responses (coarse and fine root traits) in soils managed with different species of cover crops preceding corn planting. Soils will be analyzed for traits that help us understand resource use efficiency (i.e., soil bulk density, SOC, POM-C and PMN, soil structure), C sequestration and their contribution to productivity. Objective 3: To understand how farmers can incorporate knowledge on crop and soil traits through planning to optimize cropping system functions. We will achieve this objective by engaging with an existing educational network and responding to producers' priorities and knowledge gaps to determine how to optimize management in complex rotations. We will explore farmers' mental models to identify needs, prioritize traits associated with ecosystem services of interest, and ensure relevance to participants' operations. Progress 09/01/23 to 08/31/24 Outputs Target Audience: The target audience for this project includes groups of farmers, researchers, and educators interested in systems-based breeding, rotation planning and management that support production and environmental goals in organic grain cropping systems. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? During this reporting period, the project provided training opportunities for three graduate students, two visiting scholars, and four undergraduate students from the University of Illinois. Also, two undergraduate students from the University of Puerto Rico, a partner of the University of Illinois at Urbana-Champaign participated in field and laboratory efforts during the summer of 2024. One student from Zamorano University (Honduras) and three students from EARTH University (Costa Rica) were also involved in soils and corn breeding research efforts in the fall semester. How have the results been disseminated to communities of interest? Preliminary results from YRS-1&2 were shared with participants of outreach and scientific events as follows: In February 2024, we hosted a table at the Organic Grain Conference organized by The Land Connection. We conducted a listening session to understand how farmers incorporate knowledge of crops and soil traits in their management decisions to increase productivity, farming system resistance and resilience and environmental services. In July of 2024 we hosted two field days at the Illinois Organic Systems Trial. 1) Outlined the research agenda of this project to about 200 attendees of the 2024 National Association of Plant Breeders Conference, 2) We partnered with the IDEA Farm Network and The Land Connection to host a field day at the Illinois Organic Systems Trial. We welcomed approximately 35 participants, and the program focused on understanding the range of variability in corn roots, soil organic management, soil structure and farmer on-farm experiences to optimize their systems. In November 2024, we presented our findings at the Illinois Extension Meeting for trainers that hosted 24 participants. In November 2024, we presented preliminary results at the annual tri-societies meetings in San Antonio, Texas. The presentation was titled "Maize Roots and Soil Organic Matter: Experiences from an Organic Grain Systems Trial" What do you plan to do during the next reporting period to accomplish the goals? The execution of activities for this project is on target. We are summarizing portions of the data for publication and planning to recruit new undergraduate students for the subsequent growing seasons. We will visit farmer collaborators to document farmer's rotation planning and to plan for the following year in which we will be evaluating existing germplasm at on-farm locations. Impacts What was accomplished under these goals? This multidisciplinary project has successfully completed its second year of trials designed to identify the most effective maize root ideotypes for organic agricultural practices in the Midwest. We expanded work from the 2023 growing season when we established six treatment combinations encompassing two rotation types (3- and 4-year rotations) and three fertility levels to create a gradient of soil resource conditions. These conditions are integral for evaluating a core set of maize hybrids, with the goal of pinpointing root traits that are particularly beneficial for the Midwest. Our initial phase involved assessing 15 experimental hybrids, chosen for their varied root characteristics, across these treatment combinations in 2023. We focused on evaluating plant performance traits, including root architecture, grain yield, and grain chemical composition. Additionally, we conducted agronomic performance assessments on a separate set of 150 hybrids derived from inbreds of the University of Illinois Germplasm Collection and the Elite Maize Association Mapping Panel (EMAMP). The top-performing hybrids from these assessments underwent further detailed evaluations in the summer of 2024, with a focus on root structure, function, and genetic profiles to better understand their contributions to overall performance. We assembled a set of 120 genes known to be involved in maize root architecture and physiology. Currently, we are developing a bioinformatics analysis pipeline to assess the diversity of maize root genes and determine their allele frequencies within our breeding populations. Based on root haplotype similarity, we will classify our maize germplasm into groups with distinct haplotypes and evaluate

their root performance in both greenhouse and field experiments to determine how these haplotypes influence root function and overall plant performance. Our work expanded to the evaluation of soil resource condition by quantification of the influence of the six treatment combinations on soil structure, traits associated with soil organic matter fractions that might be used to predict C storage, nitrogen and water use efficiency, and productivity. Publications Progress 09/01/22 to 08/31/23 Outputs Target Audience: The target audience for this project includes groups of farmers, researchers, and educators interested in systems-based breeding. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? This project has provided training opportunities for two graduate students, two visiting scholars, and undergraduate students from the University of Illinois. Also, two undergraduate students from the University of Puerto Rico, a partner of the University of Illinois at Urbana-Champaign participated in field and laboratory efforts during the summer of 2023. One student from Zamorano University (Honduras) and three students from EARTH University (Costa Rica) have been involved in soils and corn breeding research efforts in the fall of 2023. How have the results been disseminated to communities of interest? Preliminary results from YR-1 were shared at a workshop that included farmers, practitioners, and educators. The event was organized by the Organic Agronomy Training Service (OATS). What do you plan to do during the next reporting period to accomplish the goals? The execution of activities for this project is on target. Researchers are summarizing and curating the data collected in 2023 and are recruiting graduate and undergraduate students for the subsequent growing seasons. We will carry out exploratory listening sessions and focus groups to expand our educational network and document farmer's rotation planning and associated labor and purchasing logistics to identify improvements or alteration of plant and soil traits that can help farmers fulfill their planning objectives and deliver ecosystem services. Impacts What was accomplished under these goals? This multidisciplinary project has successfully completed its first year of trials, aiming to identify the most effective maize root ideotypes for organic agricultural practices in the Midwest. We established six treatment combinations encompassing two rotation types (3- and 4-year rotations) and three fertility levels to create a gradient of soil resource conditions. These conditions are integral for evaluating a core set of maize hybrids, with the goal of pinpointing root traits that are particularly beneficial for the Midwest. Our initial phase involved assessing 15 experimental hybrids, chosen for their varied root characteristics, across these treatment combinations in 2023. We focused on evaluating plant performance traits such as root architecture, grain yield, and the chemical composition of the grain. In addition, we conducted agronomic performance assessments on a separate set of 150 hybrids. These hybrids were derived from inbred of the University of Illinois Germplasm Collection and the Elite Maize Association Mapping Panel (EMAMP). The top-performing hybrids from these assessments will undergo further detailed evaluations in the summer of 2024. This subsequent analysis will concentrate on their root structures and functions, as well as their genetic profiles, to better understand how these factors contribute to their overall performance. Publications

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# Bio-intensified Field Crop Rotations in the Upper Midwest: Quantifying Effects Upon Pest Management and Yield.

Accession No.	1029089
Project No.	IND90028565G2
Agency	NIFA IND\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2022-51300-38056
Proposal No.	2022-04041
Start Date	01 SEP 2022
Term Date	31 AUG 2026
Grant Amount	\$1,499,944
Grant Year	2022
Investigator(s)	Krupke, C.; Johnson, WI, G.; Langemeier, MI, R.; Gruver, JO, BR.; Telenko, DA, .; Werle, RO, .; Adair, AS, .; Conley, SH, .; Silva, ER, .; Smith, DA, LE.

## NON-TECHNICAL SUMMARY

In a four year, three state study we will assess the weed, insect and pathogen pressure upon a range of field crops in a standard organic and ecologically intensified organic system. The latter approach has never been investigated in detail. We will use on-farm, replicated trials across IN, IL, and WI. We will also evaluate the economic viability of each approach and provide real-time updates on the project via a range of outreach and extension venues.

## OBJECTIVES

Our long-term goal is to develop a comprehensive understanding of the ecology of organic cropping systems. More specifically, we will develop an understanding of how key ecological factors influence the pest management considerations of organic farmers under a range of cropping regimes in the upper Midwest region. Specific objectives: Survey: in a four-year, three-state study, assess the weed, disease and beneficial and pest insect populations impacting corn, soybeans and small grains under both a "standard" and "ecologically-intensified" rotation system. Economic analysis: using data collected from both university farms and working commercial farms, assess the costs and benefits associated with these varying rotations. On-farm demonstrations: Commercial organic farmers in each state will implement these practices at their farms, including field day demonstrations, in the final two years of the project. Extension and outreach: Our team is comprised of extension specialists spanning multiple disciplines. With a combination of field days at university and grower farms, coupled with real-time SMS surveys and a regional grower advisory panel, we will transmit results and plans as the work unfolds, affording us the opportunity to course correct and alter the approach as needed. Recommendations: At the conclusion of the project, we expect to collate the results into a comprehensive set of recommendations, including university fact-sheets, that will comprise a knowledge base that will fill a current void in unbiased, research-based guidance for the most progressive organic field crop producers.

## APPROACH

This study will focus on establishing field-based research at both university-owned research farms and on commercial organic farms. The university sites include the Northeast Purdue Ag Center (NEPAC), the University

of Wisconsin Arlington Agricultural Research Station, and the Western Illinois University (WIU) Allison Organic Research and Demonstration Farm. These university-owned research farms allow us a level of fine-scale control, in-field replication and provide a location in each state to demonstrate our efforts through field days in the latter half of the project. Our network of seven farmer collaborators will allow us to collect working farm data under different management systems, soils, and contexts - including agronomic and economic data, in addition to providing all the benefits associated with larger plot work. At each university farm, a four year on-farm research trial utilizing a randomized complete block design with 8 replications/site will be implemented. NEPAC-Purdue site is a total of 30 acres, while the sites at both UW and WIU have approximately 80 acres available. All of these sites are certified organic. There are two organic cropping regimes, hereafter referred to as "treatments", placed at each location: a standard system and an ecologically-intensified system (details follow). Each treatment list includes both corn and soybeans, which are arguably the key grain crops in the region. Comparison of multiple parameters, including yields, in those cropping years will be a key determinant of how well the ecologically intensified rotations build soil N and reduce pest pressures. Standard system

At each university farm (IN, IL, and WI), the crops described below will be planted in blocks at least 60 ft. wide, with a split-plot design that includes two entries per cropping season - meaning that we will have the opportunity to document each crop twice during the life of the experiment, addressing a key weakness of our 2021 proposal. Please see the plot plan schematic below for further details. All plots will be taken to yield and harvested with a small plot combine (owned by PDs Krupke and Telenko).

Year 1 (oats are main crop): Oats will be drilled with medium red clover in early spring and harvested in July. Mowing of medium red clover will occur 1-2 times to prevent weeds from going to seed.

Year 2 (corn is main crop): Manure (target manure rate = 100 lbs available N/a) and medium red clover biomass will be incorporated with shallow tillage in early May and shallow tillage will also occur immediately prior to planting corn (32k/a on 30-in rows) in mid- to late May. Mechanical weed control will include 1-2 blind cultivations and 1-2 row cultivations. Shallow tillage will occur after corn harvest followed by drilling of cereal rye (or broadcasting of cereal rye prior to tillage).

Year 3 (soybean is main crop): Cereal rye will be terminated with tillage in early May and shallow tillage will also occur immediately prior to planting soybeans on 30-in rows in late May. Mechanical weed control will include 1-2 blind cultivations and 2-3 row cultivations. Shallow tillage will occur after harvest to level cultivation ridges.

Year 4: identical to Year 1.

Ecologically intensified system

Crops planted in 30 ft. wide strips and all legume crop and cover crop seeds inoculated with biological seed treatments (rhizobia and mycorrhizae, (USDA-NRCS 2015)) to promote root health and nutrient acquisition.

Year 1 (oats are main crop): Oat and medium red clover seed will be treated with appropriate N fixing inoculants, drilled in early spring and harvested in July. The red clover will be mowed if needed to prevent weeds from going to seed. The red clover will be terminated with shallow tillage prior to drilling cereal rye and crimson clover (both treated with appropriate N fixing inoculants).

Year 2 (soybean is main crop): Soybean seeds will be treated with an N-fixing inoculant and planted no-till on 30-in rows into standing cereal rye and crimson clover. Crimping will occur ~2 weeks after rye anthesis allowing crimson clover to develop mature seed.

Year 3 (corn is main crop): Manure (target manure rate = 100 lbs available N/a) and volunteer cover crops will be incorporated with shallow tillage in early May and shallow tillage will also occur immediately before planting corn. Corn seed will be treated with an appropriate N-fixing inoculant and planted in mid- to late May (32k/a on 30-in rows). In rows 2 and 5 (assuming 6 row plots), corn seed will be blended with a large-seeded bush cowpea @ 30% of seed count. The planter will be adjusted to deliver a full corn population, accounting for corn seed displaced by cowpea. Mechanical weed control will include 1-2 blind cultivations and 1-2 row cultivations. After corn is harvested, compost will be broadcast @ 1 ton/a followed by shallow tillage.

Year 4: identical to Year 1.

Progress 09/01/23 to 08/31/24

Outputs

Target Audience:

Purdue/ENTM: A presentation by PD Krupke at the Indiana Organic Grain Farmer Meeting (February 2024) highlighted beneficial insect trapping results. Target audience was organic/transitional growers, ca. 50 in attendance

Purdue/Weed Science/Pathology: A presentation by Telenko at the OGRAIN conference in Madison, WI (February 2024) talking about Integrated Disease Management in Organic Grains. Target audience was organic/transitional growers, ca. 100 in attendance

UW: A poster presentation by the Post Doc Tatiane Severo Silva at the Marbleseed Organic Farming Conference (February 2024), La Crosse, WI. The post highlighted crop yield, weed population, and insect diversity in both standard and eco-intensive systems.

Changes/Problems:

Purdue/NEPAC: No major issues to report. Generally good planting and growing conditions overall. Giant ragweed remains our major challenge throughout much of our study field. Cultivation and weed zapper have helped, but this is still the major issue in this field.

UW: There were issues with soybean emergence due to wet conditions, seed placement in the dense biomass of the cereal rye cover crop, and slug damage. For these reasons, the soybeans needed to be replanted. Additionally, due to the rain and wet conditions, weed control using the cultivator in the corn plots was challenging, leading to high weed pressure from mid to late season. The soybean plots also experienced high weed pressure.

What opportunities for training and professional development has the project provided?

Purdue/ENTM: One post-doctoral scholar (beginning May 2023) and one PhD student (beginning August 2022) are funded by the project and are conducting the above and below-ground insect surveys in each of the treatments, respectively.

Purdue/Weed Science/Pathology: UW: One post-doctoral scholar

How have the results been disseminated to communities of interest?

See previous section, re: Indiana

Organic Grain conference What do you plan to do during the next reporting period to accomplish the goals? Purdue/ENTM: continue ground level and above-ground insect surveys, soil samples in 2025 Purdue/Weed Science/Pathology: continue to monitor weed populations in the treatments and collect soil samples for weed seedbank evaluation. UW: continue ground level and above-ground insect assessment, soil samples in 2025

Impacts What was accomplished under these goals? Survey: in a four-year, three-state study, assess the weed, disease and beneficial and pest insect populations impacting corn, soybeans and small grains under both a "standard" and "ecologically-intensified" rotation system. Purdue/ENTM: Prior to planting, collected two sets of soil samples from each plot, one to quantify nutrient/organic matter, etc., and one to measure microbiota in the plot. Established a series of above-ground and ground level insect traps shortly after planting and monitored through crop maturity. Above-ground traps consisted of plastic intercept traps mounted at 1 m above crop canopy. Ground level traps are pitfall traps. Each trap was checked weekly and contents returned to lab for identification. We have completed identification to family level of all pitfall traps and approximately 50% of intercept traps. Purdue/Weed Science. Soil samples were collected from each plot at each field trial site (WIU, U of WI, Purdue NEPAC) at the beginning of the summer. Soil samples were shipped to Purdue, and are in cold storage. We plan to start the grow out procedure on these soil samples in December or January. Another set of soil samples will be collected at the beginning of each field season for growouts and seedbank analysis. In-field weed density and biomass samples were collected in August from each site. Data are being entered into spreadsheets and will be summarized during the spring semester. In 2024, weed seedbank growouts were completed for the soil samples collected in 2023. Additional soil samples were collected in 2024 to monitor changes in the soil seedbank induced by the treatments. These soil sample growouts will be completed during the winter of 24/25 after the samples have incubated in cold storage for a couple of months to break dormancy. Purdue/Plant Pathology. Plots were scouted all season in 2024 - by the end of the season we documented low levels of Septoria brown spot and frogeye leaf spot (FLS) in the soybeans. In corn we found very low levels of tar spot on ear leaf (EL) and 2 leaves above and below. UW: nothing to report n-farm demonstrations: Commercial organic farmers in each state will implement these practices at their farms, including field day demonstrations, in the final two years of the project. Purdue/ENTM: Two farmers (Mills and Federer) continue to work with Adair and Krupke and communicate on the project. No field days scheduled so far, tentative June/July of 2025. Purdue/Weed Science/Pathology: Nothing to report for Pathology. Nothing to report for Weed Science UW: Before planting two sets of soil samples were collected from each plot, one to quantify nutrient/organic matter, etc., and one to measure microbiota in the plot. Set up a series of above-ground (plastic intercept traps mounted at 1 m above crop canopy) and ground level (pitfall traps) insect traps at crop emergence and monitored through crop maturity. The insects were collected from each trap weekly, placed in jars with 70% ethanol and stored in a freezer until identification. We have completed identification to family level of approximately 15% of the traps. We also assessed weed pressure in mid-season (late-July), identifying and counting the main weeds using a square meter quadrat at three locations within each plot. End-of-season, soybean and corn stands were taken before harvest. Economic analysis: using data collected from both university farms and working commercial farms, assess the costs and benefits associated with these varying rotations. Purdue trial yields: Crop/System/ Yield Average/ Moisture Average Corn / Standard / 85.8 / 26.4 Oats / Standard / 49.1 / 14 Corn / Eco-intensive / 54 / 26.5 Soybean / Eco-intensive / 17.9 / 11 U of Wisconsin trial yields: Crop / System / Yield Average / Moisture Average Corn / Standard / 160 / 18 Oats / Standard / 101 / - Corn / eco-intensive / 180 / 18 Soybean / eco-intensive / 26.6 / 12.2 Publications Progress 09/01/22 to 08/31/23 Outputs Target Audience:Purdue/Pathology: NEPAC Organic Field Day on Organic disease management - target audience was organic/transitional grower and educators ~40 in attendance. UW Organic Field Day Arlington, Wisconsin Organic Field Day: August 2023 - 70 participants, in person. WIU: Summer field day attendees, undergraduate classes and twilight tour attendees this fall Changes/Problems: Purdue/NEPAC: Primary issues were related to weather, specifically precipitation. Oat planting on 04/26/23, was 3-4 weeks delayed due to wet soils. Almost no precipitation in June resulted in poor stand establishment and very low yields. Soybeans were planted in both treatments on 05/23/23. August and September combined precipitation of less than 1" resulted in poor yields. Two plots (one standard organic, one eco-intensified) were lost to giant ragweed pressure early in season. Two passes with electric weed zapper reduced giant ragweed pressure significantly. NEPAC could use some upgraded row crop cultivators for row crops, such as the AccuraFlow that WIU has for their site. UW: Significant drought conditions impacted yield in the ecointensive plots. WIU: Very poor (<25%) stands established in the ecointensive no-till soybeans plots due to moisture depletion by the cereal rye/crimson clover cover crops during serious drought conditions (0.3" over 50 days in May and June). These plots were mowed in early September with the exception of 2 small 6 row x 50' subplots that were taken to yield. Lack of student labor in summer 23 limited some field activities (e.g., insect sampling). What opportunities for training and professional development has the project provided?Purdue/ENTM: One post-doctoral scholar (beginning May 2023) and one PhD student (beginning August 2022) are funded by the project and are conducting the above and below-ground insect surveys in each of the treatments, respectively. Purdue/Weed Science: One predoctoral visiting scholar (Pedro Correa) has been recruited and is doing the weed seedbank growouts and processing the in-season weed counts and weed biomass samples. One

visiting scholar (Ivis Miranda) was recruited and monitored for diseases and soil sampled for pathogens during the 2023 season. University of Wisconsin: One post-doctoral scholar is funded by the project and is collecting all research data from our UW site. WIU: 1 undergraduate student worker assisted with plot management in summer 23. A visiting scholar from Italy will perform some biological analyses in winter 24. 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?Purdue/ENTM: continue insect surveys, soil samples in 2024 Purdue/Weed Science: Continue to collect soil samples for weed seedbank analysis, collect mid-season weed density and biomass samples. Weed seedbank growouts will be done after cold stratification. Weed biomass samples are air dried and data is processed in the fall. Continue to collect seasonal disease data in 2024 WIU: Good stands of cereal rye/crimson clover have been established in plots slated for ecointensive soybeans in 2024. Plots that had soybeans in 2023 have been shallowly tilled in preparation for ecointensive corn and standard oats in 2024. Continue to collect data into 2024. Impacts What was accomplished under these goals? Survey: in a four-year, three-state study, assess the weed, disease and beneficial and pest insect populations impacting corn, soybeans and small grains under both a "standard" and "ecologically-intensified" rotation system. Purdue/ENTM: Prior to planting, collected two sets of soil samples from each plot, one to quantify nutrient/organic matter, etc., and one to measure microbiota in the plot. Established a series of above-ground and ground level insect traps shortly after planting and monitored through crop maturity. Above-ground traps consisted of plastic intercept traps mounted at 1 m above crop canopy. Ground level traps are pitfall traps. Each trap was checked weekly and contents returned to lab for identification. We have completed identification to family level of all pitfall traps and approximately 50% of intercept traps. Purdue/Weed Science. Soil samples were collected from each plot at each field trial site (WIU, U of WI, Purdue NEPAC) at the beginning of the summer. Soil samples were shipped to Purdue, and are in cold storage. We plan to start the grow out procedure on these soil samples in December or January. Another set of soil samples will be collected at the beginning of each field season for growouts and seedbank analysis. In-field weed density and biomass samples were collected in August from each site. Data are being entered into spreadsheets and will be summarized during the spring semester. Purdue/Plant Pathology. Disease was monitored in-season and on 7 Sep disease ratings were collected that included foliar severity of frogeye leaf spot, brown spot, and downy mildew in lower and upper canopies. In addition, sudden death syndrome incidence and severity was rated and green canopy percentage. Data is currently being tabulated and will be analyzed this winter. UW. All data were collected according to protocols and samples were sent to collaborators at Purdue for analysis. WIU: Soil samples were collected (0-6") in early June 23 (after soybeans were planted but prior to emergence) and split into subsamples submitted for routine chemical analysis and subsamples stored @ -40C for biological analyses. In addition, on the same day, a larger volume of soil (~ 2 liters) was collected from each plot and transported to Purdue for weed seed bank analysis. High resolution imagery of all plots was collected with a drone in mid-June. Image analysis software was recently obtained for stand/phenotype analysis. In early June, soil moisture sensors were installed @ 6" and 24" in 1 standard and 1 eco-intensive soybean plots. Insect surveys were not performed in 2023. Economic analysis: using data collected from both university farms and working commercial farms, assess the costs and benefits associated with these varying rotations. Purdue: Yields shown below, no economic analysis performed as of yet. Crop System Yield Average Moisture Average Soybean Standard 34.23 17.7% Soybean Eco-Intensive 27.38 21.8% Oat/Clover Standard 14.60 10.8% Oat/Clover Eco-Intensive 12.84 12.7% UW: Yields shown below, no economic analysis performed as of yet. Crop System Yield Average Moisture Average Soybean Standard 75.6 11.9% Soybean Eco-Intensive 37.4 14.2% Oat/Clover Standard 56.7 18.6% Oat/Clover Eco-Intensive 57.4 19.1% WIU Oats were harvested on 7/19 and yields ranged from the low 80s to over 100 bu/a. Yield monitor data is being cleaned and analyzed to obtain variability metrics and total yields for each plot. Soybeans were harvested on 11/5 and weigh wagon and yield monitor data is being analyzed. Standard soybean plot yields ranged from the low 50s to over 70 bu/a. All field activities were documented and economic analyses will be performed using custom rates in winter 2024. Extension and outreach: Purdue/Organic Ag: The project rationale and design were introduced to various audiences during the following 2023 extension events and print materials: Northeast Indiana Organic Field Day - 32 participants, in-person Northeast Purdue Ag Center Field Day - 161 participants, in-person Huntington University Intro to Agriculture course organic field visit - 21 participants, in person Flanagan State Bank Organic Newsletter: November 2023 - 280 recipients, print and digital WIU: The project was introduced to a variety of audiences including summer field day attendees (~ 90), undergraduate classes and twilight tour attendees this fall (~20). Publications

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# Novel Sanitation Approaches to Control Listeria Biofilms in the Organic Produce Industry

Accession No.	1028972
Project No.	KS10221417
Agency	NIFA KAN\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2022-51300-37885
Proposal No.	2022-04066
Start Date	01 SEP 2022
Term Date	31 AUG 2026
Grant Amount	\$1,499,844
Grant Year	2022
Investigator(s)	Trinetta, V.; Danyluk, MI, D.; Krug, MA, DA.; Dunn, LA, .; Bhullar, MA, .; Britton, LO, .; Critzer, FA, .; Nwadike, LO, .; Rock, CH, .; Yucel, UM, .; O'Bannon, TA, LA.

## NON-TECHNICAL SUMMARY

Organic farming is an important sector of the United States of America (USA) agricultural economy and represents an alternative to conventional production systems. Consumers prefer organic products because of perceived health benefits, environmental consciousness, and animal welfare concerns. Since 2011, the fresh produce industry, including the organic market, has been linked to outbreaks and recalls associated with *L. monocytogenes* contamination: from the deadly episode connected to cantaloupe grown at the Jensen Farms in Colorado to the most recent voluntary recall for salads processed in Yuma, Arizona. Despite the recognized presence of *L. monocytogenes* in farm operations, no strict requirements are in place to prevent contamination and/or cross-contamination in facilities under the FDA Food Safety Modernization Act Produce Safety Rule. The fast-paced harvesting, handling, and processing operations, the design of equipment, the creation of debris and little time between shifts provide ideal niches for *Listeria* cell adhesion and biofilm formation. Furthermore, fresh produce distribution does not include a pathogen inactivation step and both pre-harvest and post-harvest equipment are often not designed for optimum mechanical and chemical disruption of pathogens and biofilms, e.g., cleaning and sanitation. The reality for organic growers is even more limited regarding sanitation alternatives, since the National Organic Program (NOP) Standards need to be followed to maintain "organic-certified status": few options are available to control foodborne pathogens, therefore placing the organic produce industry at a competitive disadvantage. In this project we will : (1) Assess the growth of *Listeria monocytogenes* sessile and biofilm forms on different surfaces representative of organic produce harvesting, handling and processing facilities; (2) Characterize microbial dynamics and composition by imaging, spectroscopy and genomic approaches; (3) Optimize the simultaneous use of lactic acid and UV-C in lab bench settings; (4) Develop an integrated sanitation system from lab model to industrial prototyping; (5) Validate the system on commercial harvesting equipment and in packing facilities representative of different commodities and different production capabilities; (6) Offer workshops and demonstrations on cleaning and sanitation practices for organic growers around the nation, while evaluating the economic costs for the developed prototype.

## OBJECTIVES

The overall goal of this project is to develop and validate novel sanitation strategies to control *Listeria* biofilms in the organic produce industry on food-contact surfaces and equipment and biofilm forms on different surfaces representative of organic produce handling and processing facilities; (ii) Characterize microbial dynamics and

composition by imaging, spectroscopy and genomic approaches; (iii) Develop and optimize the simultaneous use of UV-C and lactic acid from lab bench settings to industrial prototyping; (iv) Validate the system in commercial harvesting and packing facilities representative of different commodities and different production capabilities; (iv) offer workshop and demonstration on cleaning and sanitation practices targeting organic growers around the nation, and evaluate the economic costs and benefits as well as the risks and implications for the developed prototype.

## APPROACH

1. Two bacteria forms will be investigated in this project: sessile and biofilm. The sessile form is the first state of confluent growth of bacteria on agar plates, while a biofilm is a mature and more complex form of a bacterial community living in a complex matrix of EPS and nucleic acids, in addition to the microorganisms. Both conditions are relevant and practical to understand sanitizers' effectiveness and microbial susceptibility. We will measure the ability of bacteria to grow on the selected food contact and we will assess recovery yield, adhesion and repeatability of results.

2. Laser Scanning Confocal Microscopy and Spectroscopy and Whole Genome Sequencing will be performed on the biofilms to understand chemical and physical changes in bacterial structure and attachment to surfaces coupled with the investigation of gene responses to the different growth conditions and treatments will allow us to obtain a comprehensive overview of the factors that affect *Listeria* survival and growth.

3. Different antimicrobial interventions will be tested and lactic acid simultaneously with UV-C will be applied against bacteria.

4. Field testing will be performed in different locations to validate the efficacy of lab bench experiments.

Progress 09/01/23 to 08/31/24

Outputs Target Audience: For the current reporting period our audience has been mainly the scientific community, since we worked on Objective 1 (Assess the growth of *Listeria monocytogenes* sessile and biofilm forms on different surfaces representative of organic produce harvesting, handling and processing facilities) and we start working on Objective 2 (Characterize microbial dynamics and composition by imaging, spectroscopy and genomic approaches) and Objective 3 (Optimize simultaneous use of sanitizer and UV in lab setting).

Changes/Problems: Transportation of the UV tunnel system continues to be a challenge. We are investigating ways to make sure we have support when we will conduct the field trials. From an analytical point of view determining biofilm thickness was challenging due to the limitation posed by the surfaces and growing conditions. We used several techniques such as profilometry, spectral reflectance spectroscopy and fluorescent microscopy in order to understand and identify biofilm thickness.

What opportunities for training and professional development has the project provided? Nothing Reported

How have the results been disseminated to communities of interest? Yes, we had several meetings with chemical companies updating on the enhancement of sanitizing action when UV was applied simultaneously and also about the difficulty of inactivating *Listeria* on certain surfaces (e.g. PVC and Teflon). With these observations and meetings, we would like to provide scientific information to the organic produce industry regarding also the cleanability of certain materials that are commonly used in facilities and maybe encourage them to find alternatives if from our experiments those surfaces resulted very difficult to clean.

What do you plan to do during the next reporting period to accomplish the goals? In the next 6 months or so, we are planning to test the UV-tunnel system that was built and optimize the treatment choosing one or two sanitizers in combination with UV-C. We will then contact organic producers in Florida, Georgia and Arizona to set up dates to visit and perform the field trials. We will continue to study microbial dynamics and composition by imaging, spectroscopy and genomic approaches. During the field trials we will set up demonstrations for producers and start organizing workshops.

Impacts What was accomplished under these goals? Sanitizer application of lactic acid, Silver Dihydrogen Citrate, Peracetic acid alone or in combination with UV-C light at both contact times resulted in a  $>5$  log reduction of *L. monocytogenes* in all types of surfaces for sessile cells. The log reductions observed for *Listeria* biofilms were different. The best log reduction was obtained with peracetic acid ( $\sim 4$  log reduction). The simultaneous application of UV-C light and sanitizers did not significantly enhance the effectiveness of sanitizers for both sessile and biofilms cells ( $p > 0.05$ ). UV-C light only applied for 5 min was the least effective treatment, and efficacy was influenced by surface material. Teflon and PVC were the materials where inactivation activity against *Listeria* was difficult to observe. Longer application improved the effectiveness of sanitizers for biofilm control ( $p < 0.05$ ), but no difference was instead observed for sessile cells. These results help validate sanitation strategies including LA (4%), SDC (4.85% citric acid), and PAA (120 ppm) that can be utilized by the organic fresh produce industry to control *L. monocytogenes* on FCS in combination with UV to enhance the control of *Listeria*. The influence of various environmental factors, such as surface properties, shear forces, and nutrient availability influence biofilm formation and architecture. In a series of experiments (within objective 2) *Listeria* biofilms were grown under static and dynamic conditions on five different surfaces common to food processing (i.e., Teflon, plastic, nylon, steel, wood). The biofilm architecture was characterized using various chemical and biophysical techniques. Determining biofilm thickness is challenging due to limitations posed by the substrate's surface topology and varying biofilm thicknesses. We employed multiple techniques for measuring film thickness, including stylus profilometry, spectral reflectance spectroscopy, and fluorescence microscopy with staining, each

presenting unique challenges. We identified film thicknesses ranging from a few micrometers ( $\mu\text{m}$ ) up to 60  $\mu\text{m}$ , depending on the growth conditions. The spatial composition of extracellular polymeric substances (EPS) and distribution of microorganisms were analyzed using IR microscopy, which allowed for the identification of the polymeric network entrapping the microorganisms. This polymeric network determined surface hydrophobicity, assessed using an optical drop tensiometer. Additionally, small molecules responsible for quorum sensing were analyzed using GC-MS coupled with solid-phase microextraction (SPME). Overall, our integrated approach combining chemical, biophysical, and microbial information provided a systematic understanding of the formation and nature of *Listeria* biofilms under various environmental conditions, affecting their attachment to surfaces and resistance to sanitation. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Appolon, Llacsahuaga, Widmer, Mmaduabuchi, Bhullar, Yucel, Trinetta, Dunn and Critzer, 2024. Synergistic efficacy of lactic acid and UV-C in the inactivation of *Listeria monocytogenes* on soiled food contact surface materials. Technical presentation at IAFP (International Association of Food Protection) Annual Meeting, California, July 2024. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Deniz and Trinetta, 2024. Formation and Control of *Listeria monocytogenes* biofilms on various food processing surfaces. Technical Oral presentation at IAFP (International Association of Food Protection) Annual Meeting, California, July 2024. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Llacsahuaga, Trinetta, Widmer, Bhullar, Yucel, and Critzer, 2024. Effectiveness of novel sanitizer and Ultraviolet (UV-C) light to control for *Listeria monocytogenes* in the organic fresh produce industry. Technical presentation at European IAFP (International Association of Food Protection) Annual Meeting, Switzerland, May 2024. Progress 09/01/22 to 08/31/23 Outputs Target Audience: For the current reporting period our audience has been mainly the scientific community (exchange of scientific output and ideas) and the sanitation industry that was instrumental to help us building the sanitation system (Objective 4) Changes/Problems: no changes but the major challenge we encounter was to develop an integrated sanitation system that is easy to transport in all the field visit that we are planning to do. 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? We are planning to: - finalize the treatment- time concentration on both *Listeria* sessile and biofilm form on all the different surfaces - optimize the integrate sanitation system - characterize the microorganisms by image and spectroscopy Impacts What was accomplished under these goals? - Initial experiments to assess the growth of *Listeria monocytogenes* sessile and biofilms were performed. A statistical difference between microbial growth stage (sessile vs biofilm) was identified, with sessile form being generally more sensitive to treatment as compared to biofilms. A significant difference among materials was also identified with regard of efficacy of sanitizer treatment. Bacteria growth on smooth surfaces are easier to control. - Optimization of times and concentration of treatments with UV has been also accomplished and different concentration-treatment times were identified for the sessile and biofilm forms: biofilms generally require more contact time with sanitizers in order to observed a significant log reduction as compared to the sessile form - the integrated sanitation system was built Publications

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# On-farm Integration of Organic Management of Spotted-wing Drosophila in Fruit Crops

Accession No.	1029007
Project No.	ME02022-04083
Agency	NIFA ME.\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2022-51300-37890
Proposal No.	2022-04083
Start Date	01 SEP 2022
Term Date	31 AUG 2025
Grant Amount	\$2,970,079
Grant Year	2022
Investigator(s)	Fanning, P.; Daane, KE, .; Gomez, MI, I.; Zalom, FR, G.; Liburd, OS, EM.; Isaacs, RU, .; Beers, EL, .; Walton, VA, M.; Sial, AS, AH.; Rogers, MA, .; Chiu, JO, C.; Rodriguez-Saona, CE, R.; Formiga, AL, K.; Lee, JA, .

## NON-TECHNICAL SUMMARY

Spotted-wing drosophila (SWD) is a devastating pest of berries and stone fruits throughout the United States, causing significant losses (up to 100% if shipments are rejected) in crop yield and quality. This is estimated to have an economic impact of \ \$718 million annually, with associated effects on farm profitability, jobs, rural communities, and the environment. Management of SWD is achieved largely by toxic and environmentally damaging insecticide applications and is particularly challenging for organic growers due to the few effective OMRI-approved insecticides. Here we propose integrating compatible organic management approaches with biological control, monitoring, non-chemical controls, and conservation practices. This approach will enhance the adoption of integrated pest management, maintain crop quality, and support profitable berry production while developing opportunities to reduce insecticide dependence. We will pair these short-term goals to address immediate needs with outreach and training to build an organic literate extension workforce.

## OBJECTIVES

The overarching goal of this project is to reduce the economic impact of SWD on organic fruit production across the US. We will develop and evaluate decision-aid tools and thresholds based on new monitoring approaches to achieve this goal. In addition, we will integrate *G. brasiliensis* into existing crop management practices associated with organic farms. This will increase the implementation of SWD management programs compatible with the USDA National Organic Program (NOP) and true to the ethos of organic agriculture. To achieve this goal, our team has developed three research and extension objectives for organic control of SWD through careful collaboration with stakeholders, including; 1) Develop and expand monitoring and economic-based decision-aids for organic agriculture, 2) Promote beneficial insects in organic fields, and 3) Develop an integrated outreach and education training programs to implement organic SWD management strategies. These objectives will lead to greater on-farm integration of newly developed practices into NOP-compliant management programs as well as evaluating the impact of *G. brasiliensis* releases in and around organic farms.

## APPROACH

Objective 1. Develop and expand monitoring and economic-based decision-aids for organic agriculture

Obj. 1.1. Optimize sampling methods for SWD. In this objective, we will work closely with growers, including advisory board members that grow on their farms, to evaluate their management practices. These farms will overlap with those used in Obj. 2. This evaluation will be conducted by monitoring SWD adult or immature life stages (eggs or larva), or both if applicable to the region; thus, we will generate crop risk data as it pertains to the management approaches they have adopted. Measurements of SWD adults and immatures will be conducted regularly and in various locations throughout the crop. These data will be analyzed using statistical methods and will be relayed back to growers in real-time and PIs will discuss with their grower stakeholders what options exist to reduce the cost of their management strategies, particularly insecticides inputs, at specific times and locations throughout the production season.

Obj. 1.2. Assess economic impacts of organically-approved IPM-focused control strategies. An economic understanding to identify economically viable SWD control strategies is essential. In this objective, we will work closely with team members in Obj. 2 and Obj. 3 to select 4-5 areas of focus to develop SWD economic assessments where monitoring for adult and immature stages (egg/larva) will be done as described in Obj. 1.1. Each area of focus will be defined specifically for the: (1) crop, (2) region, (3) production system, (4) market channel, and (5) SWD management tactic of interest. For each area of focus, we will collect detailed data to 1) develop partial crop budgets to establish baseline profit figures (i.e., profits for a farm free of SWD infestation); 2) estimate the costs and benefits of different organic management strategies, and 3) assess economic losses due to SWD. These data will be systematically organized into databases and structured spreadsheets to conduct economic analyses of short-run (i.e., one crop cycle) and long-run (i.e., 10 years) profits to identify optimal strategies for SWD control in organic production systems. Monitoring of *G. brasiliensis* and SWD infestations will occur along a transect: adjacent habitat, field edge, 10, 50, and 100 m interior to the crop field.

Objective 2. Promote beneficial insects in organic fields

Obj. 2.1. Optimize methods for release and quantify establishment of *G. brasiliensis*. In this objective, we will release *G. brasiliensis* at organic farms across the 8 states with paired sites managed similarly or large farms (>20 acres) divided into pairs. Within a pair, one site will receive releases and the other none. Sites within a pair will be ~500 m apart to minimize the immediate overlap of parasitoids. Releases will start in late spring/early summer as fruits are infested. About 1000 - 1,500 parasitoids will be released per site per year. To quantify the establishment of *G. brasiliensis*, we will monitor wild fruit and use sentinel parasitoid traps that are suitable for in-crop monitoring but labor-intensive.

Obj. 2.2. Determine the impact of sustainable management on natural enemies and pollinators. At each of the release/control sites being sampled for *G. brasiliensis* in Obj. 2.1, we will also sample for natural enemies and pollinators along three spots of the transect: adjacent habitat, field edge, and interior ~100 m into the field. Each transect will have three sampling stations ~25 m apart. First, two unbaited yellow sticky cards will be set up per distance, and a third card baited with methyl salicylate (AgBio) to attract lady beetles, green lacewings, and parasitoids to enhance monitoring. Traps will be collected after 1 week, and sampling will occur once each month during the growing season. During the 6+ visits to deploy and collect the traps each season, we will sample the number of visiting beneficial insects. These samples will be randomized in the order of site visitation to include both morning and afternoon observations. At each transect, we will walk the border or crop row for 10 min and count identifiable beneficial insects and pollinators. Each site will also be characterized for the landscape composition surrounding the sample location (centered on the middle of the field edge transect), with the proportion of crop, natural, and other land types within 500 and 1000 m quantified using aerial photos or the USDA CDL raster file and ArcGIS or QGIS. The degree of correlation between the percent natural habitat and the abundance and diversity of beneficial insects will be determined across the whole project dataset for Years 1 and 2.

Obj. 2.3. Optimize entomopathogenic nematodes (EPNs) for control in protected environments. To establish optimal rates for use against SWD, *H. bacteriophora*, *S. carpocapsae*, and *S. feltiae* will be tested by placing SWD larval-infested caneberries on sterilized soil in Petri dishes. Nematodes may attack the larval, pupal, or teneral adult stage. Dishes will be drenched with variable rates of EPNs in water. Emerged adults, will be given diet and monitored for survival to assess if the number of eclosed adult SWD is affected. Dead pupae and adults will be dissected to confirm infection. Next, a semi-field trial will occur in organic caneberry hoop tunnels in California. Soil from hoop houses will be potted and treated with the three most promising species-doses as determined earlier. The same assessments of efficacy used in the lab bioassays will be used to assess the semi-field trial.

Obj. 2.4. Determine compatibility of organic compounds and nematodes with *G. brasiliensis*. We will conduct bioassays to determine the relative toxicity of organic-certified pesticides on *G. brasiliensis* using field-relevant rates of Entrust (spinosad), Grandevo (*Chromobacterium subtsugae*), Jet-Ag (peroxyacetic acid), Azera (azadirachtin and pyrethrins), or untreated control. Compounds at varying rates will be applied to Petri dishes and placed in a fume hood to dry. Once dry, 10 female wasps, 3-7 d old, will be introduced to each dish and covered with an untreated lid. Wasps will be observed 1, 4, and 24 h later for mortality.

Objective 3. Develop an integrated outreach and education training programs to implement organic SWD management strategies

Obj. 3.1. Develop an integrated outreach program to deliver outputs to organic growers. In this project, we will create a range of Extension and outreach products to extend the information through our team's continued partnership with eOrganic ([eorganic.info/spottedwingorganic](http://eorganic.info/spottedwingorganic)) to share updated research results and include organic-specific

research and extension articles, fact sheets, educational videos, archived webinars, newsletters, press releases, and links to other websites and blogs. In addition, we will share these resources at grower/stakeholder meetings and national webinars, on-farm demonstrations and field days, and on social media to maximize stakeholder engagement.

**Obj. 3.2. Build an organic literate extension workforce through a project-specific Research and Extension Experiences for Undergraduates.** Every year we will host 7-8 students across the project each summer in a highly supported organic IPM-based mentored undergraduate student research program to develop knowledge of scientific research and analytical skills while learning about careers in organic agriculture-related fields. PIs from participating states (CA, FL, GA, ME, MI, MN, NJ, OR, WA) will recruit and host undergraduate students in their labs for a 10-week coordinated summer research experience. Research projects will be related to the project objectives outlined in this proposal. Progress 09/01/23 to 08/31/24 Outputs Target Audience: Our target audience is organic fruit growers and processors in multiple regions (Southern, Northeastern, North Central, and Western) of the U.S., comprising small, midsize, and large-scale growers, Cooperative Extension personnel, and other stakeholders including:

- Fruit grower organizations, nationally.
- Other Entomologists nationally and internationally.
- Decision-makers at state, local, and federal levels.

**Changes/Problems:** Nothing Reported

**What opportunities for training and professional development has the project provided?** In its second year, this project has provided much training and professional development for researchers at all experience levels, from undergraduates to post-doctoral researchers. Nine undergraduates, seven graduate students, and two post-doctoral researchers received training and professional development. A vital element of this was conducted through our project-specific Research and Extension Experiences for Undergraduates, described in Objective 3.2. In this objective, undergraduates gained experience in various subjects, including conducting their research projects. Graduates and postgraduates were also trained in mentorship. In addition, or coinciding with that training in Objective 3.2, broad training in topics including rearing biological control agents, experimental design, conducting independent research, parasitoid behavior, field assessment of parasitism rates, and insect identification. Trainees, including undergraduates, will present results in state/local, regional, and national meetings organized by the Entomological Society of America. How have the results been disseminated to communities of interest? Outreach associated with this project was primarily conducted through our specific integrated outreach program objective 3.1. As seen in the Products section, dissemination to communities of interest has been through journal articles and presentations to scientific audiences at regional, national, and international meetings. Additionally, through presentations to grower groups and one-on-one contact, we have educated ~900 growers (attendance was not recorded at all talks). What do you plan to do during the next reporting period to accomplish the goals? During the next reporting period, the project teams will continue to process samples from experiments and releases (Obj. 2.1 and 2.2) of this year in advance of our planned project webinar, annual stakeholder meeting, and local, regional and national meetings to members of the scientific communities and growers under Objective 3.1. Our monthly team meetings have resumed, and we continue to complete laboratory bioassays in Objectives 2.2, 2.3, and 2.4, which will be conducted in the winter and spring. These will be the focus of our winter/spring webinar. In the spring, we will collectively start recruiting a new cohort of undergraduates for Objective 3.2 and increasing the size of our colonies for experiments with *G. kimorium* for our final year of releases.

**Impacts** What was accomplished under these goals? Objective 1. Obj. 1.1. Optimize sampling methods for SWD. To continue the effective management of SWD in organic systems, growers need reliable decision-aid tools that integrate monitoring and insecticide applications that are cost-effective and environmentally sustainable, as well as other controls. Currently, some growers monitor SWD in the field to initiate pesticide applications. In some states, experiments were conducted to determine the economic threshold of applying organic insecticides, such as in blueberries, in response to SWD captured in traps. In other states, researchers are working with organic blueberry growers to monitor SWD weekly and provide them with information on the level of infestation to guide their crop management decisions. This led to a reduction in spray applications or delay in first applications, depending on the field, through more informed IPM programs.

Obj. 1.2. Assess economic impacts of organically approved IPM-focused control strategies. A key focus of this sub-objective in the western states focuses on the commercial use of Decoy, an organic product developed by Oregon State University (OSU) for use against SWD, which has expanded from 7,630 to 11,764 acres (3,921 Organic acres). Continuing this work in 2023, research was conducted on Attract and Kill products in the laboratory, semi-field, and commercial organic grower fields; infestation data suggests similar SWD control to conventional complete cover applications. Pesticide residue is reduced by 90% and cost by ~40%. Cornell and OSU collaborated to complete a bioeconomic model (including an SWD population model and economic simulation) for evaluating the cost-effectiveness of SWD fruit sampling control strategies in organic blueberry production. Based on this, an interactive decision tool on investing in exclusion netting for NY organic blueberry growers is in development. Additionally, Cornell and UMaine are updating our previous decision support tool to incorporate perimeter applications to increase profitability.

Objective 2. Obj. 2.1. Optimize methods for release and quantify establishment of *G. brasiliensis*. In year two of the projects, they optimized and continued using standardized protocols for this objective and 2.2, described below. Nive of the eleven participating labs established and maintained colonies of *Ganaspis kimorium*, formerly *G. brasiliensis*, which is a significant

undertaking in and of itself. The project team worked at 32 sites, with 17 of these being release sites with others serving as controls. Multiple individual release events were conducted, and 36,336 adult *G. kimorium* were released. Across all sites, participating labs aimed to monitor 1 or 2 paired organic sites (2 or 4 sites total), with a site pair consisting of a "Gk release" and "control" sites. Both sites are managed organically by the grower standard. The release site has a noncrop site suitable for *G. kimorium* release; it is ideal for the control site to have a similar noncrop site. We aimed for sites large enough for transect sampling to be conducted. Notably, we are recovering primarily a separate parasitoid, *Leptopilina japonica*, an adventive population of Asian origin that also attacked SWD. Obj. 2.2. Determine the impact of sustainable management on natural enemies and pollinators. The sites and transects described above were sampled using yellow sticky cards for this sub-objective. This sampling will be conducted 3x in the season to count other natural enemies and pollinators. The timing of this sampling was at: 1) pre-release, 2) mid-season, and 3) post-release. This minimizes concern about YST capture of *G. kimorium*. Traps were hung in the canopy, preferably with shade to prevent insect degradation, for 1-2 weeks. Obj. 2.3. Optimize entomopathogenic nematodes for control in protected environments. During the last reporting period, a large multistate effort was carried out to test the compatibility of entomopathogenic nematodes (EPNs) with SWD control and parasitoids. This involved lab bioassays involving two species of EPN, *Steinernema feltiae* and *Steinernema carpocapsae*, and four species of parasitoid that are known to attack SWD, two pupal (*Trichopria drosophilae* and *Pachycrepoideus vindemiae*) and to larval parasitoids (*L. japonica* and *G. kimorium*), were conducted across labs in CA, FL, GA, MI, ME, and OR. Additionally, field trials were conducted in CA and OR to see if EPN could be dispersed through drip irrigation systems to reduce SWD populations. Obj. 2.4. Determine the compatibility of organic compounds and nematodes with *G. brasiliensis*. Establishing *G. kimorium* in organically managed agroecosystems can benefit blueberry growers by providing an additional layer of SWD management. Before fully integrating this beneficial insect into berry production systems, researchers must determine the degree to which current management tools (i.e., chemical insecticides) will affect the establishment and longevity of *G. kimorium* in the field. This year, the project team initiated a series of bioassays across multiple states to determine the impact of OMRI-approved insecticides (Entrust, Grandevo, Pyganic, PAA products) alone and in combination with an attract and kill technology in Combi-Protec, which has been demonstrated to help manage SWD while reducing the amount of insecticide. These bioassays involved both *L. japonica* and *G. kimorium*. Insecticides were tested at multiple rates and both as full cover and bait treatments.

Objective 3. Obj. 3.1. Develop an integrated outreach program to deliver outputs to organic growers. Through newsletter articles, presentations to grower groups, and one-on-one contact, we have educated organic growers on the use of classical biological control to help manage SWD and reported the project's progress. The team disseminated project information through three extension articles and multiple talks, reaching growers and researchers at state and regional levels. Additionally, we presented at conferences to national and international audiences. Obj. 3.2. Build an organic literate extension workforce through project-specific Research and Extension Experiences for Undergraduates. This project aims to train an interdisciplinary, organic, and literate community. The goals were to deepen undergraduates' knowledge and skill sets related to entomological research, organic agriculture, and professional and career development. A 10-week-long program structured around instructional workshops, mentorship, and scientific research in lab and field settings has been developed. This program utilized a network of guest lecturers and many project participants throughout the workshop to increase the understanding of basic scientific research, analytical, and communication skills. Post-program survey results from undergraduate mentees indicated increased knowledge and skills in multiple areas. Additionally, post-program surveys of undergraduate mentees indicated increased enthusiasm and confidence in the research process because of mentor-encouraged ownership of projects. At the end of the summer project, undergraduate students were given the opportunity to present their research findings during a program-wide symposium. Presentations were tailored in a research and extension-based format where undergraduates were expected to discuss their research and project findings by providing scientific literature reviews, analyzing experimental results, representing data in figures/illustrations, and answering questions based on quantitative and qualitative methods of scientific understanding. Post-program, undergraduate mentees stated they gained confidence in the research process with an improved ability to read critically and present research findings to others.

Publications Type: Peer Reviewed Journal Articles Status: Published Year Published: 2023 Citation: Mermer S, Rossi Stacconi MV, Tait G, et al. 2023. Comparing the effectiveness of different insecticide application orders for suppressing *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) infestation: experimental and modeling approaches. *Journal of Economic Entomology*. 116(3):899908. Type: Peer Reviewed Journal Articles Status: Published Year Published: 2024 Citation: Yeh DA, Dai B, Gomez MI, et al. 2024. Does monitoring pests pay off? a bioeconomic assessment of *Drosophila suzukii* controls. *Pest Management Science*. 80(2):708723. Type: Peer Reviewed Journal Articles Status: Published Year Published: 2024 Citation: Rossini L, Contarini M, Speranza S, et al. 2024. Life tables in entomology: A discussion on tables parameters and the importance of raw data. *Plos one*. 19(3):e0299598. Type: Peer Reviewed Journal Articles Status: Published Year Published: 2024 Citation: Garipey, T.D., Abram, P.K., Adams, C., Beal, D., Beers, E., Beetle, J., Biddinger, D., BrindAmour, G., Bruin, A., Buffington, M., Burrack, H., Daane, K.M., Demchak, K., Fanning, P., Gillett, A., Hamby, K., Hoelmer, K.,

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Insect management in organic production. Growers Meeting. Dresden, Maine. May 2023. (11 people). Type: Other Status: Other Year Published: 2024 Citation: Isaacs, R. and Van Timmeren, S. Larval sampling of blueberries to determine SWD infestation levels. Extension video published April 2024. <https://www.canr.msu.edu/news/new-video-on-sampling-berries-for-spotted-wing-drosophila-to-guide-your-ipm-program>. Type: Other Status: Other Year Published: 2024 Citation: Liburd, O. E. 2024. Update on SWD management including release and captures of GK. International Blueberry Meeting, April 10-12 Tampa, Florida Type: Other Status: Other Year Published: 2023 Citation: Isaacs, R. Updates on insect management in blueberries. Great Lakes Expo. Grand Rapids, Michigan. December 2023. Type: Other Status: Other Year Published: 2024 Citation: Isaacs, R. Blueberry insect management. Southwest Hort Days. Mendel Center, Benton Harbor. Michigan. February 2024. 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Type: Other Status: Other Year Published: 2024 Citation: Van Timmeren, S., Li, S-L., and Vander Weide, J. September 4, 2024. Effect of ground covers on Bluecrop photosynthesis, fruit quality, and SWD pressure. Blueberry Field Day 2024. Trevor Nichols Research Center, Fennville, Michigan. Type: Other Status: Other Year Published: 2024 Citation: Van Timmeren, S. and Isaacs, R. September 6, 2024. Technique for monitoring SWD and BBM larvae and notes on BBM emergence and infestation in blueberries. Blueberry Field Day 2024. Trevor Nichols Research Center, Fennville, Michigan. Type: Other Status: Other Year Published: 2023 Citation: Miguel I. Gomez, Bingyan Dai and R. Karina Gallardo. Moving from crisis response to long-term integrated management of SWD: A keystone pest of fruit crops in the United States. Feb 12-13, 2024. Type: Other Status: Other Year Published: 2024 Citation: Miguel I. Gomez and Bingyan Dai. Global threat to agriculture and food industry from invasive species. North American Horticulture Advisory Council. May 22-24, 2024. Progress 09/01/22 to 08/31/23 Outputs Target Audience: Our target audience is organic fruit growers and processors in multiple regions (Southern, Northeastern, North Central, and Western) of the U.S., comprising small, midsize, and large-scale growers, Cooperative Extension personnel, and other stakeholders including: Fruit grower organizations, nationally. Other Entomologists nationally and internationally. Decision-makers at state, local, and federal levels. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? In its first year, this project has provided a rich

amount of training and professional development for researchers at all levels of experience, from undergraduates to post-doctoral researchers. Nine undergraduates, nine graduate students, and three post-doctoral researchers received training and professional development. A key element of this was conducted through our project-specific Research and Extension Experiences for Undergraduates, described in Objective 3.2. In this objective, undergraduates gained experience in a wide array of subjects, including conducting their own research projects. Graduates and postgraduates were also trained in mentorship. In addition, or coinciding with that training in Objective 3.2, broad training in topics including rearing of biological control agents, experimental design, conducting independent research, parasitoid behavior, field assessment of parasitism rates, and insect identification. Trainees, including undergraduates, will present results in state/local, regional, and national meetings organized by the Entomological Society of America. How have the results been disseminated to communities of interest? Outreach associated with this project was primarily conducted through our specific integrated outreach program objective 3.1. As seen in the Products section, dissemination to communities of interest has been through journal articles and presentations to scientific audiences at regional, national, and international meetings. Additionally, through presentations to grower groups and one-on-one contact, we have educated ~1100 growers (attendance was not recorded at all talks). What do you plan to do during the next reporting period to accomplish the goals? During the next reporting period, the project teams will continue to process samples from experiments and releases (Obj. 2.1 and 2.2) of this year in advance of our planned project webinar, annual stakeholder meeting, and local, regional and national meetings to members of the scientific communities and growers under Objective 3.1. Our monthly team meetings have resumed, and plans for laboratory bioassays in Objectives 2.2, 2.3, and 2.4 will be conducted in the winter and spring. In the spring, we will collectively start recruiting a new cohort of undergraduates for Objective 3.2 and increasing the size of our colonies for experiments with *G. brasiliensis*. Impacts What was accomplished under these goals? Objective 1. Obj. 1.1. Optimize sampling methods for SWD. To continue the effective management of SWD in organic systems growers need reliable decision-aid tools that integrate monitoring and insecticide applications that are cost-effective and environmentally sustainable, and other controls. Currently, some growers monitor SWD in the field to initiate pesticide applications. In some states, experiments were conducted to determine the economic threshold of applying organic insecticides, such as in blueberries in response to SWD captured in traps such as the Trécé Pherocon SWD traps in Florida or the yeast sugar trap in Maine. In other states, researchers are working with organic blueberry growers to monitor SWD weekly and provide them with information on the level of infestation to guide their crop management decisions. This led to a reduction in spray applications or delay in first applications, depending on the field, through more informed IPM programs. Obj. 1.2. Assess economic impacts of organically approved IPM focused control strategies. A key focus of this sub-objective in the western states focuses on the commercial use of Decoy, an organic product developed by Oregon State University (OSU) for use against SWD which has expanded from 7,630 to 11,764 acres (3,921 Organic acres) in Western (California, Oregon and Washington) production regions. Additionally, a collaboration between Cornell and OSU developed a bioeconomic model (including a SWD population model and economic simulation) for evaluating the cost-effectiveness of SWD fruit sampling control strategies in organic blueberry production. From this, an interactive decision tool on investing in exclusion netting for NY organic blueberry growers is in development. Objective 2. Obj. 2.1. Optimize methods for release and quantify establishment of *G. brasiliensis*. In year one, the team developed standardized protocols for this objective and objective 2.2, described below. Ten of the eleven participating labs established and maintained colonies of *G. brasiliensis*, which is a significant undertaking in and of itself. The project team worked at a total of 37 sites, with 19 of these being release sites, with others serving as controls. 47 individual release events were conducted and a total of 25,645 adult *G. brasiliensis* were released. Across all sites, participating labs aimed to monitor 1 or 2 paired organic sites (2 or 4 sites total), with a site pair consisting of a "Gb release" and "control" sites. Both sites are managed organically by the grower standard. The release site has a non-crop site suitable for *G. brasiliensis* release, it is ideal for the control site to have a similar non-crop. We aimed for sites large enough for transect sampling to be conducted. For the release sites, we selected sites with some wooded or non-crop vegetation so *G. brasiliensis* need wouldn't get sprayed; and ones that ideally had other SWD host fruit so Gb can reproduce (ie. wild blackberry). *G. brasiliensis* adults were to be released as mated and younger wasps, over multiple weeks per release site. The timing of releases was to start when SWD was found to be infesting fruit to support Gb, and the window (i.e., June-Aug) will depend on fruit phenology. Three transects were set up at each farm along which fruit and sentinel traps were set up at the wood line, 1m, 10m, and 25m into the crop. Records of release locations (GPS) and numbers were kept along with habitat descriptions of non-crop areas (i.e., wild Himalaya blackberry along conifers near a river, blackberry about 40% cover). Obj. 2.2. Determine the impact of sustainable management on natural enemies and pollinators. For this sub-objective, the same sites and transects as described above were sampled using yellow sticky cards. This sampling will be conducted ~3x in the season to count other natural enemies and pollinators. The timing of this sampling was at: 1) pre-release, 2) mid-season, and 3) post-release. This minimizes concern about YST capture of *G. brasiliensis*. Traps were hung in the canopy, preferably with some shade to prevent insect degradation, for 1-2 weeks. Obj. 2.3. Optimize entomopathogenic nematodes for control in protected

environments. This objective's work focused on nematodes and focused on small-scale organic growers/stakeholders because we have limited protected agricultural production in small fruits in Florida. Preliminary trials indicate that *Steinernema feltiae* had the highest pathogenicity against and the highest reproduction within SWD. *Steinernema riobrave* had the least pathogenicity against SWD and lowest reproduction within this insect. Obj. 2.4. Determine compatibility of organic compounds and nematodes with *G. brasiliensis*. Establishing *G. brasiliensis* in organically managed agro-ecosystems has the potential to benefit blueberry growers by providing an additional layer of SWD management. Prior to the full integration of this beneficial insect into berry production systems, researchers must determine the degree to which current management tools (i.e., chemical insecticides) will affect the establishment and longevity of *G. brasiliensis* in the field. In the first year of the project, experiments in FL tested the response of adult *G. brasiliensis* to three organically labeled insecticides (Grandevo, Entrust, and Pyganic) directly in residue-response assays and indirectly against fruit sprayed-response assays in the lab. Objective 3. Obj. 3.1. Develop an integrated outreach program to deliver outputs to organic growers. Through newsletter articles, presentations to grower groups, and one-on-one contact, we have educated organic growers on the use of classical biological control to help manage SWD, reporting the project's progress. In total, the team disseminated project information through three extension articles and multiple talks, reaching growers and researchers at state and regional levels. Additionally, we presented at conferences to national and international audiences. Obj. 3.2. Build an organic literate extension workforce through project-specific Research and Extension Experiences for Undergraduates. This project has a specific goal of training an interdisciplinary organic literate community, the goals were the deepening of undergraduates' knowledge and skill sets related to entomological research, organic agriculture, and professional and career development. A 10-week-long program structured around instructional workshops, mentorship, and scientific research in lab and field settings has been developed. For this program, a network of guest lecturers, many of whom are project participants, was utilized throughout the workshop schedule to increase the understanding of basic scientific research, analytical, and communication skills. Post-program survey results from undergraduate mentees indicated increased knowledge and skills in multiple areas. Additionally, post-program surveys of undergraduate mentees indicated increased enthusiasm and confidence in the research process as a result of mentor-encouraged ownership of projects. At the end of the summer project, undergraduate students were given the opportunity to present their research findings during a program-wide symposium. Presentations were tailored in a research and extension-based format where undergraduates were expected to discuss their research and project findings by providing scientific literature reviews, analyzing experimental results, representing data in figures/illustrations, and answering questions based on quantitative and qualitative methods of scientific understanding. Post-program, undergraduate mentees stated they gained confidence in the research process with an improved ability to critically read and present research findings to others. Publications Type: Journal Articles Status: Accepted Year Published: 2022 Citation: Panthi, B. et al. (2022). Using red panel traps to detect spotted-wing drosophila and its infestation in US berry and cherry crops. *Journal of Economic Entomology*. 10.1093/jee/toac134. Type: Journal Articles Status: Published Year Published: 2022 Citation: Rossi-Stacconi, M. V., Wang, X.-G., Stout, A., Fellin, L., Daane, K. M., Biondi, A., Stahl, J. M., Buffington, M. L., Anfora, G., Hoelmer, K. A. 2022. Methods for rearing the parasitoid *Ganaspis brasiliensis*, a promising biological control agent for invasive *Drosophila suzukii*. *JoVE Journal* 184 e63898. doi 10.3791/63898 (online: <https://www.jove.com/v/63898>) Type: Journal Articles Status: Accepted Year Published: 2022 Citation: Beers, E. H., Beal, D., Smytheman, P., Abram, R., Schmidt-Jeffris, R., Moretti, E., Daane, K. M., Looney, C., Lue, C.-H., Buffington, M. L. 2022 First records of adventive populations of the parasitoids *Ganaspis brasiliensis* and *Leptopilina japonica* in the United States. *Journal of Hymenoptera Research*. 91: 1125. doi: 10.3897/jhr.91.82812 Type: Journal Articles Status: Submitted Year Published: 2023 Citation: Hopper, K. R., Wang, X.-G., Kenis, M., Seehausen, L., Abram, P., Daane, K. M., Buffington, M., and Hoelmer, K. A., 2023. Genome divergence and reproductive incompatibility between populations of *Ganaspis nr. brasiliensis*. *Evolutionary Applications* (submitted) Type: Journal Articles Status: Published Year Published: 2023 Citation: Sriram, A., Voyvot, S., Johnson, B. C., Chowdhury, S. M., Fanning, P. D., & Lee, J. C. (2023). Mesh covers on sentinel parasitoid traps prevent *Drosophila suzukii* movement and impact parasitism by *Ganaspis brasiliensis* and *Pachycrepoideus vindemiae*. *Biocontrol Science and Technology*, 1-11. Type: Other Status: Other Year Published: 2023 Citation: Rogers, M. Integrated pest management in organic horticulture. Guest lecture for ENT 3211/5211: Insect Pest Management. 5 April 2023. Type: Other Status: Other Year Published: 2023 Citation: Rogers, M. Organic management strategies for insect pests in small fruit. MN Agriculture Experiment Station Seminar, Dept. of Horticultural Science Seminar Series. 19 April 2023. Type: Other Status: Other Year Published: 2023 Citation: Rogers, M. Organic research and teaching update. Minnesota Dept. of Agriculture Organic Taskforce. 29 June 2023. Type: Other Status: Other Year Published: 2023 Citation: Jones, C. and A. Sial. 2023. Releases of *Ganaspis brasiliensis* In South Georgia to Help Control Spotted-Wing *Drosophila* Populations (Poster). 2023 Georgia Blueberry Growers Meeting (~180 attendees) Type: Other Status: Other Year Published: 2023 Citation: Lee, J., and Rendon D. (2023) Updates on research at the USDA to growers. North Willamette Research and Extension Center, Aurora, OR. July 26, 2023. Type: Other Status: Other Year Published: 2023 Citation: Lee, J.

2023. Predators and pathogens for SWD control presentation by J. Lee. to OREI student webinar series. June 29, 2023. Type: Other Status: Other Year Published: 2023 Citation: Lee, J. 2023. SWD control with stingers, sterility and sweets presentation to growers. Oregon Caneberry Workshop, Salem, OR. Mar 10, 2023. ~100 people Type: Other Status: Other Year Published: 2023 Citation: Walton, V.M. 2023. Presentation to growers at the Oregon Caneberry Workshop, Salem, OR. Mar 10, 2023. ~100 people Type: Other Status: Other Year Published: 2023 Citation: Walton, V.M. 2023. Presentation to growers at the Oregon Blueberry Workshop, Clatskanie, OR. ~80 people Type: Other Status: Other Year Published: 2023 Citation: Fanning, P. 2023. New tools in the management of spotted-wing *Drosophila*. Talk to growers at the Blueberry Hill Farm day, Jonesboro, Maine, 6/22/2023. (132 people). Type: Other Status: Other Year Published: 2023 Citation: Fanning, P. 2023. Insect management in organic production. Talk to growers at the Dresden ME, 5/12/2023. (11 people). Type: Other Status: Other Year Published: 2023 Citation: Fanning, P. 2023. Controlling springtime insects. Fanning, P., Talk to growers at the Union ME, 5/10/2023. (16 people). Type: Other Status: Other Year Published: 2023 Citation: Fanning, P. 2023. Insect IPM in blueberry. Talk to growers at the Downeast IPM and Crop Management Field Meeting, 4/17/2023. (9 people). Type: Other Status: Other Year Published: 2023 Citation: Johnson, B., and Fanning, P. 2023. Spotted-wing *Drosophila* and Biological Control. Maine Invasive Species Network meeting. 23 March 2023. Oral Presentation. (47 people). Type: Other Status: Other Year Published: 2023 Citation: Fanning, P., 2023. Insect Pest Management in Wild Blueberry. Wild Blueberry Conference Bangor Maine. 22 February 2023. Oral Presentation. (53 people). Type: Other Status: Other Year Published: 2023 Citation: Fanning, P., and Annis, S. 2023. Climate change and pest observations. 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Type: Conference Papers and Presentations Status: Other Year Published: 2023 Citation: Brown, J., and O. E. Liburd. 2023. Towards the biocontrol of *Drosophila suzukii* in North-central Florida natural areas using *Ganaspis brasiliensis*, March 12-15. SEB-ESA, Little Rock Arkansas. Type: Conference Papers and Presentations Status: Other Year Published: 2023 Citation: Lambert, A., J. Brown, L. Daucan , and O. E. Liburd. 2023. Biological control potential of SWD with natural enemies including entomopathogenic nematodes. March 12-15. SEB-ESA, Little Rock Arkansas. Type: Other Status: Other Year Published: 2023 Citation: Isaacs, R., Perkins, J. and Van Timmeren, S. Preparing for blueberry insect management in 2022. SW Michigan Hort Days. Benton Harbor, MI. February 3, 2022. 15 growers (online) Type: Other Status: Other Year Published: 2023 Citation: Rodriguez-Saona, C., P. Prade, K. Hamby, D. Biddinger, K. Regan, and K. Demchak. 2023. Efforts to establish a natural enemy of spotted-wing *drosophila* in the Mid-Atlantic and beyond. Proceedings of the Mid-Atlantic Fruit & Vegetable Convention. Hershey, PA. Type: Other Status: Other Year Published: 2023 Citation: Rodriguez-Saona, C., and P. Prade. 2023. Efforts to release a spotted-wing *drosophila* parasitoid in New Jersey. Proceedings of the Atlantic Coast Agricultural Convention and Trade Show. Atlantic City, NJ. Type: Conference Papers and Presentations Status: Other Year Published: 2022 Citation: Hogg, B. N. et al. Releases of *Pachycrepoideus vindemiae* for augmentative biological control of spotted wing *drosophila*. Annual Entomological Society of America meeting. Vancouver, BC, November 2022. Type: Conference Papers and Presentations Status: Other Year Published: 2022 Citation: Stahl, J. M. et al. Setting the stage: An overview of spotted-wing *drosophila* biocontrol efforts. Entomological Society of American & Entomological Society of Canada Annual Meeting. Vancouver, Canada. Nov 2022 Type: Conference Papers and Presentations Status: Other Year Published: 2022 Citation: Lee, J. et al. Areawide releases of *Ganaspis brasiliensis* in the West Coast. Entomological Society of American & Entomological Society of Canada Annual Meeting. Vancouver, Canada. Nov 2022 Type: Conference Papers and Presentations Status: Other Year Published: 2022 Citation: Daane et al. Classical biological control of the spotted-wing *drosophila* in USA. Entomological Society of American & Entomological Society of Canada Annual Meeting. Vancouver, Canada. Nov 2022 Type: Conference Papers and Presentations Status: Other Year Published: 2022 Citation: Fanning, P., Johnson, B., Prade, P., Rodriguez-Saona, C. and Loeb, G. Progress towards classical biological control for spotted wing *drosophila* in the northeastern United States. Invited talk at ESA annual meeting in Vancouver, Canada on 15 November, 2022 as part of symposium titled Biocontrol of *Drosophila suzukii*: status updates on natural enemies and ecological considerations. Type: Conference Papers and Presentations Status: Other Year Published: 2023 Citation: A Bioeconomic Model for Sustainable Control of *Drosophila suzukii* in Organic Blueberry Production. Agricultural and Applied Economics Association (AAEA) Annual Meeting (Selected Paper Presentation). Washington D.C. 2023. Type: Conference Papers and Presentations Status: Other Year Published: 2023 Citation: Prade, P., and C. Rodriguez-Saona. 2023. Survey of *Drosophila suzukii* parasitoids in

New Jersey prior and after the release of *Ganaspis brasiliensis*. Entomological Society of America, Eastern Branch Meeting. Providence, RI, March 20, 2023.

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# Impact of Long-term Cover Cropped Organic Farming Practices on the Development of Disease Suppressive Soils

<b>Accession No.</b>	1028968
<b>Project No.</b>	MO.W-2022-04046
<b>Agency</b>	NIFA MO.W\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37883
<b>Proposal No.</b>	2022-04046
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2025
<b>Grant Amount</b>	\$749,331
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Eivazi, F.; Patel, JA, .; Bardhan, SO, .; Lin, CH, .
<b>Performing Institution</b>	LINCOLN UNIVERSITY, 820 CHESTNUT ST, JEFFERSON CITY, MISSOURI 651023537

## NON-TECHNICAL SUMMARY

Current organic systems depend on various management practices such as crop rotation, cover crops, use of composts and other soil amendments to develop healthy soils that are supposedly able to suppress pathogens and plant diseases. While there is extensive information about the individual amendments and practices and their role in soil health and disease suppression, there is very little information about the systemic role of organic farming systems to maintain soil health and suppress diseases. Moreover, the long-term organic management practice might also have a significant impact on these properties. In this project, we propose to fill this gap in knowledge and evaluate a chronosequence of soils maintained under organic farming using cover cropping practices for their ability to preserve or improve soil health and also impart resistance to pathogens and diseases. We expect that the benefits of long-term use of cover crops will create a dynamic and biodiverse habitat for beneficial soil microbial populations within the rhizosphere of such production systems. Diverse lab analysis and field studies information on soil-plant relationship systems will be collected to develop tools to quantify supporting (nutrient fixation and recycling, soil health), and regulating (disease suppression) functions of organic cover cropped soils. Educational and outreach programs will include providing internship opportunities, training minority undergraduate and graduate student, attendance and presentations at annual meetings, train the trainer workshop, field days, publications, and a presence on the internet and in social media.

## OBJECTIVES

Our long-term goal is to utilize an integrated approach for research, education, and extension to develop a deeper understanding of disease suppressive ability imparted by a chronosequence of organic farming systems. This project will generate science-driven knowledge, guidance, and tools to better assess the capability of long-term organic management to impart disease suppressibility. Specific objectives are to: 1. Quantitatively measure soil physico-chemical properties, and soil health indicators under cover cropped plots using different spring termination methods (Research objective) 2. Generate a complete soil chemical profile using metabolomics approach (Research objective). 3. Assess the disease suppression capability of soil maintained under a chronosequence of organic farming practices (Research objective). 4. Develop and conduct educational and outreach programs to enhance stakeholder knowledge of innovative organic systems that improve soil health and

ecosystem services; provide internship opportunities for high school students; train undergraduate and graduate students (Education and Extension objective).

## APPROACH

Objective 1: Quantitatively measure the soil physico-chemical properties and soil health indicators under cover cropped plots using different spring termination methods (Research objective). Treatments: The following cover crop termination treatments and two controls will be evaluated: (1) crimper rolled, (2) occultation (3) flail mowed, (4) sickle bar mowed, (5) rotary mowed (6) black plastic mulch on tilled soil (control 1) and (7) bare tilled soil (control 2). Each treatment will be replicated four times following a randomized block design. Site Preparation, Fertility, Irrigation management: The research site has been cover cropped over the past six years. In early September each year, an area will be disked and tilled as necessary to facilitate a seeding bed for cover crops in treatment plots. Each treatment unit will comprise a 20 feet-long raised bed created with a bed hiller/shaper at 48" width. In control plots for summer squash transplant beds, a mixture of hairy vetch and rye will be broadcasted and harrow-incorporated to simulate standard organic grower practices. In treatment plots, hairy vetch and cereal rye will be planted with a push seeder in September down the length of each bed seeded in alternating rows on 5" centers. The area between the beds will be 72" wide to facilitate tractor mowing, and it will be planted with rye and a biennial clover. These bed alleys will be broadcast-seeded and incorporated through harrowing. Bed alleys will be planted in fall and mowed the following spring and as needed throughout the growing season to reduce competition with the cash crop and ease foot traffic. Soil physical, chemical, and health indicator parameters including microbial diversity and enzymatic activities will be measured under each treatment using appropriate methods. Objective 2: Generate a complete soil chemical profile using metabolomics approach (Research objective). The chemicals in the rhizospheres will be extracted by MeOH and analyzed by ultra-high pressure liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS). The ion chromatograms will be submitted to XCMS platform operated by the Center for Metabolomics at the Scripps Research Institute. The peak detection, peak grouping, spectra extraction, and retention alignment will be processed by XCMS. The spectra will be annotated, and the compounds identified and categorized by integrating the METLIN, the world's largest metabolite database. Multivariate analysis and principal component analysis (PCA) will be performed by XCMS to compare the chemical profiles of the treatments. Each identified compound will be assigned and related to its biological pathway through XCMS biological pathway/network analysis. Objective 3: Assess the disease suppression capability of various soils maintained under a chronosequence of organic farming practices (Research objective). Experiments will be conducted in 2023, 2024, and 2025 to evaluate disease suppressibility of soils maintained under organic cover crop management for five, six, and seven years respectively. To accurately measure the effects of organic management, we will divide split plots in three portions, first portion for assessing effects of organic management on reducing tomato soil-borne diseases in 2023, second portion in 2024, and third portion in 2025 (see attached supplemental plot layout). Such field design allows us to assess the impact of a chronosequence of organic farming management on soil's suppressing ability of diseases. The dependent variables for this objective will be disease incidence, disease severity, fruit yield, and fruit weight. We will collect data for all soil-borne diseases detected in susceptible varieties. Disease incidence and severity of root and stem diseases of tomato will be evaluated every two weeks after transplanting. The tomato yield will be assessed at the end of the crop season. The number of fruits and weight of fruits will be recorded. We will analyze the interacting effects of the main plot and split-plots on disease incidence, disease severity, and fruit yield. The statistical analysis will be performed using two-way ANOVA to test whether or not the independent variables have a statistically significant ( $p < 0.05$ ) relationship with the dependent variables. Objective 4. (Extension and Education): Lincoln University has served the needs of underserved Missourians since 1866, and its role in education and service to stakeholders throughout the state and the nation has long been recognized. In accordance with the mission of the 1890 Land Grant Extension System, the ultimate goal of Lincoln University Cooperative Extension is "to help diverse audiences with limited resources improve their quality of life through the application of educational and research-based information focused on critical issues and needs". Because our clientele is very diverse to include (but not limited to) beginning, limited resources, and minority farmers, then we use culturally-appropriate approaches to deliver research-based information to our stakeholders. One of the vital aspects of this project is to prepare outstanding future farmers, educators, and researchers in agriculture. This proposal supports the experiential learning of students in organic agriculture. The project director will be working with two certified organic growers in Missouri who have internship programs for high school students. Farm 1: Earth Dance Organic Farm and School <https://earthdancefarms.org> Farm 2: Happy Hollow Farm <https://www.happyhollowfarm-mo.com/> Both farms offer 10-week summer internships to high school students. The program offers apprentices the following: Composting and methods of soil fertility management Crop planning and rotations Propagation (both in a greenhouse and direct seeding) Organic pest and disease management An introduction to farm equipment use and maintenance Proper harvest and post-harvest handling techniques Selling and marketing via farmer's markets

and through the CSA (community supported Agriculture)How to work effectively as a team player and moreThis project will support internships of two students per farm (for 3 Summers) by means of internship stipend payments. , The involvement of students in hands-on learning activities in the farm will help them to learn and experience using the latest developments in organic farming. The main goal of this project is to prepare the younger generation for a successful transition to the agricultural workforce, which is in alignment with this proposal's goal. Progress 09/01/22 to 08/31/23 Outputs Target Audience:Organic grower organizations, Small farmers with limited resources, Agronomy faculty, Students majoring in agriculture. Changes/Problems:Extreme heat and drought in the summer of 2023 in central Missouri had a negative effect on our field experiments. What opportunities for training and professional development has the project provided?One graduate student and a post-doctoral fellow were identified and hired. Two minority undergraduate students are hired to assist with research objectives in the field and laboratory analysis of soil samples.? How have the results been disseminated to communities of interest?Two Field days and visits by different community clubs such as Master Gardeners, etc. What do you plan to do during the next reporting period to accomplish the goals?The field experiments will be repeated and better managed to prevent deer and wildlife damage. New organic pesticides will be used to prevent outbreaks of insects. Impacts What was accomplished under these goals? Objective 1:In mid-September 2022, the field experiment started at the research plot located at Lincoln University's Busby farm which is a certified organic farm. The plot was divided into two areas; the first area is a long-term cover-cropped area by cereal rye and hairy vetch since 2018, and the second area is the first year for cover crop cultivation. Each area contained six replications of a different cover crop termination method, including the no cover crop bare tilled, cover cropped bare tilled, rotary mowed, and flail mowed. A total of 480 zucchini (Dunja variety), 240 per area were transplanted after cover crop termination during the last week of May 2023. In addition to zucchini, tomato plants were also planted as test crop for investigation of soil disease suppressive capacity. Each area received two different tomato varieties, 60 Luci 2103, a variety less susceptible to soil-borne pathogens, and 60 Skyway F1, a variety more susceptible to soil-borne pathogens. The tomatoes were transplanted into rows allowing for three replicates per cover crop termination method per area. Although plants were irrigated by drip irrigation method, because of severe drought conditions and extreme heat in the central Missouri area during the summer of 2023, we did not have a good yield for Zucchini and tomato plants. Also, it is important to note that almost all the tomatoes harvested were still immature and not yet ripe. In our opinion, the severe drought and hot weather may be the reason for tomatoes not getting ripe. In addition, we encountered problems with insects and wildlife damaging the plants and eating the produce. In general, the results for yield indicated that the area with a history of cover cropping since 2018 produced a significantly higher yield than the area that had just received one year of cover crop. Soil samples were collected in Spring 2023 before cover crop termination and again in early September 2023 and processed. Soil physical, chemical, and health indicator parameters including microbial diversity and enzymatic activities are being measured under each treatment using appropriate methods.Objective 2:Soil samples were collected, and analysis is in progress at the University of Missouri laboratory.Objective 3:.. The disease incidence (monocyclic diseases) and disease severity for polycyclic diseases were recorded at weekly intervals in the different treatments under field conditions. For the assessment of disease incidence total number of infected plants was counted among the population irrespective of disease severity. The percent disease incidence was calculated using the following formula, Percent Disease incidence = No. of infected plants X 100 / Total no. of plants assessed.The percentage of disease severity was assessed by recording the percentage of infected tissue over the total crop canopy. The percent disease severity was calculated by using the following formula, Percent Disease Severity = Infected tissue X 100 / Total crop tissue/area.?It was observed that two fungal diseases Alternaria leaf blight caused by (*Alternaria solani*) and Septoria leaf spot caused by (*Septoria lycopersici*) were noticed in the trial throughout the cropping season. However, the severity of the disease was low to medium. Damping off caused by (*Pythium* spp.) and Vascular wilt caused by (*Fusarium oxysporum*f.sp.lycopersici) were observed in a few plots with low disease incidence during the early and later stages of the crop, respectively. Sun-scorching symptoms were also noticed in almost all the plots after transplanting the seedlings to the main field. In Demo Area-1, Alternaria blight severity varied from a minimum (3.70%) in Bare Tilled Skyway-F1 plots to a maximum of 6.90%) in Control Bare Tilled Luci-2103 plots. In Demo Area-2, minimum disease severity (3.58%) was recorded from Bare Tilled Skyway-F1 treatment to maximum (4.95%) in Rotary Moved Skyway-F1 treatment. There was no significant difference was observed among the treatments, Demo Areas, and varieties due to low disease pressure throughout the cropping season. Maximum Septoria leaf blight severity was observed on Control Bare Tilled Luci-2103 plots and minimum (3.01%) in Bare Tilled Luci-2103 plots. Similarly, in Demo Area-2, maximum disease severity 5.86%) was observed in Control Bare Tilled Luci-2103 plots and minimum (3.22%) Bare Tilled Luci plots. There was no significant difference was recorded between the treatments due to low disease pressure.Damping off disease incidence was observed only in a few plots. The remaining plots were found free from disease. Among the Demo Areas, Area-2 had the maximum disease incidence (5.93%) compared to Area-1 with 0.74% damping off incidence. Demo Area-1 was found free from wilt incidence, while a few plots in Demo Area-2 exhibited the wilt incidence. Only Bare Tilled Luci-2103 plots in Demo Area-2 exhibited 3.70% wilt incidence. Results of damping-off and wilt incidence among

the Demo Areas boost our hypothesis of the disease suppressiveness effect of long-term cover cropping. Tomato seedlings exhibited sun scorch injury soon after transplanting to the main field due to high sunlight. However, the seedlings were recovered from injury and symptoms disappeared around three weeks after transplanting. Zucchini crop was affected by different fungal and bacterial diseases during the cropping season. Among the fungal diseases Alternaria blight, bud rot, and damping off, and among the bacterial diseases wilt was a major constraint during the cropping season. In Demo Area-1 Alternaria blight severity among the different treatments varied. Among the Demo Areas, Area-2 exhibited maximum disease severity (9.27%) compared to Demo Area-1. Damping off disease severity in Demo Area-1 was maximum (3.15%) in Flail Moved plots and minimum (0.19%) in control bare-tilled plots. Similarly, in Demo Area-2 maximum disease incidence (7.41%) was recorded from Flail Moved plots and minimum (0.37%) in control Bare Tilled plots. Among the Demo Areas, Area-2 had a maximum (7.41%) damping-off incidence compared to Demo Area-1 with 3.15% disease incidence. Bud rot disease incidence was observed only during the early stage of flowering. In Demo Area-1 maximum bud rot incidence (2.28%) was recorded in Rotary Moved plots and minimum (1.45%) in Control Bare Tilled plots. In Demo Area-2, maximum disease incidence (3.1%) was recorded from Flail Moved plots and minimum in Rotary Moved plots. The disease incidence level was comparatively higher in Demo Area 2. Disease incidence was noticed during flowering to the final harvest stage of the crop. Wilt incidence was very high during the later stage of the crop, it may be due to infestation of higher populations of cucumber beetle and squash bug. In Demo Area-1 maximum wilt incidence (23.0%) was recorded from Flail Moved plots and minimum (15.33%) from Control Bare Tilled plots. Similar results were obtained from Demo Area-2 with a maximum (27%) disease incidence in Flail Moved plots and a minimum (12.33%) in Control Bare Tilled plots. Demo area-2 had more wilt incidence than Demo Area-1. Demo Area 1 exhibited less disease incidence (damping off, bud rot, and wilt) and severity (Alternaria blight) compared to Demo Area 2. These results clearly indicate the positive impact of long-term cover cropping on improving soil health and induction of disease suppressiveness in soil.

Objective 4. (Extension and Education) One graduate student and a post-doctoral fellow were identified and hired. Two minority undergraduate students are hired to assist with research objectives in the field and laboratory analysis of soil samples. The outreach activities included field days in June and July 2023 and a visit to the experimental plots by Boys and Girls Club/Master Gardeners in August. Publications

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# Building a Resilient Organic Hemp Industry from Seed to Market: Assessing Research, Extension, and Education Needs

<b>Accession No.</b>	1028982
<b>Project No.</b>	NYC-149530
<b>Agency</b>	NIFA NY.C\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37879
<b>Proposal No.</b>	2022-04032
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2023
<b>Grant Amount</b>	\$50,000
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Moore, V.; Cebert, ER, .; Baltensperger, DA, D.; Ellison, SH, .; Suchoff, DA, H.; Sosnoskie, LY, M.; Grab, HE, .

## NON-TECHNICAL SUMMARY

Hemp is a versatile and productive crop; it is widely adapted, and has many potential uses including textiles, construction, food, animal feed, health, and personal care. The 2018 Farm Bill legalized hemp production across the US, paving the way for rapid growth in cultivation and industry. While there is tremendous excitement about hemp, there is also uncertainty and risk, with major gaps in research, extension, and education due to previous legal barriers, especially for organic producers. As the industry grows and develops rapidly in the coming years, there is a critical need to invest resources in high-impact research, extension, and education activities that will guide organic hemp production in a sustainable, profitable, and equitable direction. This project seeks to understand gaps in knowledge and resources limiting organic hemp production for both farmers and hemp industry professionals, assess market potential for organic hemp products, and prioritize research, extension, and education needs to develop an equitable, resilient, productive, and profitable organic hemp industry. Collaborators will accomplish these objectives by engaging with farmers, industry stakeholders, and educators through a survey and focus groups. Through these activities, the project will directly address legislatively-defined OREI goals 1, 2, 4, 5, and 8 by: improving organic hemp production, breeding, and processing methods (Goal 1), evaluating potential economic benefits of organic hemp production (Goal 2), determining desirable traits for organic hemp products (Goal 4), identifying market and policy constraints on organic hemp production (Goal 5), and guiding development of improved hemp varieties for organic agriculture (Goal 8).

## OBJECTIVES

The goal of this project is to identify research, extension, and education priorities that will support the development of the organic hemp industry to be resilient, sustainable, profitable, and equitable. Project Objectives: Understand the critical gaps in farmer knowledge and resources limiting the equitable participation in and resilience, productivity, and profitability of organic hemp production. Assess the market potential for organic hemp products and identify the major barriers limiting access to markets, and how these factors vary across farmer identity. Identify knowledge gaps of hemp professionals regarding organic production systems. Prioritize identified research, extension, and education needs and develop further collaboration and development of grant proposal(s) to address these needs.

## APPROACH

**Objective 1: Farmer Survey & Focus Groups.** In January-April 2023, a survey will be distributed to both organic hemp producers and organic grain producers who do not currently produce hemp. Topics will include farmer demographics, current acreage, barriers to adoption, variety selection and seed sourcing, management challenges, end-use products, markets, future plans, perceived training needs, and other topics identified by key stakeholders. Survey questions will be determined by the project team in consultation with key stakeholders. The survey instrument will be designed and distributed using the Tailored Design Method (Dillman et al., 2009). The survey will be piloted with a group of farmers before distribution, the finalized questionnaire will be distributed via both paper mail and email, and each participant will receive multiple contacts including the survey, a reminder, and a second copy of the survey. Participants will be identified through the USDA Organic Integrity Database as well as through farmer organizations, listservs, and conferences. Focus groups will also be conducted with farmers at regional organic farming conferences, pending public health and safety limitations. Questions will focus on key topics identified through a preliminary analysis of the survey data. Focus groups will be planned and facilitated according to the best practices identified by Nyumba et al. (2018). Each focus group will include up to twelve participants and will last one to two hours. Focus groups will be led by a facilitator and an assistant; the facilitator will introduce the goals of the study, obtain consent from participants, ask open-ended questions, and draw out participants as needed. The assistant will take notes on non-verbal interactions, group dynamics, and general discussion content. Focus groups will be recorded for future transcription and analysis.

**Objective 2: Industry Focus Groups.** In January-April 2023, one or more focus groups will be conducted with representatives of companies currently buying organic hemp fiber, grain, or cannabinoids, and companies with potential to become buyers in the future. Participants will be recruited by reaching out directly to companies known to be purchasing organic hemp, or who are perceived as potential future buyers. These individuals will be identified through the networks of project collaborators, stakeholders (see letters of support), and by direct outreach to companies. Focus groups will take place either virtually or in-person in conjunction with a relevant industry conference, pending public health and safety limitations. Topics will include their current use of organic hemp, challenges with quality and sourcing, future plans, perceived training needs, and perceptions of the organic hemp market broadly. Focus groups will be conducted according to the methods described in Objective 1.

**Objective 3: Education Focus Groups.** In January-April 2023, one or more focus groups will be conducted with educators teaching courses related to hemp production and processing. Participants will be identified through researcher networks and publicly available course listings. Participants will represent courses taught in diverse modalities (online, in-person, degree- and certificate-granting) offered by a range of institutions including minority-serving institutions, public land grant universities, tribal colleges and universities, and community colleges. Topics will include current inclusion of organic principles and practices in their curriculum and the types of knowledge they feel would be most helpful to future professionals participating in hemp-related degree programs and informal education (e.g., workshops, educational materials, development of online certificate programs), and what, if any, barriers prevent the adoption of this content. Focus groups will take place virtually and will be conducted according to the methods described in Objective 1.

**Objective 4: Researcher Meetings.** Researchers will meet virtually for a project initiation meeting in October 2022. Thereafter, the group will meet bimonthly to coordinate project activities and provide feedback on stakeholder engagement tools, analysis methods, and other project activities. A final, in-person meeting will take place in September 2023. Results of the stakeholder needs assessment will be synthesized and presented at the meeting, and a series of intensive brainstorming and planning sessions will take place with a target outcome of developing ideas and concrete plans for one or more grant proposals by the end of the meeting.

Progress 09/01/22 to 08/31/24  
Outputs  
Target Audience: Target audiences reached during the duration of the project include organic farmers, professionals involved in the hemp industry, and researchers, educators, and extension professionals working on hemp- or organic agriculture-related topics.

Changes/Problems: PD Moore had an unplanned medical leave (due to a serious car accident), which slowed progress on the project for several months. This in particular led to delayed progress on the white paper and a follow-on grant submission. What opportunities for training and professional development has the project provided? Communications Assistant Emily Fratz was trained in development of survey and focus group instruments, focus group facilitation, and survey and qualitative data analysis. During the project, she took courses on qualitative data analysis and adoption of innovations. Fratz was accepted to Cornell University's PhD program in plant breeding and started the program in June 2024. How have the results been disseminated to communities of interest? Results were presented at an academic conference and have been accepted for publication in a scientific journal. A public-facing white paper is in process and expected to be published in 2025. We also plan to submit a grant to follow up on the identified research, education, and extension priorities, which will lead to additional dissemination avenues. What do you plan to do during the next reporting period to accomplish the goals? This is the final reporting period. However, we expect the accepted paper to be published by the end of 2024 and a white paper and grant submission to be finalized in 2025.

Impacts  
What was accomplished under these goals? The project team met in September 2023 to discuss the contents of an internal project report and plan future activities, including additional analysis and writing of a peer-reviewed publication, white paper, and follow-on grant

proposal. Survey and focus group data analysis was finalized in Winter/Spring 2024. A peer-reviewed manuscript was drafted and revised in Spring/Summer 2024, and submitted for publication in June 2024. Results from the project were presented at a scientific meeting in November 2024. The manuscript was also accepted for publication in November 2024, but as of the date of this submission the paper has not yet been published by the journal. The project team continues to work on a white paper and follow-on grant submission. The survey received a total of 140 responses and 39 participants engaged in focus groups. Respondents and participants included current and prospective hemp growers as well as other professionals engaged in relevant industries. Among current hemp growers, survey respondents were mostly growing hemp for cannabinoid products while focus group participants skewed towards grain and fiber production. The most significant challenges were related to marketing, sales, and regulations. Based on the results of the survey and focus groups, we identified the following research, extension, and education priorities to support organic hemp production in the US: Evaluate and improve suitability of organic hemp for new and emerging end-uses; Develop enterprise budgets for organic hemp operations; Evaluate and develop extension and education materials on best management practices related to organic hemp production, including: organic seed production, organic weed/pest/disease management options, the roles of tillage, cover crops, and crop rotation in organic hemp production, and post-harvest handling; Breed hemp cultivars that are (a) compliant with current THC limits, (b) uniform and stable especially in terms of sex ratio, cannabinoid production, and flowering time, (c) regionally adapted, (d) resistant to key diseases and insect pests, and (e) competitive with weeds (d and e being particularly important specifically for organic production systems); Develop information hubs with clear and evidence-based information about hemp-related regulations, markets and supply chains, and best management practices; Support farmer-to-farmer learning opportunities and farmer-led organizing efforts; and Develop shared curriculum resources and peer learning communities for hemp educators, including an increased emphasis on sustainability and organic agriculture topics in hemp curricula. Publications Type: Other Journal Articles Status: Accepted Year Published: 2024 Citation: Moore, V., E. Fratz, D. Baltensperger, S. Ellison, H. Grab, L. Sosnoskie, D. Suchoff, and D. Vergara. 2024. Building a resilient organic hemp industry: survey and focus groups assess research, extension, and education needs. *Agrosystems, Geosciences & Environment*. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Moore, V., E. Fratz, D. Baltensperger, S. Ellison, H. Grab, L. Sosnoskie, D. Suchoff, and D. Vergara. 2024. Building a Resilient Organic Hemp Industry: Survey and Focus Groups Assess Research, Extension, and Education Needs. ASA, CSSA, SSSA International Annual Meeting (San Antonio, TX). Progress 09/01/23 to 08/31/24 Outputs Target Audience: Target audiences reached during this reporting period include organic farmers, professionals involved in the hemp industry, and researchers, educators, and extension professionals working on hemp- or organic agriculture-related topics. Changes/Problems: PD Moore had an unplanned medical leave (due to a serious car accident), which slowed progress on the project for several months. This in particular led to delayed progress on the white paper and a follow-on grant submission. What opportunities for training and professional development has the project provided? Communications Assistant Emily Fratz gained experience in survey and qualitative data analysis, and has since applied these skills to another survey and focus group project on another topic related to organic agriculture. Fratz was accepted to Cornell University's PhD program in plant breeding and started the program in June 2024. How have the results been disseminated to communities of interest? Results were presented at an academic conference and have been accepted for publication in a scientific journal. A public-facing white paper is in process and expected to be published in 2025. What do you plan to do during the next reporting period to accomplish the goals? We expect the accepted paper to be published by the end of 2024 and a white paper and grant submission to be finalized in 2025. Impacts What was accomplished under these goals? The project team met in September 2023 to discuss the contents of an internal project report and plan future activities, including additional analysis and writing of a peer-reviewed publication, white paper, and follow-on grant proposal. Survey and focus group data analysis was finalized in Winter/Spring 2024. A peer-reviewed manuscript was drafted and revised in Spring/Summer 2024, and submitted for publication in June 2024. Results from the project were presented at a scientific meeting in November 2024. The manuscript was also accepted for publication in November 2024, but as of the date of this submission the paper has not yet been published by the journal. The project team continues to work on a white paper and follow-on grant submission. Publications Type: Other Journal Articles Status: Awaiting Publication Year Published: 2024 Citation: Moore, V., E. Fratz, D. Baltensperger, S. Ellison, H. Grab, L. Sosnoskie, D. Suchoff, and D. Vergara. 2024. Building a resilient organic hemp industry: survey and focus groups assess research, extension, and education needs. *Agrosystems, Geosciences & Environment*. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Moore, V., E. Fratz, D. Baltensperger, S. Ellison, H. Grab, L. Sosnoskie, D. Suchoff, and D. Vergara. 2024. Building a Resilient Organic Hemp Industry: Survey and Focus Groups Assess Research, Extension, and Education Needs. ASA, CSSA, SSSA International Annual Meeting (San Antonio, TX). Progress 09/01/22 to 08/31/23 Outputs Target Audience: Target audiences reached during this reporting period include: Organic hemp farmers, Organic farmers who previously produced hemp but are not currently doing so, Organic farmers not currently producing hemp but interested in doing so in the future, Processors, buyers, and other

stakeholders engaged with the hemp industry, and Researchers, educators, and extension professionals working on hemp- or organic agriculture-related topics. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? Communications Assistant Emily Fratz was trained in development of survey and focus group instruments, focus group facilitation, and qualitative data analysis. During the reporting period, she took courses on qualitative data analysis and adoption of innovations, and plans to pursue a PhD integrating plant breeding and social science research methods. How have the results been disseminated to communities of interest? Relevant stakeholders participated in the survey and focus groups. Project results will be disseminated during the next reporting period. What do you plan to do during the next reporting period to accomplish the goals? The project team plans to finalize data analysis and write/submit a publication for peer review. They also plan to draft a white paper for public distribution summarizing research, extension, and education priorities. Finally, they plan to develop a grant proposal for submission to the USDA OREI program. Impacts What was accomplished under these goals? Objective 1: Understand the critical gaps in farmer knowledge and resources limiting the equitable participation in and resilience, productivity, and profitability of organic hemp production. A farmer survey was conducted both by paper mail and online, using the USDA AMS Organic Integrity Database as well as organic farmer listservs and organizations. The survey solicited input from organic farmers currently growing hemp and from other organic grain producers not currently growing hemp but who may be interested in doing so in the future. The survey asked questions related to current crop production in terms of scope, scale, and methods, and about the major challenges limited farmers ability to grow and market hemp. In addition to the survey, two focus groups were conducted with organic hemp producers to understand the same topics in greater depth. Objective 2: Assess the market potential for organic hemp products and identify the major barriers limiting access to markets, and how these factors vary across farmer identity. Topics related to market access were included in both the survey and farmer focus groups. In addition, two industry focus groups were conducted to assess the perceived barriers by potential buyers and processors of organic hemp. Objective 3: Identify knowledge gaps of hemp professionals regarding organic production systems. Topics related to organic production systems were included in the industry focus groups and in two additional focus groups conducted with hemp educators. Objective 4: Prioritize identified research, extension, and education needs and develop further collaboration and development of grant proposal(s) to address these needs. Data collected through the survey and six focus groups were analyzed and summarized in a report, which was distributed to all project collaborators. After receiving a no-cost extension, the project team met in September 2023 to discuss the contents of the report and prioritize research, extension, and education needs. These prioritize will guide future grant writing and publication activities during the next reporting period. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Moore, V. and E. Fratz. 2023. Producer Roundtable: Sharing Perspectives on Growing Organic Hemp. Organic Commodities & Livestock Conference (Raleigh, NC).

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# Enhancing Organic Dry Bean Production in the Northeast and Upper Midwestern United States

<b>Accession No.</b>	1028984
<b>Project No.</b>	NYG-621812
<b>Agency</b>	NIFA NY.G\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37881
<b>Proposal No.</b>	2022-04036
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2026
<b>Grant Amount</b>	\$2,999,204
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Pethybridge, S.; Gomez, MI, I.; Darby, HE, MA.; Ryan, MA, R.; Moore, VI, .; Mallory, EL, .; Silva, ER, .; Smith, DA, LE.

## NON-TECHNICAL SUMMARY

This aim of this Integrated Tier 1 Research and Extension project is to enhance the sustainability of the organic dry bean industry in the Northeast and Upper Midwest by developing improved management practices that build soil health and resilience to climate change. Yield and quality of organic dry beans is routinely, deleteriously affected by a lack of information on variety selection, inadequate management of diseases and weeds, and suboptimal agronomic recommendations for no-till production. Due to these production challenges, the exponential increase in consumer demand for organic dry bean have not been realized. The first project objective will identify and develop varieties best suited to organic production through variety trials and breeding. Objective two will develop best practices for organic dry bean production by identifying optimal no-till seeding rates that balance potential tradeoffs in weed and disease management, quantifying the efficacy of OMRI-listed materials for seedborne pathogens and selected foliar diseases, and evaluating an interrow mower for weed management in no-till production. Objective three will explore the interactions between tillage and nitrogen fertility to devise a productive and profitable cover crop-based no-till system that improves soil health and increases resilience to weather fluctuations. Objective four will strengthen the connections and collective skillsets among organic dry bean farmers, processors, and advisors through a multifaceted outreach plan. Our project aligns with FY22 USDA-OREI program priorities: (1), (2), (4), and (7), and addresses two of the research priorities for organic food and agriculture outlined by The National Organic Standards Board.

## OBJECTIVES

Dry beans are a lucrative crop with potential for organic field crop farmers, and yet despite strong regional market opportunities, production is limited by biotic stressors (e.g., diseases and weeds), knowledge gaps that prevent new farmer entry, and lack of optimized management strategies. Dry beans also represent an opportunity to diversify crop rotations, reduce external inputs and regenerate soil health, especially when grown as part of a system with cover crops and reduced tillage. For example, the growth of organic small grains for regional supply chains in the Northeast and Upper Midwestern regions has created a need for high-value legume crops to increase rotational diversity and farm profitability and has fostered new markets for regionally grown staple crops. However, regional knowledge of dry bean production, once plentiful within farming communities, has diminished greatly, and investments in research and extension have been minimal within these regions. The major goal of this project is to increase the sustainability of the organic dry bean industry in the Northeast and Upper

Midwestern regions by overcoming production challenges and developing improved management practices that build soil health and resilience to climate change. To attain this goal, there are four objectives: (1) identify dry bean types and varieties best suited to organic production; (2) develop best practices for the management of diseases and weeds in organic dry bean; (3) devise a productive and profitable cover crop-based no-till system that improves soil health and increases resilience to weather fluctuations for organic dry bean production; and (4) support adoption of management strategies to increase and stabilize organic dry bean profitability and provide decision-making tools to farmers through a broad range of outreach and educational methods.

## APPROACH

**Objective 1: Activity 1.1.** During Years 1-3, dry bean variety evaluation trials will be planted in ME, NY, VT, and WI. The trials will include 15 to 25 commercially available varieties and lines from breeders in high priority market classes including black, pinto, and specialty varieties. In Year 3, we will identify best-performing varieties within each market class and compare to any newly identified material. Trials will be planted in four-row plots in a randomized complete block (RCB) design with four replications. **Activity 1.2.** Results of the Year 1 variety trial will be used to identify specialty varieties that would benefit from improvement (e.g., increased yield, disease resistance) and commercial varieties with desirable agronomic traits. Initial crosses between specialty and commercial material will be made in the greenhouse in winter 2023-2024 (NY). Seed from these crosses will be planted for seed increase in NY in Year 2. In Year 3, seed from the F2 generation will be planted in the field for evaluation and selection. **Activity 1.3.** A side-by-side trial will compare the performance of six selected black bean varieties in rolled-crimped cereal rye mulch and bare soil cultivation-based systems to assess differential performance across these systems (i.e., genotype × management interactions). The trial will take place in Years 2 and 3 in all states. **Objective 2: Activity 2.1.** This trial will be conducted on certified organic land at two locations in NY (Geneva and Ithaca), and single locations in ME, VT, and WI, in Years 1 and 2. The location in Geneva, NY, has a history of white mold, as *S. sclerotiorum* sclerotia have been homogeneously distributed across the field annually since 2016. Cereal rye will be seeded at 128 kg/ha across the entire trial area in fall of Years 1 and 2. The cereal rye will be rolled-crimped at 30% anthesis in each year with a water-filled crop roller. Organic black bean seed (cv. Zorro) will be seeded at five rates (247,000; 370,500 [current organic farmer practice]; 494,000; 617,500; and 741,000 seeds/ha) to an optimum depth of ~4 cm in each spring using a tractor-mounted variable rate planter. The experimental design will be a RCB with five replicates of each treatment. Incidence and severity of white mold and other diseases also will be recorded where they occur at all locations. Pod and bean yield will be evaluated. **Activity 2.2.** To evaluate the efficacy of interrow mowing in organic no-till planted dry bean we will conduct a field trial in all states in Years 1-3. Across the trial area, cereal rye will be seeded each fall and rolled-crimped in spring to coincide with dry bean planting. Organic black bean seed (cv. Zorro or similar class) will be seeded at the current conventional farmer practice (370,500 seeds/ha). The trial design will be a RCB trial with four replications. The experiment will include four treatments: 1) no interrow mowing [negative control]; 2) interrow mowing early (as most weeds reach a susceptible height); 3) interrow mowing late (prior to canopy closure), and 4) interrow mowing early and late [positive control]. **Activity 2.3.** Two black bean rejected seedlots infested with *C. lindemuthianum* and a noninfested seedlot (in-hand) will be used to quantify the effect of seed treatments on the incidence of *C. lindemuthianum* and other fungi, seed germination, and seedling emergence. Trials will be conducted in vitro and in planta in Year 1. A field trial including one infested dry bean seedlot will be conducted in each of Years 2 and 3 at the Gates West certified organic farm facility, Cornell AgriTech, Geneva, NY. Four of the most efficacious treatments identified in Year 1 will be soak-applied to the seed, in addition to a nontreated control. The field trial will be planted in mid-May of each year with a single row Jang JP-1 push seeder at a within row spacing of 5.1 cm, and 76 cm between rows. Irrigation by overhead sprinklers will be provided to optimize crop growth as required. The experimental design will be a RCB with five replications of each treatment. The severity of anthracnose and other foliar diseases will be evaluated at flowering and harvest. **Activity 2.4.** This trial will be conducted on certified organic land at two locations (NY and WI) in Years 1 and 2. Organic black bean seed (cv. Zorro) will be planted in spring using a Monosem planter at a rate of 36 seeds/m with 76 cm between rows. Sodium nitrate (125 kg/ha; North Country Organics) will be spread across the trial area and incorporated before planting. Weed management will be conducted by cultivation. Treatments including microbial biopesticides and plant defense activators (decided with the Stakeholder Advisory Panel) will be arranged in a RCB design with five replications, including a nontreated control. The incidence of white mold on pods and plants and yield in each plot will be evaluated at harvest. **Objective 3: The Tillage × Nitrogen Trial (TNT)** will be conducted on certified organic land in each state in each of two years. Plots will be established so cereal rye is planted (200 kg/ha) at an early seeding date (before 7 September across locations). Tillage will be conducted in one direction, and dry bean planting and weeding will be perpendicular. Black bean (cv. Zorro; Rhizobium-inoculated organic seed) seeding rate will be 556,000 seeds/ha. Planting date will be location-specific and optimized for tilled soil. The TNT will be established in Year 1 and repeated at a different location in Year 2, both following wheat harvest. The experimental design will be a split-plot, RCB with four replications. Tillage treatments (main blocks) are: 1) cereal

rye tilled prior to stem elongation and dry bean planted into bare soil; and 2) cereal rye rolled-crimped and dry bean no-till planted into mulch. N treatments are a) no N applied; b) 29 kg N/ha in fall; c) 57 kg N/ha in fall; d) 29 kg N/ha in spring; e) 57 kg N/ha in spring; and f) 29 kg N/ha in fall and spring. N in fall will be broadcast applied. N in spring will be applied to plots as a starter fertilizer during planting. Aggregate stability will be quantified using the standard wet aggregate stability test. For the root disease bioassay, soil will be placed in four cone tube holders, and one bean seed (var. Hystyle) will be planted and maintained in the glasshouse at 20°C for 4 weeks. Plants will be removed, and root disease severity (1 to 9). Isolations will also be conducted from symptomatic roots to quantify the isolation frequency of fungi associated with root rot. Microbial abundance and diversity. Five plants will be collected from each plot within each of the trials by removing entire plants. Bulk soil will be collected to a depth of 10 cm within 1 m of the selected plants. Samples will be stored at 4°C and processed within 24 h. To collect rhizosphere soil, large soil aggregates will first be removed before sample collection. Roots will be cut into small pieces using a sterile scalpel. Rhizosphere and bulk soil will be sieved to a 2 mm particle size and stored at -20°C. Bacterial community composition will be captured using the V4 region of the 16S rRNA gene. Fungal communities will be captured using the internal transcribed spacer 1 region (ITS1). The 16S rRNA gene and ITS1 region will be sequenced separately on the Illumina MiSeq Platform. At the end of each cropping sequence, we will use a spring barley uniformity trial to evaluate tillage and nitrogen legacy effects. Objective 4: A vital component of this project is to strengthen the connections and collective skillsets among organic dry bean farmers, processors, and advisors throughout these regions. We will work with our Stakeholder Advisory Panel to generate information surrounding production and profitability questions. We will use multiple methods to share outcomes and highlight accomplished dry bean farmers. Progress 09/01/23 to 08/31/24 Outputs Target Audience: The target audience reached during this project period (September 1, 2023 to August 31, 2024) were organic dry bean, field, and specialty crop growers that include dry beans in their rotations and conventional growers considering shifting to organic production. Growers also considering a shift to organic dry beans were also reached as the industry is expanding and there is considerable interest in incorporating dry beans into rotations. Dry bean industry stakeholders (personnel of companies that receive and process organic dry beans) were also engaged in the project. For example, a formal consultation with the stakeholder advisory panel (8 organic growers; extension educators and industry personnel from each collaborating state) was held in February 2024. This group provided advice on the fine-tuning of treatments in the tillage x nitrogen trial, cultivars of desirable traits for inclusion in the breeding/cultivar assessment trials across multiple states, and OMRI-listed products for evaluating efficacy in the white mold trials in WI and NY. Stakeholders (~150) also participated in field days of the field trial demonstrations in the various states during July to September 2024. Results have been incorporated into multiple extension avenues including presentations and project reports that were detailed at extension events and placed on the project (ECOBEAN) website. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? In this reporting period, five graduate students received training in organic crop production and field and laboratory research. The graduate students are located at Cornell University (2), the University of Vermont (1), University of Maine (1), and the University of Wisconsin (1). There are also two postdocs involved in the program at Cornell University (one recently hired to concentrate on plant pathology and soil microbiomes within the TNT trial \Objective 3\). Three undergraduates have also been involved in research in 2024. These early career researchers have been involved in all aspects of the extension and outreach activities. How have the results been disseminated to communities of interest? The Stakeholder Advisory Panel has met with collaborators in each state and then with the PD/co-PD team in 2024. They have provided advice on research but also venues and methods to leverage and maximize the investment for extension and outreach to communities of interest, specifically advising on the webinar times and formats, and the target extension conferences. There have been strong involvement in the multiple field days and especially the main extension activity for 2024 - the four part Beans for Lunch Webinar Series. Results are also regularly posted on the project website: Field Research ECOBEAN: East-Central Organic Dry Bean Collaborative (cornell.edu). What do you plan to do during the next reporting period to accomplish the goals? Objective 1. Identify dry bean types and varieties best suited to organic production. In Year 3, each state, will identify best-performing varieties within each market class and compare to any newly identified material. Trials will be again be established in small plot replicated trials to evaluate the dry bean types and suitability for organic production in each state. Breeding crosses are also planned for the greenhouse in winter 2024-25 (NY) to generate F1 seed. This seed will be increased in 2025 and the F2 will be planted in 2026. Objective 2. Develop best practices for the management of diseases and weeds in organic dry bean production. Findings from Activity 2.1 over the first two years of the project will be combined and analyzed together for a journal article and extensions/outreach programming. A side by side grower demonstration trial of the outcomes will be conducted in Year 4. Inter-row mowing trials will continue in all states (Activity 2.2) in Year 3. Efficacious treatments and equipment refinements will be established for grower demonstration trials in Year 4. Seed treatment research will progress into the glasshouse in Activity 2.3. Activity 2.4 will be completed within the trials conducted in 2024. Objective 3. Tillage x Nitrogen Trial (TNT). Replications of this trial have been planted in 2024 for the main cropping season, which will involve dry bean planting into the treatments in 2025. Samples will again be taken from selected plots for root rot

severity and microbiome analyses in fall 2025 from selected plots. Objective 4. Extension and Outreach. We will continue to work with our stakeholder advisory meeting (annual February meeting scheduled) to achieve our integration of farmer-driven demonstrations and networks. An in-person project investigator meeting is scheduled for 7 February 2025 in Ithaca, NY. A Stakeholder Advisory Panel by zoom has also been planned in late March 2025. The feature of extension/outreach activities for 2025 will be the factsheets from our research, and the development of the dry bean short course. The project team website will also be regularly updated with our findings. The Beans for Lunch Webinar Series continues to be available on Youtube and will continue to be promoted as a grower resource over 2025. Impact evaluations will also continue embedded within each extension and outreach activity.

Impacts What was accomplished under these goals? Objective 1. Identify dry bean types and varieties best suited to organic production. Dry bean variety trials were again conducted in VT, NY, ME, and WI (second year of these trials). These trials were harvested in October/November 2024. In each of the trials, up to 25 dry bean varieties from the market classes of black, light red kidney, navy, pinto, small red, specialty and yellow eye were evaluated. Varieties for evaluation and inclusion in the trial were based on results from Year 1 (2023) and recommendations of our stakeholder advisory panel. Results from Years 1 and 2 will be combined for publications in each collaborating state/region. The trial in NY was also featured in a dry bean extension event for growers in late summer. This information has also been levered into the Dry Bean 'Pan-Genome Selection' Consortium including researchers throughout the mid-western United States for the development of a genomic prediction model for dry bean for use by organic and conventional dry bean breeders.

Objective 2. Develop best practices for the management of diseases and weeds in organic dry bean production. Activity 2.1. Dry bean seeding rate. This trial has now been conducted in VT and NY in each of two years (2023 and 2024). The 2024 trials were harvested in October and data is being analyzed and combined with that from Year 1. Preliminary data analysis has suggested that black bean emergence is deleteriously reduced in no-till plots compared to tilled soil. The trade-offs between seeding rate, tillage and optimal yield, weed suppression, and foliar disease management is being explored. We anticipate this will lead to a refereed journal article including data from both VT and NY in 2025. Activity 2.2. Inter-row mowing. In Year 2 (2024), inter-row mowing trials were conducted in ME, VT, NY, and WI. Treatments in the small plot, replicated trials were inter-row mowing early, late, and as-needed were compared to a control where no inter-row mowing occurred to understand how the timing of the weed management impacted black bean yields and weed biomass. Discussions within the collaborator team meetings have discussed how best to optimize this new equipment to set up on the tractor and decrease the potential for substantial canopy damage following canopy closure. This research has also identified some organic dry bean varieties which are not suitable for inter-row mowing due to significant vining and early canopy closure making mowing of weeds and not the dry beans challenging. In each state, demonstration style plots on research farms are planned to facilitate farmer discussions at field days. Activity 2.3. OMRI-listed seed treatments for *C. lindemuthianum* control. Repetition of experiments evaluating OMRI-listed seed treatments have again demonstrated significant reductions in seedborne fungal populations from a copper soak. Additional experiments have optimized the rate and exposure timing for the copper soak in *C. lindemuthianum*-infested dry bean seed of one variety without substantial phytotoxicity (seedling emergence). The effect of these optimized treatments on shelf-life of the seed (i.e., emergence and seedling vigor) are being evaluated. Discussions with an interested industry partner have also identified two additional microbial formulations for evaluation. Additional OMRI-listed treatments will provide rotational benefits to growers for seed treatments. The most efficacious of these treatments will be included in field trials planned for 2025 and 2026 to evaluate disease control, seedling emergence, and plant vigor. Activity 2.4. Evaluate OMRI-listed treatments for white mold control in organic dry beans. Small plot, replicated trials evaluating the efficacy of ~15 OMRI-listed products for white mold control have now been conducted in WI and NY in 2023 and 2024. In NY in 2024, the trial quantified the efficacy of selected Rovensa Next products (OR-079B, OR-329H, and OR-009 EPA) in comparison to the OMRI-listed (Double Nickel LC, Badge X2, Howler and Theia, all included in 2023). All products significantly reduced the incidence of white mold in plants and pods compared to the nontreated control plots. OR-079B applied to the soil increased green leaf area (as measured by the Normalized Difference Vegetative Index; NDVI) by 8.9% and decreased the incidence of white mold in pods compared to nontreated plots. Application of OR-079B (soil) followed by OR-329H at flowering, NDVI was increased by an additional 4.3% but there were no additional reductions in white mold incidence. The combined treatment of OR-079B + OR-329H (soil) followed by OR-329H + OR-009 EPA at flowering resulted in a 12.2% increase in NDVI compared to nontreated plots but was significantly less than the soil application only. The incidence of white mold on plants was not significantly different from nontreated plots and there was no additional benefit in disease control from the flowering treatment compared to the soil only products. Fungicide treatment had no significant effect on pod number and weight, and average pod weight. The entire experiment in 2024 was repeated in WI but again had low disease pressure and therefore did not identify any significant white mold control from these products.

Objective 3. Tillage x Nitrogen Trial (TNT!). This trial was initiated in all states in 2023 (fall treatments) and this season (2024) was the first cropping season. Harvest data was collected in all trials and the effect of treatment is being analyzed. Soil and root samples were also collected from the same selected treatments within trials located in NY and ME. The

severity of root rot was evaluated and fungal isolations were made from all samples at Cornell AgriTech. DNA was extracted from the fungal isolates and multilocus sequencing was conducted for species identification. The isolation frequency of species differed between locations. For example, *Macrophomina phaseolica* was present at high abundance at the NY location but not in ME. This is a new disease report for dry bean in NY. *Fusarium* spp. (*F. oxysporum* and *F. solani*) were present at high frequency in both locations. The effect of treatment on fungal root rot severity and isolations is being analyzed. Bulk soil and rhizosphere samples were also used for DNA extraction for analysis of the microbiome through 16S and ITS sequencing to quantify the diversity and abundance of bacterial and fungal species, respectively. 16S sequencing has been completed, and PCR reactions for the ITS sequencing are being optimized. These trial locations will be planted in spring 2025 with barley to evaluate legacy effects of the treatments. This trial has also been established for the second time at a different location in each state in fall 2024, with a replication of the cropping season in 2025.

**Objective 4. Extension and Outreach.** Results were presented to members of the stakeholder advisory panel (twice throughout this reporting period) and to the broader organic farming community. We estimate a reach of over 750 growers within our combined multistate activities. The four part webinar series was very successful and reached ~400 growers alone. The format of having the webinar over the lunch period was also highly regarded so growers did not need to take time out of their day. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Loria, K., Brockmueller, B., Darby, H., Diggins, K., Everest, E., Gomez, M., Krezinski, I., Mallory, E., Molloy, T., Moore, V., Murphy, S., Pelzer, C., Pethybridge, S. J., Ryan, M., Sharifi, A., Smith, D., and Youngerman, E. 2023. Expanding productivity and resilience of organic dry bean systems in the Northeast and upper Midwest. Bean Improvement Cooperative Conference, Greenville, SC. (Poster Presentation). 6-8 November 2023. Progress 09/01/22 to 08/31/23 Outputs Target Audience: The target audience reached during this project period were organic dry bean and field crop growers that include dry beans in their rotations and conventional growers considering shifting to organic production. Dry bean industry stakeholders (personnel of companies that receive and process organic dry beans) were also reached and engaged in multiple ways including as members of the stakeholder advisory panel providing advice on experimental design and treatments, land and crops for on-farm trials, and giving feedback on the results and findings of the project. The stakeholder advisory panel, consisting of 8 growers (representing each state involved in the project), extension educators and industry personnel, met twice during this period (February 2023 and February 2024). Stakeholders also visited the trials personally during July 2023. Results have been distributed through multiple avenues including factsheets, extension commodity presentations, and research articles.

**Changes/Problems:** Nothing Reported What opportunities for training and professional development has the project provided? In this reporting period, five graduate students received training in organic crop production and field and laboratory research. The graduate students are located at Cornell University (2), the University of Vermont (1), University of Maine (1), and the University of Wisconsin (1). There are also two postdocs involved in the program at Cornell University. Two undergraduates have also been involved in research in 2023. These early career researchers have also involved in all aspects of our extension and outreach activities. How have the results been disseminated to communities of interest? Results have been disseminated to the stakeholder advisory panel (organic table beet growers and industry stakeholders) on the two occasions they have met during this last project period, and a broader grower and industry stakeholder audience across the northeast and mid-western United States. As listed in the 'Other Products' section of this report, the activities of this project have been included in multiple field days in each state, and a four-part dry bean webinar series hosted by the University of Vermont. Results are also being regularly posted on the project website: Field Research ECOBEAN: East-Central Organic Dry Bean Collaborative (cornell.edu).

**What do you plan to do during the next reporting period to accomplish the goals?** Objective 1. Identify dry bean types and varieties best suited to organic production. In Year 3, in each state, will identify best-performing varieties within each market class and compare to any newly identified material. Trials will be planted in four-row plots in a randomized complete block (RCB) design with four replications. In addition, activity within 1.2 and 1.3 will be initiated. In activity 1.2, specialty varieties will be identified from the Year 1 and 2 results, that will benefit from improvement by crossing. This crossing is targeted for the greenhouse in winter 2024-25 (NY). This seed will be increased in Year 3 and the F2 will be planted in Year 4. In activity 1.3, in Year 4, side-by-side trial will compare the performance of six selected black bean varieties in rolled-crimped cereal rye mulch and bare soil cultivation-based systems to assess differential performance across these systems (i.e., genotype × management interactions).

**Objective 2. Develop best practices for the management of diseases and weeds in organic dry bean production.** Findings from activity 2.1, will be incorporated into the side-by-side trial in activity 1.3. Inter-row mowing trials will continue in all states (activity 2.2). Seed treatment research will progress into the glasshouse in activity 2.3. Activity 2.4 will be completed within the trials conducted in 2024.

**Objective 3. Tillage x Nitrogen Trial (TNT!).** This trial will be replicated in 2025 (established in fall 2024) and therefore harvested in fall 2025. The trial will be again conducted in all states.

**Objective 4. Extension and Outreach.** We will continue to work with our stakeholder advisory meeting (annual February meeting scheduled) to achieve our integration of farmer-driven demonstrations and networks. An in-person project investigator meeting is also planned for this winter in NY. Field days are also planned for

August and September 2024 and our research will be integrated into our state-based extension programming over winter (one per state in summer and winter). Following up from the webinar series, factsheets will be developed as planned on four different dry bean topics including the results of our research. These will also support the dry bean short course curriculum planned for Year 4 over a 5-week period. The project team website will also be regularly updated with our findings. The Youtube series has resulted from the webinar series and is now completed. Impact evaluations will also continue embedded within each extension and outreach activity.

Impacts What was accomplished under these goals? Objective 1. Identify dry bean types and varieties best suited to organic production. Small plot replicated dry bean variety trials were conducted in VT, NY, ME, and WI. A summary of variety research conducted in VT is available online: Table 6 (uvm.edu). In individual trials, up to 27 dry bean varieties from the market classes of black, light red kidney, navy, pinto, small red, specialty and yellow eye were evaluated. Apart from providing local suitability information that was highlighted in extension and outreach events, and will form the basis for publication after combining with the 2024 trial results, this information will also be levered through the dry bean 'pan-Genome Selection' consortium. This consortium aims to use phenotype and genotype data across many dry bean breeding programs to create a giant genomic prediction model for the species. This information will also lever the genomic resources of the species to make the organic dry bean breeding program (Activities 1.2 and 1.3) more intuitive and powerful. This model will be used in the short-term to inform decisions about what to cross and potentially which plants to select among early-generation breeding lines. Objective 2. Develop best practices for the management of diseases and weeds in organic dry bean production.

2.1. Dry bean seeding rate. Planting black beans at increasingly higher seeding rates increased emergence populations, but the effect varied in tilled versus no-till plots. Black bean emergence was below the target population for all seeding rates in both tillage treatments, but no-till planting black beans into rolled down rye resulted in even lower emergence. At the highest seeding rate (300,000 seeds ac<sup>-1</sup>), emergence in the no-till plots was only 144,680 plants ac<sup>-1</sup>, nearly half the target population, compared to 237,660 plants ac<sup>-1</sup> in the tilled plots. This research is being repeated in VT and NY in 2024.

2.2. Inter-row mowing. In 2023, the first year of a research trial to determine if inter-row mowing can provide adequate weed management while minimizing damage to the organic no-till dry bean crop was conducted in ME, VT, NY, and WI. Inter-row mowing early, late, and as-needed were compared to a control where no inter-row mowing occurred to understand how the timing of the weed management impacted black bean yields and weed biomass. Results from this year's trials suggested that the inter-row mower can be a valuable tool for weed management in an organic no-till system, but the timing of inter-row mowing is very important. There is a risk of inter-row mowing too late in the season once plants have approached canopy closure or if the plants have significant vining. These trials are being repeated in 2024.

2.3. OMRI-listed seed treatments for *C. lindemuthianum* control. To date, in vitro testing has identified an OMRI-listed copper based soak with promise to control seedborne infection by *C. lindemuthianum* on organic dry bean seed. In vitro experiments are continuing to optimize the concentration of copper, differences between formulations, duration of treatment, and to evaluate any effects on seed shelf-life.

2.4. Evaluate OMRI-listed treatments for white mold control in organic dry beans. Small plot, replicated trials have been conducted in WI and NY in 2023, and are replicated in 2024. In NY, all products (Theia, Howler, BF009-03, Kocide 3000-O, and Double Nickel) were all highly and equally efficacious for white mold control in the organic blackbean var. Zorro. The location at WI had low disease pressure and therefore did not identify any significant white mold control from these products. The same treatments are also replicated in each state again in 2024 and will be harvested in September.

Objective 3. Tillage x Nitrogen Trial (TNT!). This trial was initiated in all states in 2023 (fall treatments) and is in the first cropping season in 2024. Data collection is multidisciplinary ranging from soil health attributes, root disease testing, nutrient analyses, and yield. This trial will also be established for the second time at a different location in fall 2024, with a replication of the cropping season in 2025.

Objective 4. Extension and Outreach. Results were presented to members of the stakeholder advisory panel (twice throughout this reporting period) and to the broader organic farming community. We estimate a reach of over 1,000 growers and industry stakeholders just within the last project period across our four states (see Other Products). The four part webinar series has been completed.

Publications Type: Journal Articles Status: Published Year Published: 2023 Citation: Diggins, K. R., Murphy, S., and Pethybridge, S. J. 2023. Efficacy of fungicides for white mold control of black bean in New York, 2023. *Plant Dis. Manage. Rep.* 18:V020. Type: Journal Articles Status: Published Year Published: 2023 Citation: Diggins, K. R., Murphy, S., and Pethybridge, S. J. 2023. Efficacy of OMRI-listed fungicides for white mold control of black bean in New York, 2023. *Plant Dis. Manage. Rep.* 18:V021. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Pethybridge, S. J., and Ryan, M. R. 2023. Breaking down the barriers to organic no-till soybean and dry bean production through improved white mold management. USDA NIFA Organic Programs Project Directors Meeting (Poster and Oral Presentation). Pp. 73-75. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Loria, K., Brockmueller, B., Darby, H., Diggins, K., Everest, E., Gomez, M., Krezinski, I., Mallory, E., Molloy, T., Moore, V., Murphy, S., Pelzer, C., Pethybridge, S. J., Ryan, M., Sharifi, A., Smith, D., and Youngerman, E. 2023. Expanding productivity and resilience of organic dry bean systems in the Northeast and

upper Midwest. Bean Improvement Cooperative Conference, Greenville, SC. (Poster Presentation). 6-8 November 2023.

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# Building Resilient Organic Weed Management Systems with Precision Smart Sprayer Technologies

<b>Accession No.</b>	1029058
<b>Project No.</b>	PENW-2022-04051
<b>Agency</b>	NIFA PENW\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-38008
<b>Proposal No.</b>	2022-04051
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2026
<b>Grant Amount</b>	\$2,997,383
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Mirsky, S. B.; Schomberg, HA, .; Leon, RA, G.; Reberg-Horton, S., .; Skovsen, SO, .; Mortensen, AN, .; Dyrmann, MA, .; Bagavathiannan, MU, .; Melander, BO, .
<b>Performing Institution</b>	Agricultural Research Service, 600 E. Mermaid Lane Rm 2023, Glenside, PENNSYLVANIA 19038-8551

## NON-TECHNICAL SUMMARY

Weed control has always been one of the top management constraints for organic grain producers, largely due to variable weather impacting cultivation. These challenges are exacerbated by climate change. We will adapt smart, precision micro-volume sprayers for organic herbicides deployed by tractors and autonomous robots. Our long-term goal is to develop resilient weed management solutions that organic row-crop producers use to meet growing US demand. Our objectives are to: 1) expand and adapt existing machine vision and learning systems for dominant weeds in organic corn, soybean, and cotton in South Central, Southeast, and mid-Atlantic US; 2) develop a modular, scalable high concentration, micro-volume smart organic herbicide spray system that targets intra-row weeds; 3) field test the smart sprayer system on tractor and robotic platforms; 4) have a camera-vision based reassessment system for continuously retraining and improving performance; and 5) facilitate knowledge exchange about alternative weed control tactics using robotics among farmers, researchers, and educators. We will address these objectives through an integrated multi-institutional, multi-state interdisciplinary research and outreach project. Our project addresses the FY 2022 OREI priorities of 1) Conducting advanced on-farm crop, livestock, or integrated livestock-crop research and development that emphasizes observation of, experimentation with, and innovation for organic farms, including production, marketing, and socioeconomic considerations, 2) Developing and demonstrating educational tools for Cooperative Extension personnel and other professionals who advise producers on organic practices, and 5) Exploring technologies that meet the requirements of the National Organic Program (NOP) and protect soil, water, and other natural resources.

## OBJECTIVES

Project Objectives.1. Expand and adapt existing machine vision and learning systems for dominant weeds in organic corn, soybean, and cotton in South Central, Southeast, and mid-Atlantic US.2. Develop a modular, scalable micro-volume smart organic herbicide spray system that targets intra-row (0-15 cm from row) weeds (cotyledon size and larger).a. Build the smart micro-jet pulse array prototype system.b. Evaluate and optimize organic herbicide concentrations and combinations, in greenhouse, for use with smart applicators.3. Field test and iteratively improve the smart sprayer system on multiple robotic and tractor mounted platforms.a. Test and

improve smart micro-volume sprayer interoperability and integration for tractor and autonomous robot platforms. b. Evaluate field efficacy of smart micro-volume sprayer in replicated field trials. c. Evaluate economics of the organic herbicides and the smart micro-volume sprayer compared to standard operations. 4. Have a vision based "look back" or reassessment system(s) within 1.5 years to enable a camera to assess accuracy of the targeting system and provide system optimization data to continuously retrain the system. 5. Facilitate knowledge exchange about alternative weed control tactics using robotics among farmers, researchers, and educators.

## APPROACH

Our machine vision sub module will provide real-time intra-row detection of individual cotyledon-size weeds at travel speeds up to 2 m/s. High-speed cameras and artificial lighting will be used to ensure short exposure times required for fast travel speeds and detection accuracies adequate for the in-crop detection and classification of small monocots and dicot weeds. The high-speed image processing camera located near the weed control spray unit will allow for millimeter-level positioning accuracy, sufficient for the smart spray weed-targeting sub module to successfully treat the detected weed. At each site, we will establish trials using a split-plot design with four replicates in certified organic fields with a long history of good weed control; all US sites have significant organic acreage for field crop research (>5 hectares). Weed seeds will be sown at typical seedbank population densities in the fall of the preceding year at each location to permit vernalization of seeds to break primary dormancy. Each trial will include +/- irrigation as the main treatment and three fertilization levels as the split-plot treatment. Varying water and nutrient availability will increase the number of weed species and the amount of variation in individual weed phenotypes present in the field, thereby increasing the quality of the dataset used to train detection algorithms. Weed images will be collected daily with the computer vision sub module to be developed. High concentrations of non-synthetic herbicides will be targeted to the growing point on individual weeds using pressurized short pulses from micro-jet nozzles. An array of micro-jet nozzles will be mounted on a boom, with an electric solenoid valve controlling each nozzle in the application system. The nozzle/solenoid combination has the proven ability to deliver a single droplet of herbicide to a target. The efficacy of a selection of NOP approved non-synthetic herbicides and potential combinations available on the market will be compared under micro-volume applications in greenhouse experiments. Based on our previous research, commercial formulations of acetic acid, clove oil, and caprylic plus capric acid, will be tested in these greenhouse trials for controlling common broadleaf and grass weed species with micro-volume applications. Effective concentrations with micro-volume application will be tested for various weed species and sizes. Adjuvants to be tested will include organic foams and gels to insure adhesion of herbicides to the target. In addition to control efficacy, the herbicide research will also include evaluation of fluid dynamics, viscosity, and other spray solution factors that might influence the micro-jet spray pulses. Multiple wide ranging platform designs are being proposed and commercially introduced as autonomous ground-based vehicles for future crop production systems. To ensure that the smart sprayer system developed here can be used across these platforms, including existing tractors of any make and model, the weed recognition and control systems will be combined in a self-contained, scalable modular unit that can be fitted to multiple platform types. Standardized structural support attachments and power supply couplings will be incorporated into the design to ensure flexibility in platform use. A prototype module will be field tested on autonomous and manned platforms to confirm functionality as well as weed control efficacy. The micro-jet spray system will be evaluated in replicated field trials on certified organic ground in each of the four US sites and two international partners for assessing their field performance. Within each site, the spray modules integrated with a tractor or autonomous robot will be evaluated in the three major target crops corn, soybean, and cotton. The treatments will include side-by-side comparisons of the smart technology, a standard weed management program specific to each region, and a non-treated control. The study will be arranged in a split-plot design, with the crop species as the main-plot factor and the management approach as the sub-plot factor, with four replications. Each sub-plot will measure 6-m wide and 25-m long. Crop planting and general crop management will be implemented based on recommended best practices for the specific location. Both tractor- and robot-mounted systems will be tested at various field sites to evaluate any differences in efficacy, cost, and operation of the system on different platforms. Any weeds that escape early spray applications will be relatively larger than the 5-cm stage at later application times, but the smart spray technology will automatically adjust to account for weed size. Concurrently, the inter-row weeds will be removed by mechanical cultivation when they reach 7- to 10-cm tall. In the standard program, periodic inter-row cultivation and a hooded-organic herbicide application will be carried out as-needed, reflecting a typical organic row-crop weed management program practiced by growers in each region. Control evaluations will be carried out on five dominant weed species important to each region; if the natural seedbank is low for these species, additional seed will be introduced across the entire study site during the fall season prior to the study year. Weed control efficacy will be rated on a scale of 0 to 100% in the middle four rows at 14 days after each pass. Further, plant height distribution of all uncontrolled escapes in the intra-row area will be measured at each observation timing from five random quadrats placed within the middle 4 rows of each plot. Total weed biomass will be obtained from four random quadrats within the middle four rows of each plot.

separately for the intra-row and inter-row areas during the mid-crop growth stage, and again at crop maturity. Crops will be harvested using a small-plot combine. For cotton, seed cotton yield as well as ginning percentage will be calculated. All input costs and other expenses incurred while implementing the field treatments will be documented for utilization in economic analysis. Economic assessment includes collecting pertinent information on cash crop yields, input applications, weed pressure, and other relevant costs will be collated from the field trial results, and published reports as appropriate. Then we will develop short-run partial budgets by attaching values to the yield and input application data in the first step. We will assess whether adoption of the micro-volume precision organic herbicide approach would result in lower overall costs relative to the 'standard' organic herbicide approach. We will construct partial budgets for different scenarios of soil/weather conditions that impact weed control operations, enabling the assessment of net profitability of the sprayer systems under various weather conditions and profitability in wetter versus dryer spring conditions and no-till versus reduced till environments. At each study location, we will conduct field days and workshops that will facilitate knowledge exchange among stakeholders. Participant surveys for each event will be used to determine the quality of information shared in terms of relevance and delivery. Survey information will be used to adjust subsequent field day activities and workshop presentations. Field days will be designed to prompt in-depth discussions between producers, extension educators, and researchers. In addition to field days and workshops, we will publish journal articles, extension publications, conference presentations and popular press articles. For all train-the-trainer activities, exchanges with industry, and direct communication with farmers, we will focus on the subjects of multi-tactic weed management and the benefits of integrating autonomous robots for mechanical intra-row weed control and low-rate precision organic herbicide application. Progress 09/01/23 to 08/31/24 Outputs Target Audience: The target audience of this project includes farmers, researchers, and private industry. Organic farmers are in critical need of robust precision weed management solutions that limit excessive tillage and increase the competitiveness of the cash crop. These technologies must be affordable and accessible. By building image repositories for organic field crops and making them available to the research community, we will accelerate innovation in the research community around organic weed management. Lastly, by taking proof-of-concept technologies and operationalizing them for organic farmers, we will de-risk innovations that can be expanded on by private industry. In the second reporting period of this project, team members presented at events including state and national soybean boards workshops, the Weed Science Society of America annual meeting, and USDA ARS leadership meetings and departmental seminars. PI Mirsky gave keynote presentations at All of the aforementioned events were attended by varying combinations of farmers, researchers, and private industry personnel. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? There are several graduate students involved in the project as well as post docs. We also present at annual conferences and host hackathons for professional development to graduate students. How have the results been disseminated to communities of interest? Objective 5. Facilitate knowledge exchange about alternative weed control tactics using robotics among organic farmers, researchers, and educators. Team members presented at events including state and national soybean boards workshops, , the Weed Science Society of America annual meeting, and USDA ARS leadership meetings and departmental seminars. PI Mirsky gave keynote presentations at several commodity based farmer focused events, technology conferences, government agency events, and farmer field days . All of the aforementioned events were attended by varying combinations of farmers, researchers, and private industry personnel. Work was also disseminated through journal publications as noted in the Products section of this report. What do you plan to do during the next reporting period to accomplish the goals? Continue research and development of training data, sprayer technology, and professional development activities. Impacts What was accomplished under these goals? We designed and built a computer vision system, including machine vision camera, lens, artificial light, computational platform, gps receiver, 5g modem, and height sensor. We used novel integration of system firmware and software with hardware components to create a working prototype. Iterative optimization of the camera parameters was then carried out that focused on travel speed, availability of light, and ranges of weed sizes. Finally, we installed server infrastructure and set up remote access into the camera system when in operation, as well as automatic data upload. With the system above, we collected a representative dataset of crops (maize, beets) under Danish field conditions. The system was mounted on four different tractors and two full size autonomous robots (Robotti and Farmdroid). The collected dataset consists of approximately 200,000 images of 16 megapixel each. We then annotated 2,500 of these images on a per-plant basis using bounding boxes and per-pixel masks with human-in-the-loop methods. Specifically, an existing YOLO-based detection model was used to populate the images with suggestive plant bounding boxes. After manual refinement of the boxes, a modified SAM (segment anything model) was used to create species-specific instance masks of all the plants in the annotated dataset. Based on the training dataset above, a series of instance segmentation models were developed and trained. These models were trained to differentiate between major species groups within the annotated dataset, specifically monocot weeds, dicot weeds, maize, beets, cereals, beans, lupins, buckwheat, and peas. Utilizing the computational platform within the camera and optimization libraries (Pytorch, nVidia tensorRT), the trained models were optimized to be able to run in real-time in field conditions with larger than 100% overlap at the designated travel

speed of 6 km/hour, thus providing an accurate map of all plants within the traversed area. With the developed camera system we are able to capture approximately 10 images per second. Depending on the driving speed, each plant can be imaged at least three times, thus providing varied perspective views of each plant instance. To implement this functionality, we have developed a tracking module to 1) detect crop rows and interrows to boost appropriate classification accuracy, 2) keep track of each individual plant for accurate mapping and spraying, and 3) make use of multi-perspective views of plants to increase detection and species classification accuracy. With this tracking system, we have shown an increase in the stability of the computer vision algorithm, as well as solved the issues stemming from detection uncertainty from overlapping image regions. At the Beltsville, Maryland location we conducted image collection of corn and common summer annual weed species relevant to the Maryland region for the image repository. At the North Carolina State University location, we extended evaluation of a mobile inkjet sprayer for targeted application of microvolume herbicides. We also conducted image collection of soybean and common summer annual weed species relevant to the North Carolina region for the image repository. At the Texas A&M University site, we developed of a multi-nozzle system, including electronic controller for the sprayer and sprayer output analysis. We also conducted image collection of cotton and common summer annual weed species relevant to the Texas region for the image repository. Services you provided, E.g. consulting, counseling, tutoring N/A Objective 1. Expand and adapt existing machine vision. See the description of activities for Denmark (above) for an overview of machine vision work progress. Supporting efforts continued among the Maryland, North Carolina, and Texas partners to build and expand upon a national image repository. This data collection involved the use of a high-resolution camera system mounted on a robotic platform arm that, on a daily or bi-daily basis, traversed a large outdoor potting table containing corn, cotton, soybean, and common summer annual weed seedlings. By seeding crops and weeds into sterile soil, we know the correct species of each emerging plant from seedling and onwards which ensures accurate image annotation. Objective 2. Develop a low-volume organic herbicide sprayer prototype that targets intra-row (0-15 cm) weeds (cotyledon size and larger). The Denmark team has constructed a field-deployable cell sprayer with 25 nozzles. The working width of the sprayer is 1 meter, corresponding to the field of view of the camera system, orthogonal to the driving direction. It will be field-tested in the 2025 spring season in combination with the computer vision system. The Texas A&M collaborator has developed a multi-nozzle sprayer system. Each nozzle provides approximately one-centimeter spray resolution, and the valve response time is two milliseconds. The system integrates a computer vision system for real-time weed detection, nozzle control circuits, and accompanying hardware, including the nozzle mount, accumulator tank, and power supply. The entire setup is mounted on an Amiga farm-ng® robot to test its functionality. Experiments were conducted to synchronize the vision system, ground robot, and sprayer for precise microdose application of herbicides. The system will be tested in varied agricultural systems in 2025. Objective 3. Field test and iteratively improve the smart sprayer system on multiple robotic and tractor mounted platforms. As noted above, the current computer vision part of the smart sprayer system has been successfully deployed on two full-size robots as well as four different tractors without issues. This was achieved with minimal dependency from the tool carrier, where the only external dependencies are 12V power and an A-mount. To make things simpler, field tests were carried out with a mobile car battery, leading to an installation time of about five to ten minutes on any of the tractors or robots. Objective 4. Create a vision based "look back" or reassessment system(s) within 1.5 years to enable a camera to assess accuracy of the targeting system and provide system optimization data to continuously retrain the system. With the chosen components (dual RTK GPS, lidar-based height sensor) we have demonstrated the ability to georeference each individual plant in the traversed area of the field. Because of GPS positioning capabilities, plants can then later be reidentified across multiple runs. With this in mind, we have not yet installed a second "look back" camera. The system, however, is designed with the option of expanding the number of cameras from one to two. The team has experimented with another approach involving the extension of the field of view of the single camera in the driving direction to overlap with the spray zone. This could allow a single camera to be used for crop and weed detection, and simultaneously use the lowest part of the images to inspect sprayed areas. ? Publications Type: Conference Papers and Presentations Status: Submitted Year Published: 2024 Citation: Jørgensen, R. N., Skovsen, S. K., Green, O., Sørensen, G. 2024. Enhancing Precision Agriculture Through Dual Weed Mapping: Delineating Inter and Intra-row Weed Populations for Optimized Crop Protection. 16th International Conference on Precision Agriculture Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Madsen, M.S.N., Skovsen, S.K., Melander, Bo., Jørgensen, R.N. 2024. Enhancing Precision Weeding with YoloV11 Object Tracking for Robust Early Crop Detection as a Foundation for Future Organic Precision Spraying Trials. 5th NJF - EurAgEng - Agromek Joint Seminar. Type: Conference Papers and Presentations Status: Submitted Year Published: 2024 Citation: Gurjar, B., Kumar, S., Johnson, J., Hardin, R. G., & Bagavathiannan, M. (2023, October). Designing and Testing a Micro-Volume Spray System for Site-Specific Herbicide Application Using Ground Robots. In ASA, CSSA, SSSA International Annual Meeting. ASA-CSSA-SSSA. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Mirky et al. Precision organic weed management; 18 seminars, webinars, government agency presentations, stakeholder groups, and private industry. Progress 09/01/22 to 08/31/23 Outputs Target Audience: The target audience of this

project includes farmers, researchers, and private industry. Organic farmers are in critical need of robust precision weed management solutions that limit excessive tillage and increase the competitiveness of the cash crop. These technologies must be affordable and accessible. By building image repositories for organic field crops and making them available to the research community, we will accelerate innovation in the research community around organic weed management. Lastly, by taking proof-of-concept technologies and operationalizing them for organic farmers, we will de-risk innovations that can be expanded on by private industry. In the first reporting period of this project, team members presented at events including state and national soybean boards workshops, the August 2023 Keck/NASA Jet Propulsion Lab carbon workshop, the Weed Science Society of America annual meeting, the American Chemical Society Spring 2023 conference, a Northeast Climate Hub meeting, a Southeast Climate Hub meeting, and USDA ARS leadership meetings and departmental seminars. PI Mirsky gave keynote presentations at the National Predictive Modeling Tool Initiative (Feb. 2023) and the International Agro-Geoinformatics 2023 conference; he also participated in the Sustainable Precision Agriculture in the Era of IoT and AI BARD/NSF workshop and the Center for AI Image Repo workshop. All of the aforementioned events were attended by varying combinations of farmers, researchers, and private industry personnel. Changes/Problems: Work was delayed for some project objectives due to difficulty putting in place the agreement between USDA and Aarhus University. However, we still expect to meet our objectives by the end of the project. What opportunities for training and professional development has the project provided? Project leadership and staff participated in an intensive five-day webinar series on the beta-testing of technology led by user-testing experts from Game Theory, a Vermont-based software development collective. This webinar series was followed by an in-person training event at North Carolina State University in June, 2023. The project facilitated a visiting Fulbright postdoctoral researcher from Australia. This enabled professional development of the Australian Fulbrighter working with the US and Danish team, as well promoting cross cultural exchange and further international collaboration. How have the results been disseminated to communities of interest? In the first reporting period of this project, team members presented at events including state and national soybean boards workshops, the August 2023 Keck/NASA Jet Propulsion Lab carbon workshop, the Weed Science Society of America annual meeting, the American Chemical Society Spring 2023 conference, a Northeast Climate Hub meeting, a Southeast Climate Hub meeting, and USDA ARS leadership meetings and departmental seminars. PI Mirsky gave keynote presentations at the National Predictive Modeling Tool Initiative (Feb. 2023) and the International Agro-Geoinformatics 2023 conference; he also participated in the Sustainable Precision Agriculture in the Era of IoT and AI BARD/NSF workshop and the Center for AI Image Repo workshop. All of the aforementioned events were attended by varying combinations of farmers, researchers, and private industry personnel. Work was also disseminated through journal publications as noted in the Products section of this report. What do you plan to do during the next reporting period to accomplish the goals? During the next reporting period we plan to expand on the computer vision work to develop a real-time capable computer vision model. The model will initially be developed and tested for corn, and will be able to differentiate between corn and emerging weeds at the early stages of the crop growth (0-15 cm tall). Using the Nvidia Jetson Orin AGX platform, we intend to train a yolov8 model, capable of analyzing a high resolution color image fast enough for spraying directly after detection. We plan to integrate weed detection logging using RTK-gps, which later will support logging of spray patterns as well. We also intend to conduct field testing, which would put us ahead of the official project timeline. This field testing will refine real-time detection of weeds using the developed computer vision system. With regards to sprayer-centric objectives, greenhouse experiments will be carried out to evaluate sprayer efficacy on emerging weeds and fine tune the integration of the spray system, machine vision, and targeting algorithm. This includes 1) choice of organic herbicide, 2) hit rate of the nozzle system, and likely 3) accuracy of the computer vision system for delivering control signals to the nozzles. Impacts What was accomplished under these goals? Objective 1. Expand and adapt existing machine vision Efforts continued among the Maryland, North Carolina, and Texas partners to build and expand upon a national image repository. This data collection involved the use of a high-resolution camera system mounted on a robotic platform arm that, on a daily or bi-daily basis, traversed a large outdoor potting table containing corn, cotton, soybean, and common summer annual weed seedlings. Using this system, an extensive image dataset of the three crops and weeds was created. By seeding crops and weeds into sterile soil, we know the correct species of each emerging plant from seedling and onwards. Denmark has been part of this effort on the image processing side to transform the collected images into a readily usable dataset. The Denmark and NCSU collaborators worked together to develop a computer vision system prototype consisting of a weatherproof machine vision camera, machine vision lens, and LED-based ring flash. The computational platform, power supply, and GPS components must still be ruggedized. The Denmark, Maryland, and North Carolina team members collaborated to collect field images in the three different climates/regions. While each camera system was built upon the same principle, they differed in the expected image quality and field of view. Objective 2. Develop a low-volume organic herbicide sprayer prototype that targets intra-row (0-15cm) weeds (cotyledon size and larger) NCSU explored several off-the-shelf hardware options for precise, low-volume application of liquid products that could be adapted to fulfill project needs. Most work has revolved around adapting an inkjet printhead (from a large character drop on-demand

printing system used for coding and marking systems for texts, dates, or logos) to apply herbicide. The printhead consists of 32 linearly arranged inkjet valves and nozzles, spaced 4.5 mm apart, that can be individually controlled. Experiments have been conducted to 1) evaluate the ability to control the nozzles accurately, in real time, from a computational platform, including integration with a camera and Machine Vision system; 2) characterize the performance of the printhead across a range of pressures, valve open times, ground height, and ground velocity; 3) determine the accuracy at which the printhead can hit various arrangements of targets; and 4) characterize the dispersed droplet morphologies (with high speed imagery) and the effectiveness of different spray configurations on target coverage. This prototype test system was mounted on a Farm-ng Amiga platform for lab and field experiments. The Texas A&M collaborator worked extensively to design a multi-nozzle sprayer from the ground up using off-the-shelf nozzles. This included development of control circuits and the accompanying hardware (nozzle mount, accumulator tank, power supply). Experiments were then carried out to evaluate the usability of the sprayer for microdose application of herbicides. Objective 3. Field test and iteratively improve the smart sprayer system on multiple robotic and tractor mounted platforms As noted above, field tests of the computer vision system were carried out as part of field image collection. This included evaluation of the camera-related components, specifically related to investigating whether the artificial light source provided enough light to 1) reliably collect images at a fast shutter speed to avoid motion blur while driving and 2) brighten hard shadows caused by sunlit conditions sufficiently to detect weeds under said bright conditions. We also evaluated the performance of the camera, lighting, and computing integration hardware when operating under field conditions. Objective 4. Have a vision based "look back" or reassessment system(s) within 1.5 years to enable a camera to assess accuracy of the targeting system and provide system optimization data to continuously retrain the system We haven't yet progressed to this objective, which relies on observing a field-deployed sprayer that will be deployed in upcoming reporting periods. Objective 5. Facilitate knowledge exchange about alternative weed control tactics using robotics among organic farmers, researchers, and educators. In the first reporting period of this project, team members presented at events including state and national soybean boards workshops, the August 2023 Keck/NASA Jet Propulsion Lab carbon workshop, the Weed Science Society of America annual meeting, the American Chemical Society Spring 2023 conference, a Northeast Climate Hub meeting, a Southeast Climate Hub meeting, and USDA ARS leadership meetings and departmental seminars. PI Mirsky gave keynote presentations at the National Predictive Modeling Tool Initiative (Feb. 2023) and the International Agro-Geoinformatics 2023 conference; he also participated in the Sustainable Precision Agriculture in the Era of IoT and AI BARD/NSF workshop and the Center for AI Image Repo workshop. All of the aforementioned events were attended by varying combinations of farmers, researchers, and private industry personnel. Work was also disseminated through journal publications as noted in the Products section of this report. Publications Type: Journal Articles Status: Published Year Published: 2022 Citation: Dobbs, A.M., Ginn, D., Skovsen, S.K., Bagavathiannan, M.V., Mirsky, S.B., Reberg-Horton, C.S. and Leon, R.G. 2022. New directions in weed management and research using 3D imaging. *Weed Science* 70:641-647. Type: Journal Articles Status: Published Year Published: 2022 Citation: Hu, C., Xie, S., Song, D., Thomasson, J.A., Hardin IV, R.G. and Bagavathiannan, M. 2022. Algorithm and system development for robotic micro-volume herbicide spray towards precision weed management. *IEEE Robotics and Automation Letters* 7(4): 11633-11640. Type: Journal Articles Status: Published Year Published: 2022 Citation: Coleman, G.R., Bender, A., Hu, K., Sharpe, S.M., Schumann, A.W., Wang, Z., Bagavathiannan, M.V., Boyd, N.S. and Walsh, M.J. 2022. Weed detection to weed recognition: reviewing 50 years of research to identify constraints and opportunities for large-scale cropping systems. *Weed Technology* 36(6): 741-757. Type: Journal Articles Status: Published Year Published: 2022 Citation: Sapkota B.B., Popescu, S., Rajan, N., Leon, R.G., Reberg-Horton, C., Mirsky, S. and Bagavathiannan, M.V. 2022. Use of synthetic images for training a deep learning model for weed detection and biomass estimation in cotton. *Scientific Reports* 12(1):19580.

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# The Future of Organic Beekeeping: Increasing Opportunities for Beekeepers Through Assessment of Honey Bee Foraging Patterns on Organic Farms

<b>Accession No.</b>	1028980
<b>Project No.</b>	PENW-2022-04054
<b>Agency</b>	NIFA PENW
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37884
<b>Proposal No.</b>	2022-04054
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2026
<b>Grant Amount</b>	\$1,500,000
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Lopez-Uribe, M. M.; Fuentes, JO, .; Underwood, RO, MA.; Couvillon, MA, .; Wilson, JA, M.; Urbina, JU, V.; Walters, EM, .
<b>Performing Institution</b>	PENNSYLVANIA STATE UNIVERSITY, 408 Old Main, UNIVERSITY PARK, PENNSYLVANIA 16802-1505

## NON-TECHNICAL SUMMARY

Honey bees are the most important agricultural pollinators and generate commercial products such as honey and wax that contribute over \$300 million to the US economy annually. Despite their higher market value, organic products are currently not produced in the continental United States because meeting recommendations for certification is not feasible. Specifically, beekeepers cannot meet the pesticide-free zoning requirements (at least a 3 km radius around the colonies) that were established based on maximum foraging distance of honey bees. Our preliminary data and published research indicate that honey bee foraging is context-dependent and can be significantly smaller in colonies of smaller size and surrounded by high-quality landscapes. Here, we propose integrating data from automated tag readers, harmonic radars, and waggle dance decoding to characterize honey bee foraging patterns in colonies of different population sizes and placed in landscapes with varying floral quality. We will develop high-quality floral maps around the apiaries based on satellite imagery, ground observations, and pollen metabarcoding to develop a seasonal landscape quality index that will be used as a predictor of the estimated average foraging distances of the colonies in different landscapes. Our goal is to assess whether the recommendations for pesticide-free zones can be reduced based on empirical data that incorporates information about landscape quality and colony size. The project will count with the input of an advisory panel composed of scientists, beekeepers, farmers and policy makers. The extension teams will disseminate our results to stakeholder groups and the national organic program.

## OBJECTIVES

Objective 1: Assess the role of temporal variation in landscape quality on the foraging distances of honey bees. Objective 2: Determine the impact of colony size on foraging distances of honey bee colonies placed on organic farms. Objective 3: Develop extension materials and education programs to disseminate the outcomes of this study to beekeepers, organic farmers, and advisory boards.

## APPROACH

A. Landscape quality estimation: To generate a spatial prediction of the density of honey bee foraging and landscape quality, we will utilize data from pollen metabarcoding to adjust seasonal forage maps to reflect visitation preferences of honey bees. Specifically, we will re-generate forage maps excluding species that were not detected in pollen samples and apply a distance-weighting algorithm (Lonsdorf et al. 2009) to develop a foraging quality index based on these weighted average distances. Floral resources closer to the colony will have greater weight, thus the floral index will be positively correlated with the proximity and the floral availability in the landscape.

B. Honey bee foraging estimation: We will integrate data from QR code automated readers, harmonic radars, and waggle dance decoding to describe the distance, duration, and direction of foraging flights in honey bees. Data sets from QR code tags, harmonic radar and waggle dances will be analyzed to ascertain whether bees mainly forage within the radius of the organic farms throughout the year. We will develop foraging kernels from each of these three methods to determine the percent of the foraging trips that occur outside of the boundaries of organic farms. Radar data will be analyzed to determine bee flight direction, length of each step bees take, outbound and inbound bee flying patterns, and length of time for each trip bees can take over the course of a given day. The resulting data sets will be used to develop a numerical model (to be improved from previous formulations as, for example, outlined in Fuentes et al. 2016, see Equation 5) to determine the foraging patterns of bees as a function of meteorological conditions (e.g., wind speed) and floral resources distributed throughout the landscape. Data collected from the QR code automated readers will include flight length for 100 individuals per colony to robust distribution of the foraging distances in each colony. The QR code data sets will serve as the basis to generate a numerical model to estimate the distances bees travel based on the duration of the foraging per individual. These numerical models will be calibrated with the more accurate data collected from harmonic radars and waggle dance decoding. Radar data will provide the necessary parameters (e.g., minimum step lengths taken by bees, ensemble range distances traveled from hives, etc.). We will compare the results of the waggle dance data (distance and forage location, as per Schürch et al. 2013; 2019) with the data obtained through the automated methods. Specifically, we will convert dance duration into distances and correlate individual flight distance with flight duration, as determined by QR code surveillance. A highly significant positive correlation between the different methods ( $r^2 > 0.9$ ) would indicate that QR code methods can provide a cost-effective and automated methodology to study foraging distances of honey bee foragers. The verification of this method would also provide a technology that could be easily incorporated to determine whether the organic certification of honey bee products is possible in different organic farms.

C. Pesticide residue analysis: In addition to quantitatively assessing honey bee foraging distances with three methods, we will validate the feasibility of the development of organic beekeeping certification protocols through the quantification of pesticide residues in honey, pollen, and wax from the experimental colonies. We will collect pollen samples in May, June, and September (as described in Obj 1). Honey and wax samples will be collected each fall in years 1 and 2. During the honey extraction procedure, containers of 50 ml of honey will be collected from each selected hive. Wax samples (15g) will be scraped from a frame in the center of each hive and placed in a 50 ml container for storage. All samples will be collected on dry ice and stored at  $-80^{\circ}\text{C}$  until analysis. These samples will be sent to Cornell University for analysis of the quantities of 93 pesticides by LCMS (<https://blogs.cornell.edu/cccef/>). Results of pesticide residues from all bee products will be used to indicate the presence of chemical contaminants and whether they contain levels that would be acceptable to qualify them for organic certification. We will calculate the total abundance and the total number of compounds to generate summary statistics for the diversity of pesticides in all samples. We will use a GLM to statistically test whether the diversity of pesticides in honey, wax, and pollen is significantly different in colonies foraging on different organic farms. All statistical analyses will be done in R (R project 2021).

Progress 09/01/23 to 08/31/24  
Outputs  
Target Audience: Our main targeted audiences are beekeepers and policy-makers. The information that we are generating with this project is of interest to beekeepers who may be interested in transitioning to organic beekeeping management and/or seeking an economic opportunity to commercialize organic honey bee products. Additionally, we hope to have conversations with regulatory agencies (e.g., EPA, NOP) to use the information from this project to change regulations that can possibly support the development and marketing of organic beekeeping products in the US. We are also developing instruments for studying honey bee foraging that will be of interest to scientists and practitioners working with honey bees. This past year, we talked to several beekeeping groups about this project including the American Beekeeping Federation. The engineering team offered a workshop to students, professors, and USDA scientists on how to implement the instruments that they developed to study honey bee foraging. Additionally, we are training the new generation of honey bee scientists (two PhD students and one postdoc at Penn State, one MSc student at Cornell, and one MSc student at Virginia Tech).

Changes/Problems: The research objectives of the grant are following the proposed timeline but we have had some setbacks. For the calibration experiment led by VT, QR codes are not being used because it was difficult to optimize its implementation for that application (i.e., needing to individually identify in real time each forager bee in the field). Another setback this year was that the postdoc from Penn State who was leading the field crew in Pennsylvania and the integration of the different datasets of the project has moved on for a permanent position at Auburn University. As such, we have modified

the project to limit the data collection component to only one year. We will start looking for a new postdoc who can lead the data analysis of the project this year. The completion of all the proposed outcomes of the grant in a timely manner will depend on our ability to successfully find a qualified person in this role. What opportunities for training and professional development has the project provided?The VT and Cornell teams have been working with MSc students who have supported the research activity of this grant. The PSU engineering team supported two PhD students on this grant, who worked on the development of the BeeCam-AprilTag system. These graduate students have attended scientific conferences to give talks about the results of their research. This year we can successfully report that the entomologists and engineers worked effectively on the development of the BeeCam-AprilTag (which resulted in a paper submitted for peer-review). This is an important achievement given that transdisciplinary research can be difficult to develop effectively. The postdoc hired at Penn State (Selina Bruckner) moved on to a permanent position at Auburn University as an Assistant Extension Professor of Apiculture at Auburn University. How have the results been disseminated to communities of interest?Please see the list of talks reported under \"other products\". We published 5 extension articles and gave at least 9 presentations to beekeeper audiences. What do you plan to do during the next reporting period to accomplish the goals? Development of models to correlate foraging time and foraging distance. Results of pollen metabarcoding data from corbicular pollen samples collected in 2023 and 2024. These data in combination with the landscape analyses will allow us to associate floral resources to types of habitats where the bees are foraging. Pollen, honey, and wax pesticide analysis from all the colonies in the study during year 2. Comparison of the foraging behavior of colonies in single vs doubles. Impacts What was accomplished under these goals? Below, we report on the specific accomplishments for each goal: Objective 1: Assess the role of temporal variation in landscape quality on the foraging distances of honey bees -We completed the landscape analysis of floral resources for all the organic farms included in PA, and NY. These data will be used to validate the results of the pollen metabarcoding from pollen samples of the experimental colonies. In 2024, the newly developed BeeCam-AprilTag system (Peñaloza-Aponte et al submitted) was successfully deployed in the field to collect data on the foraging behavior of all the experimental colonies. We have been collecting foraging data since April 2024 from 6 sites (3 in PA and 3 in NY) by marking 100 bees per colony every two weeks. The calibration experiment at VT also started this year, and it will be completed in spring 2025. Objective 2: Determine the impact of colony size on foraging distances of honey bee colonies placed on organic farms-The PSU and Cornell teams have been collecting data on colony sizes every two weeks. This will allow us to estimate the effect of colony size on the foraging distance of bees. Objective 3: Develop extension materials and education programs to disseminate the outcomes of this study to beekeepers, organic farmers, and advisory boards-This past year, we published 5 extension articles and gave at least 9 presentations to beekeeper audiences. Additionally, we met with Brenda Foss (Director of the Monitoring Programs Division) and Roger Simonds (Lab Chief of the National Science Gastonia Laboratories) about our project and the potential utility of our results to inform changes in policy. We met twice with our advisory board to share updates about the project and seek their input on some of the experimental problems that we encountered. Publications Type: Journal Articles Status: Submitted Year Published: 2024 Citation: Peñaloza-Aponte D, Brandt S, Dent E, Underwood RM, DeMoras B, Bruckner S, López-Urbe MM, Uribina JV (Submitted) Automated entrance monitoring to investigate honey bee foraging trips using open-source wireless platform and fiducial tags. HardwareX Type: Other Status: Published Year Published: 2024 Citation: MJ Couvillon. Colony management impacts success, but organic beekeeping remains sidelined in the USA. The Beekeepers Quarterly. No 156, 28-29. Type: Other Status: Published Year Published: 2023 Citation: López-Urbe MM, Underwood RM, Bruckner S (2023) The benefits of organic beekeeping and how management affects honey bee colony health, survival, and productivity. American Bee Journal 163(7):753-755. Type: Other Status: Published Year Published: 2024 Citation: LC McHenry, I McKellips, LE Johnson, and MJ Couvillon. The worst and the best of bee research: a story in four snapshots. The Beekeepers Quarterly. No. 155, 26-27. Type: Other Status: Published Year Published: 2024 Citation: Bruckner S, López-Urbe MM, Underwood RM (2024) Current challenges for the production of certified organic honey bee products. American Bee Journal 164(1):79-81. Type: Other Status: Published Year Published: 2023 Citation: Bruckner S, López-Urbe MM, Underwood RM (2023) Organic and Treatment-free Colony Management They are not the same. American Bee Journal 163(10):1123-1125. Progress 09/01/22 to 08/31/23 Outputs Target Audience:Our main targeted audiences are beekeepers and policy-makers. The information that we are generating with this project is of interest to beekeepers who may be interested in transitioning to organic beekeeping management and/or see an economic opportunity with organic honey bee products. Additionally, we hope to have conversations with regulatory agencies (e.g., EPA, NOP) to use the information from this project to change regulations that can possibly support the development and marketing of organic beekeeping products in the US. The engineering arm of the team held a workshop about the instrumentation of raspberry pi for studying honey bee foraging that was attended by government employees, scientists, and students. Changes/Problems:The original grant had this work starting in Fall 2022, but because the funds came in in the Fall, student recruitment had to be postponed for one year. Getting the honey bees to establish in new equipment and draw enough comb has been the biggest issue so far. Thus, comparisons between the two management regimes (single and double brood chamber

management) cannot begin until spring 2024. We think that the difficulty of starting organic colonies with new equipment will be a major hurdle for anyone wanting to transition to organic beekeeping. It is a huge investment to start colonies in hives with no existing comb and to have to build them. After a full season of effort, the singles are full but the doubles still have many frames to fill. Very little honey has been produced this year, so most colonies will likely need to be fed to ensure winter survival. Next year, the colonies need to build comb in the honey supers, so honey production probably will not be very high. For a beekeeper, this probably means it will take 3 years before a decent honey crop can be harvested. This is likely a steep entry fee for organic beekeeping that few beekeepers can afford. This is probably worth discussing with USDA to see if an existing comb (or Bettercomb) can be used when starting an organic beekeeping operation. What opportunities for training and professional development has the project provided? The VT and Cornell teams have recruited MSc students who will be trained during the activities associated with this project. These graduate students are planning to attend scientific conferences to present the results of their research. The PSU team hired a postdoctoral researcher. Scientists (professors and students) are learning to incorporate technology into studies of ecology and biology. Engineers are learning how to work with living things. In addition to the training workshop that was publically open in June 2023, we offered an internal Raspberry Pi Workshop with Electrical Engineering Students and collaborators from Virginia Tech and Cornell. 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? Data collection to develop models to correlate QR code data and waggle dance should have been completed. Results of pollen metabarcoding data from corbicular pollen samples collected from May - October 2023 will allow us to associate floral resources to types of habitats where the bees are foraging. Collect corbicular pollen samples from March - August 2024, and begin to compare foraging for large (doubles) and small (single) colonies. Pollen, honey and wax pesticide analysis from all the colonies in the study during year 2. Impacts What was accomplished under these goals? Objective 1: Assess the role of temporal variation in landscape quality on the foraging distances of honey bees. We completed a preliminary analysis of the landscape quality (based on floral resources and pesticide exposure) surrounding all organic farms present in PA, NY, and VT. These analyses helped us identify the farms that were chosen for the study. Since the establishment of the colonies in April 2023, we have started collecting pollen samples from returning forager bees over the season. These samples will allow us to determine the plants that the bees are visiting to collect pollen. The data will be used to show variation in plant diversity over time, and to correlate these data with information about available landscape types. Additionally, Raspberry Pi computers were installed on a subset of the colonies to test and troubleshoot the QR code tagging and detection process. Objective 2: Determine the impact of colony size on foraging distances of honey bee colonies placed on organic farms. The team from Virginia Tech has successfully recruited a new MS student (Lindsay Johnson), who began this Fall 2023. This student will generate the data to correlate and model how the information from flight duration from the QR code data can be associated with the distance and directionality that the bees are flying to based on estimates from waggle dance decoding. The PSU and Cornell teams established colonies and assigned them to either the double or single deep treatment groups. Colonies are still drawing out wax foundations in either Deep brood boxes or medium honey supers. Objective 3: Develop extension materials and education programs to disseminate the outcomes of this study to beekeepers, organic farmers, and advisory boards. We do not have any outcomes to share at this time. This ongoing project has been mentioned when talking about previous work related to organic beekeeping management. Publications Type: Other Status: Published Year Published: 2023 Citation: Lopez-Uribe MM, Underwood RM, Bruckner S (2023) The benefits of organic beekeeping and how management affects honey bee colony health, survival, and productivity. American Bee Journal 163:753-755.

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# Native Warm Season Perennials: an Enduring Solution to Summer Drought and Slump for Fescue Belt Organic Forage Production

<b>Accession No.</b>	1028995
<b>Project No.</b>	TEN2022-04076
<b>Agency</b>	NIFA TEN\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37887
<b>Proposal No.</b>	2022-04076
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2026
<b>Grant Amount</b>	\$750,000
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Jagadamma, S.
<b>Performing Institution</b>	UNIVERSITY OF TENNESSEE, 2621 MORGAN CIR, KNOXVILLE, TENNESSEE 37996-4540

## NON-TECHNICAL SUMMARY

Assuring high-quality forage availability for an extended grazing period is crucial to closing the gap between the demand and domestic production of organic livestock products. The forage systems in the southeastern US, dominated by cool-season grasses, especially tall fescue, do not thrive well in the hot summer months and also produce toxic alkaloids. We propose to complement summer forage availability in the Fescue Belt by utilizing native warm-season perennial grasses (NWSGs). While these grasses are well adapted to the local growing conditions and support environmental stewardship by requiring less external inputs and preventing the overgrazing of fescue-based systems, they are slow to establish. Our discussions with organic livestock producers in the Fescue Belt identified the need for technical guidance to organically establish NWSGs as very limited information is currently available. We propose to conduct three on-station and on-farm trials on the certified organic lands in Tennessee and Arkansas to evaluate four establishment methods and three NWSG species treatments on forage yield and nutritive value, weed suppression, soil health, and economic benefits. We will develop and disseminate Extension and education activities to improve producers' awareness and knowledge of improved organic forage production strategies. Our interdisciplinary and multi-institutional team is highly qualified to conduct this research with the support of an Advisory Panel and stakeholders' engagement. Our project is expected to provide critical information to enhance the profitability and sustainability of the organic forage systems in the Fescue Belt by integrating NWSG.

## OBJECTIVES

The major goal of this project is to build interdisciplinary research, education, and Extension framework across multiple institutions in the southeastern U.S. to help organic ranchers and stakeholders establish native warm-season grass integrated forage systems to overcome summer slump, summer drought stress, and overgrazing challenges and enhance animal performance. The specific objectives of this project are :1) Assess the forage yield, nutritive value, and weed suppression capacity of three NWSG species treatments relative to a common non-native warm-season perennial grass under four establishment methods2) Assess the effects of different establishment methods and forage systems on soil health3) Conduct a comparative economic analysis of different establishment methods and forage systems to identify the most profitable approach for organic

production systems) Integrate the knowledge generated under Objectives 1-3 into Extension and education programs that improve producers' awareness of organic forage production

## APPROACH

We will conduct a 4-year project that includes three on-station and three on-farm trials on certified organic lands by leveraging strong support from multiple stakeholders in the southeastern US. The on-station experiments will take place at 1) the University of Tennessee's Organic Crops Unit, in Knoxville, TN, 2) Middle Tennessee State University's Experiential Learning and Research Center in Lascassas, TN, and 3) USDA-ARS Dale Bumpers Small Farms Research Center in Booneville, AR. The on-station experiments will include four forage species and four establishment strategies arranged in a randomized complete block design with four blocks. We will measure a suite of agronomic (forage yield and quality) and soil health attributes for four years. Based on our preliminary results from years 1 and 2 of the on-station experiments, we will choose the most promising establishment strategy and NWSG species combination from each location and test them on farmers' fields in years 3 and 4. We will collect all the necessary data to conduct an economic evaluation of the tested practices in years 3 and 4. A comprehensive Extension and education program will be implemented to disseminate the research experiences and knowledge to a wide range of audiences. Progress 09/01/23 to 08/31/24 Outputs Target Audience: In 2024, our project continued to target the following primary audiences: 1) farmers in the southeastern U.S. managing Fescue-dominated pastures during the summer; 2) conventional forage farmers interested in transitioning to organic farming practices; 3) students and agricultural professionals focused on organic farming techniques; and 4) consumers and the general public who support organic meat and dairy products. This year, we expanded our outreach by conducting workshops, conference presentations, and field days tailored for farmers, students, extension agents, and agricultural professionals. These events provided practical demonstrations and discussions on establishing organic native warm-season grasses (NWSG). The students recruited for this project gained valuable hands-on experience in field experiment setup and maintenance, data collection, soil sampling, lab analysis of soil and plant samples, and preliminary data processing. Notably, the Ph.D. student supervised by lead PI Jagadamma at the University of Tennessee-Knoxville (UTK) presented posters on the short-term soil health impacts of various organic NWSG establishment methods at the Soil and Water Conservation Society (SWCS) annual conference and the University of Tennessee-Knoxville (UTK) Beef and Forage annual meeting. Additionally, the M.S. student, supervised by Co-PIs Philipp and Nieman at the University of Arkansas (UARK), presented an overview of our project at the Organics in the Ozarks Field Day held at the University of Missouri Southwest Research and Extension location in Mt. Vernon, MO, on April 12, 2024. This event attracted around 100 participants from Missouri and northern Arkansas interested in learning about organic crop and livestock production. Co-PI Cui also organized the Digital Agriculture Summer Camp and Digital Agriculture Academy events in the summer of 2024, introducing 28 high school students and 20 non-formal educators from the Middle Tennessee area to scientific experimentation concepts and the benefits of organic NWSG production. Through these efforts, we successfully reached and engaged a diverse audience interested in sustainable agriculture and organic farming practices. Changes/Problems: We encountered an armyworm outbreak at both the UARK and MTSU sites, which we managed to control effectively through pest scouting and application of Entrust insecticide (OMRI approved). Middle Tennessee experienced its most severe flooding event in the past 50 years, which inundated our research plots at the MTSU site. Fortunately, despite this setback, our seeds could still germinate successfully following the event. What opportunities for training and professional development has the project provided? PI Jagadamma is mentoring a Ph.D. student responsible for tasks under Objective 2. For research activities related to Objective 1, an M.S. student is co-advised by Co-PIs Philipp and Nieman at UARK, and a research associate has been recruited by Co-PI Cui at MTSU. Additionally, a part-time postdoctoral researcher under Co-PI Keyser will be undertaking tasks related to Objective 1 at UTK. Three undergraduate students are actively participating in lab and field work at the UTK site. All students and early-career professionals involved in the project are gaining critical knowledge on organic forage production as well as soil and plant sampling and analysis. These individuals will continue to have access to valuable networking opportunities and other professional development resources throughout the project's duration. How have the results been disseminated to communities of interest? This year, we were able to present our research to farmers, agricultural professionals and students at different conferences. We were also able to introduce this project and the status of our research to the attendees of the field day event at the Organics in the Ozarks at the University of Missouri Southwest Research and Extension location in Mt. Vernon, MO. What do you plan to do during the next reporting period to accomplish the goals? Our primary focus for Year 3 will be to closely monitor NWSG growth across all four establishment strategies. We will continue collecting and analyzing soil and plant samples from the NWSG plots. Additionally, we plan to initiate the economic analysis by the end of Year 3. Team members will also carry out outreach demonstrations throughout the next reporting period. Impacts What was accomplished under these goals? 1. Assess the forage yield, nutritive value, and weed suppression capacity of three NWSG species treatments relative to a common non-native warm-season perennial grass under four

establishment methods Field experiments are ongoing on certified organic land at three locations: the University of Tennessee-Knoxville (UTK), Middle Tennessee State University (MTSU), and the University of Arkansas (UARK). At the Knoxville site, we were able to accomplish all activities expected this season. After the harvest of the first smother crop, pearl millet, we planted our second smother crop, cereal rye, in October 2023. NWSGs were planted on time; March 21 for dormancy planting (TRT 3), April 30 for smother crop harvesting treatment (TRT 1), May 24 for smother crop rolling-crimping treatment (TRT 2) and June 15 for till treatment (TRT 4). All tillage passes were done for the tillage treatment. Treatments 1 to 3 were clipped on June 28 and August 15 to control weed growth. The second clipping in August was done because continuous rain after the first clipping led to more weed proliferation. At the MTSU site, the second smother crop was planted in September 2023. NWSGs were planted as per schedule: March 18 for dormancy planting (TRT 3), May 1 for the smother crop harvest treatment (TRT 1), May 3 for the smother crop rolling-crimping treatment (TRT 2), and May 16 for the tillage treatment (TRT 4). All tillage activities were conducted as outlined in the experimental plan. Clipping of Treatments 1 through 3 was done on June 28 and August 8 to control weed growth and reduce competition. Similarly, at UARK, the second smother crop, cereal rye, was also planted in September 2023. NWSGs were subsequently planted as follows: March 5 for dormancy planting (TRT 3), April 24 for the smother crop harvest treatment (TRT 1), May 21 for the smother crop rolling-crimping treatment (TRT 2), and June 20 for the tillage treatment (TRT 4). Clipping for Treatments 1 through 3 occurred on June 25 and August 14. Cereal rye that was planted 2023 Fall was harvested in Spring 2024. We recorded cereal rye plant and tiller counts and weed rating data (mid to late April 2024) at all three sites. Before harvesting rye, we also took plant samples using three 0.25 m<sup>2</sup> quadrats per plot to assess the yield and forage value of cereal rye as a potential forage while allowing NWSGs to establish. Samples were clipped at 7.5 cm stubble heights, the samples were separated, weighed and dried at 55 °C for 72 hours, weighed again to determine dry matter content. Six samples were selected at each site randomly, including three samples that are only for cereal rye and three samples that are mixed with dominant weeds in each plot, to be used for NIRS analysis. At the same time, we visually recorded data on weed pressure per plot. Native warm-season seedlings were counted at 42 days after planting in Knoxville because rain made it difficult to do that activity at 35 days after planting. Treatments 1 and 3 (Smother crop harvesting and Dormant season planting, respectively) were counted at the same time while treatments 2 and 4 were counted at the same time. Data analysis is currently ongoing. Weed growth in the Smother Crop Rolling-Crimping treatment was lower compared to the other treatments due to the residue layer on the soil surface. The Tillage-only treatment received occasional tillage passes as needed, with all tillage passes documented at each site.

2. Assess the effects of different establishment methods and forage systems on soil health Baseline samples were collected from 0-60 cm in each plot and divided into three depth increments (0-10 cm, 10-30 cm, and 30-60 cm). These samples were analyzed for bulk density, inorganic nitrogen, microbial biomass carbon, extracellular enzymes, pH, permanganate-oxidizable carbon, Mehlich I-extractable nutrients, soil texture, soil organic carbon, and total nitrogen. The Ph.D. student supervised by the lead PI, along with part-time undergraduate assistants, completed the laboratory analysis of baseline soil samples collected from three experimental sites. The Ph.D. student also performed statistical analyses on each soil health metric. Since the baseline soils have not yet undergone treatment, differences among parameters were only assessed by soil depth. At the Knoxville site, bulk density ranged from 0.96 to 1.76 Mg m<sup>-3</sup>. Inorganic nitrogen was highest in the top 0-10 cm layer (40.06 mg kg<sup>-1</sup>), followed by 10-30 cm (20.69 mg kg<sup>-1</sup>) and 30-60 cm (19.74 mg kg<sup>-1</sup>). All soil health parameters followed a similar trend, with values generally higher at the surface (0-10 cm > 10-30 cm > 30-60 cm). Microbial biomass carbon content ranged from 82 to 359 mg kg<sup>-1</sup>, and soil organic carbon stock varied from 12 Mg C ha<sup>-1</sup> at lower depths to 23 Mg C ha<sup>-1</sup> in the topsoil. Average pH was 6.33, with a clay loam texture. Enzymatic activities (alpha-glucosidase, beta-glucosidase, cellobiohydrolase, N-acetyl glucosaminidase, and leucine aminopeptidase) were highest in the 0-10 cm depth and lowest in the 30-60 cm depth, a trend also observed in Mehlich I-extractable nutrients, total nitrogen, and permanganate-oxidizable carbon. At MTSU site, bulk density values ranged from 0.93 to 1.73 Mg m<sup>-3</sup>. Inorganic nitrogen levels varied between 4.27 and 24.03 mg kg<sup>-1</sup>. Microbial biomass carbon ranged from 63 to 427 mg kg<sup>-1</sup>, and soil organic carbon stock ranged from 22 Mg C ha<sup>-1</sup> at greater depths to 25 Mg C ha<sup>-1</sup> in the topsoil. Average soil pH was 6.49, with a silty clay loam texture. Enzyme activities were highest in the 0-10 cm depth and lowest at 30-60 cm. Mehlich I-extractable nutrients, total N, and permanganate-oxidizable carbon levels followed the same depth-dependent trend. At the UARK site, bulk density ranged from 0.96 to 1.76 Mg m<sup>-3</sup>, increasing from topsoil to subsoil. Inorganic nitrogen levels peaked in the 0-10 cm layer (5.7 mg kg<sup>-1</sup>) and decreased with depth to 2.56 mg kg<sup>-1</sup> at 30-60 cm. Microbial biomass carbon ranged from 114 to 509 mg kg<sup>-1</sup>, and soil organic carbon stock varied from 28 Mg C ha<sup>-1</sup> in surface soils to 24 Mg C ha<sup>-1</sup> at deeper levels. The soil's average pH was 5.74, with a silt loam texture. Enzyme activities, Mehlich I-extractable nutrients, total nitrogen, and permanganate-oxidizable carbon showed a depth-dependent decline, with the highest activity in the 0-10 cm layer and the lowest at 30-60 cm. Soil samples at the end of Year 1 were collected from all three sites, from the 0-30 cm depth, divided into two increments (0-10 cm and 10-30 cm) to assess how NWSG establishment methods influenced soil health and nutrient status. The Ph.D. student is currently analyzing these samples in the lead PI's lab at UTK.

3. Conduct a comparative economic analysis for different

establishment methods and forage systems to identify the most profitable approach for organic production systems. The Ph.D. student has been systematically recording farm management activities at the UTK field site to support the economic analysis led by Co-PI Griffith. In addition, this student is documenting similar data from other project sites to ensure a comprehensive dataset. The economic analysis is set to begin in Year 3 of the project.

4. Integrate the knowledge generated into Extension and education programs that aim to improve producers' awareness of organic forage production. At MTSU, Co-PI Cui included a module where he used task-based learning (TBL) and project-based learning (PBL) approaches to teach the organic establishment of NWSGs in two courses he is offering: PLSO 3330 "Field Crop Production" and PLSO 4310 "Forage Crops". While teaching both courses, Cui talked about challenges associated with organic NWSG production, cropping system concepts and regulation issues. He also introduced the benefits of NWSG production to high school students and educators during their summer camp events.

Publications Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Kallingal, B., and Jagadamma, S. 2024. Soil health impact of different methods of establishment of organic native warm season grasses. 79th Soil and Water Conservation Society International Annual Conference, July 21-24, Myrtle Beach, NC (Poster) Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Kallingal, B., Keyser, P., and Jagadamma, S. 2024. Sustainable forage solutions: organic native warm season grass establishment methods and soil health in the Southeast U.S. 2024 University of Tennessee Beef and Forage Center Annual Research Meeting, October 8, Knoxville, TN (Poster) Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Kallingal, B., Keyser, P., and Jagadamma, S. 2024. Organic native warm season grasses: soil health impacts of conventional and sustainable land preparation methods. 2024 ASA, SSSA, and CSSA Annual International Meeting, November 10-13, San Antonio, TX (Poster) Progress 09/01/22 to 08/31/23

Outputs Target Audience: The primary target audiences for this study are 1) farmers in the southeastern U.S. who manage Fescue-dominated pasture in the summer, 2) farmers following conventional forage systems who would like to transition to organic farming, 3) students and agricultural experts who are interested in organic farming techniques, and 4) consumers and the general public who prefer organic meat and dairy products. During the first year of this project, we focused mainly on student training. We recruited a part-time postdoctoral scientist, one MS student, one Ph.D. student, one research associate, and three undergraduate students. These students are gaining experience in field experiment establishment and maintenance, field data collection, soil sampling, laboratory analysis of soil and plant samples, and preliminary laboratory data processing/analysis.

Changes/Problems: Organic pearl millet seeds were not available to purchase. Hence, we used untreated non-GMO seeds of pearl millet in all three locations after receiving approval from organic certifiers at all three locations. Due to the limitation of available certified area at the MTSU field experiment location, the plot size was reduced to accommodate all treatments and replications. What opportunities for training and professional development has the project provided? PI Jagadamma recruited a Ph.D. student, who conducts the tasks outlined in Objective 2. For undertaking research proposed under Objective 1, an MS student is recruited by Co-PI Philipp at UARK and a research associate is recruited by Co-PI Cui at MTSU. A part-time postdoc under Co-PI Keyser will be undertaking the tasks under Objective 1 at UTK. Three undergraduate students have been participating in lab and field work at the UTK location. All these students and early career professionals have been receiving critical knowledge about the production of organic forages as well as soil and plant sampling and analysis. These individuals will also receive many opportunities for networking and other professional development opportunities in the coming years of the project.

How have the results been disseminated to communities of interest? This year, our focus was to recruit the students and other personnel and establish the field experiment. Once we collect data, we will begin the outreach activities as described in the proposal. We received one opportunity to introduce this project to the attendees of the field day event at the UARK field experiment location. What do you plan to do during the next reporting period to accomplish the goals? Our main focus in Year 2 will be to complete implementing the weed control strategies (by growing cereal rye as a smother crop and completing the tillage activities in the tillage-only treatment) and plant native warm season grasses in all treatments. We will continue collecting soil samples as well as plant samples (from cereal rye smother crop and native warm season grasses) and analyze them. The team members will start conducting outreach demonstrations towards the end of the next reporting year.

Impacts What was accomplished under these goals? 1. Assess the forage yield, nutritive value, and weed suppression capacity of three NWSG species treatments relative to a common non-native warm-season perennial grass under four establishment methods. The field experiments were started in the certified organic land at three locations: University of Tennessee-Knoxville (UTK), Middle Tennessee State University (MTSU), and University of Arkansas (UARK). The fields were tilled in March 2023 and planted with pearl millet as smother crop in three out of four treatments. The fourth treatment is a tillage-only treatment to represent the conventional seedbed and weed control technique, where we performed three rounds of tillage. In the pearl millet plots, we recorded pearl millet tiller count and weed rating data in July 2023 to characterize the pearl millet stand. Before harvesting pearl millet, we collected plant samples using a quadrat to assess the yield and forage value. The pearl millet was harvested from all three sites by August 2023. Overall, we noticed that the weed growth in smother crop-planted plots was less compared to the tillage-only

treatment. After the smother crop harvest, all plots received tillage to further reduce the weed seed bank. The second smother crop, i.e., cereal rye, is scheduled to be planted in September 2023. The tillage-only treatment has been receiving occasional tillage passes as and when needed (the number and type of tillage passes have been documented in all sites).

2. Assess the effects of different establishment methods and forage systems on soil health To assess the baseline soil health and nutrient status, we took soil samples from 0 to 60 cm depth from all plots and divided them into three depth increments (0-10 cm, 10-30 cm, and 30-60 cm). Samples from all three sites were sent to the lead PI's lab in UTK, where the Ph.D. student has been analyzing the samples for a suite of soil health parameters. Upon arrival, core sections were weighed and sub-samples (~10 g) were oven-dried at 105°C to determine the gravimetric soil moisture content. Using the wet weight and gravimetric moisture contents, bulk density was determined. The rest of the samples were portioned into two. One part (~150 g) was stored under 4°C for inorganic N, microbial biomass C, and extracellular enzymes measurements, and the rest was air-dried, sieved through a 2-mm sieve, and retained for the analysis of a suite of soil physical and chemical properties. All the time-sensitive analyses such as gravimetric moisture content, microbial biomass, inorganic N, and extracellular enzymes have been completed. The Ph.D. with the support of two part-time undergraduate students is currently working on measuring other properties using the air-dry soils.

3. Conduct a comparative economic analysis for different establishment methods and forage systems to identify the most profitable approach for organic production systems The Ph.D. student has been recording the farm management activities at the UTK field site to enable economic analysis by Co-PI Griffith. This student is also documenting similar information received from other sites. The economic analysis will start in Year 3 of the project.

4. Integrate the knowledge generated into Extension and education programs that aim to improve producers' awareness of organic forage production Outreach activities are proposed to start from Year 2 only. However, the project PIs have been taking advantage of any opportunities to make producers and other stakeholders aware of the initiation of this project. Co-PI Keyser received a Partnership for Climate-Smart Commodities grant to work on the production and marketing challenges faced by livestock producers in the Fescue Belt region. The producer and other stakeholder meetings as part of this large project will also be a platform to discuss the outcomes of our project. The PIs have also been providing one-on-one educational training to the participating postdoc and students, and staff at the research centers where field experiment is being implemented. At MTSU, Co-PI Cui included a module on the organic establishment of native warm season grasses in two courses he is offering: ABAS 3330 "Field Crop Production" and ABAS 4310 "Forage Crop Production". Publications

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# Systems Approach to Maximize Organic Spinach Productivity

<b>Accession No.</b>	1028983
<b>Project No.</b>	TEX09960
<b>Agency</b>	NIFA TEX\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-37886
<b>Proposal No.</b>	2022-04068
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2025
<b>Grant Amount</b>	\$453,789
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Joshi, V.
<b>Performing Institution</b>	TEXAS A&M UNIVERSITY, 750 AGRONOMY RD STE 2701, COLLEGE STATION, TEXAS 77843-0001

## NON-TECHNICAL SUMMARY

Despite the soaring demand for organic spinach in the US and worldwide, production is not keeping the same pace, especially during the pandemic. Nutrient acquisition, abiotic stresses like extreme temperature (heat), nutritional attributes, and limited seed availability are critical challenges in organic spinach production. The proposal focuses on a systems approach designed to streamline the efforts to maximize spinach productivity by developing varieties with improved nitrogen use efficiency and nutraceuticals, understanding the role of soil and spinach root and leaf associated microbes adapted exclusively to low-input organic production, and boosting the seed production of organic spinach through optimal genetics. Organic spinach growers need varieties adapted to organic growing conditions and qualities demanded by organic consumers. This project will lay a foundation to develop spinach varieties for organic producers and consumers with an improved nutrient acquisition, improve our understanding of how spinach leaf and root-associated microbiomes interact with organic fields dedicated to spinach production, and create a breeding model to develop varieties with higher organic seed productivity.

## OBJECTIVES

Spinach, the most popular nutrient-rich staple, has notoriously high pesticide residues when grown conventionally. Despite the soaring demand for organic spinach in the US, production is not keeping the same pace. Most varieties currently used for low-input organic production are poorly adapted and cause substantial yield gaps relative to conventional farming. The proposed project aims at addressing the concerns with organic spinach productivity by focusing on the following objectives - (1) Enhancing the nitrogen use efficiency (NUE) by identifying suitable genetics for low-input organic spinach production and speed breeding (2) Characterizing the role of the microbiome in nutrient uptake adapted explicitly to organic spinach by metagenomic analysis of rhizosphere (soil) and endosphere (root) specific microflora (3) Enhancing seed production and availability of organic spinach using the natural variation in spinach germplasm via genome wide-association analysis and genomic prediction. The outcomes of this project will benchmark efforts to develop organic spinach varieties to enhance productivity and quality through improved nutrient acquisition and expand our understanding of soil microbial interactions in organic spinach production. Project results will be disseminated nationally through Organic, Organic Seed Alliance PD, Co-PD initiated outreach programs, agricultural publications, and field days.

## APPROACH

Methods: For Objectives 1 and 2; we will be undertaking NUE screens, metabolite/nutraceutical analysis, soil and plant microbial analysis, and GWAS/GS of traits. PD and Co-PD, will participate in the multi-location trial to grow and evaluate spinach germplasm in a certified organic farm; collect physiological data, measure chemical traits and micronutrients, sample tissue for metabolite analysis, and perform genome-wide association study and genomic prediction analysis for various traits such as NUE, heat stress, nutritional qualities, and seed yield. Co-PD will be leading the spinach seed yield and quality trials at the primary research site in Chimacum, WA and collaborate with the project team on evaluation protocols, data management, development of trial reports, outreach, and dissemination of project results. Project team members will support the project outreach and communications, including working with eOrganic on the website, webinars, and project promotion efforts. Another Co-PD will support the outreach component of this project to bring the findings to a national audience. eOrganic will provide peer- and National Organic Program compliance review and editorial support for at least three publicly available articles by project members, which will be published on the eOrganic website and announced in the newsletter and on social media. eOrganic will also conduct at least three webinars and evaluate them for quality and utility. It will also assist by creating a public project website on the eOrganic website.

Progress 09/01/23 to 08/31/24 Outputs Target Audience: Organic vegetable growers, vegetable seed companies, vegetable plant breeders, plant science researchers in horticulture, plant breeding, plant pathology, soil science, and botany. Changes/Problems: The Arkansas location had weather issues during our winter 2023 trial in Fayetteville, AR. We expect to have fewer issues with the weather during our Fall trial. What opportunities for training and professional development has the project provided? The project provides training and professional development opportunities for graduate students, postdoctoral positions at Texas A&M, and research internship positions at OSA. As part of education activities, interns, graduate students, and post-doctorates are being trained in genomics, crop and seed production, disease protection and diagnosis, and soil science with a focus on organic systems as they participate in research projects critical to the project activities, and present in professional conferences. This project also provides external training (Extension) and professional development for farmers, seed producers, and seed companies through educational resources, webinars, field days, and conference presentations. Educational outreach is delivered through the project website on eOrganic ([www.eorganic.info/spinach](http://www.eorganic.info/spinach)) and in year 1 spinach seed production resources were posted and promoted through the website and partner-organization newsletters. Collaborating farmers gained first-hand experience evaluating crop diversity through on-farm trials and gained training and professional development through coordination of field research with the project team. Trials were conducted with organic spinach farms in WA, . Each trial provided training on carrot trialing and seed production for employees and interns. These trials allowed expansion of project impacts, testing materials in new regions, and soliciting input on evaluations from two organic seed companies. Project collaborators also hosted public farmer field days with trials at the project research sites. Dr. Gehendra Bhattarai, a postdoctoral researcher at the University of Arkansas, led this project. Under the supervision of Dr. Ainong Shi (Co-PD) and Dr. Vijay Joshi (PD), Dr. Bhattarai played a crucial role in executing project tasks. Dr. Haizheng Xiong, a research associate in Dr. Shi's lab, was responsible for field management and contributed to the project. As a senior researcher in Dr. Shi's lab, Dr. Bhattarai provided guidance and mentorship to a Ph.D. student (Ibtisam Alatawi) and two MS students (Teresha Phiri and Kenani Chiniwa) in completing their research projects and gained valuable experiences through their involvement in the lab's ongoing activities. The graduate students gained valuable experiences by participating in the lab's ongoing activities. All three graduate students were involved in field trial activities and data collection for this project to enhance the nitrogen use efficiency (NUE) and nutrient components. How have the results been disseminated to communities of interest? Educational events and conference presentations delivered include: Article: Formiga, A., V. Joshi, M. Colley. 2023. Challenges in the market for organic spinach seed. eOrganic article. Available at <https://eorganic.org/node/35758> ?ASHS conference: Oral presentation and a poster Exploiting the Natural Variation in Spinach Germplasm to Enhance Organic Productivity D Thompson, M Moreno, M Ramezani, A Shi, V Joshi - 2023 ASHS Annual Conference, 2023 Outreach: eOrganic resources: Guide to organic spinach seed production, video of field evaluations at Texas A&M, photo gallery of spinach seed production morphological stages. Field Days and Training: OSA Washington Farmer Field Day, Sep 11, 2023 Blogposts/ Media: Organic Seed Alliance, June 19, 2023: Spinach trial underway to inform organic spinach seed production. <https://seedalliance.org/2023/spinach-trial-launch/> What do you plan to do during the next reporting period to accomplish the goals? In year 2, the OSA research team will repeat the seed production trials in Chimacum, WA. Trial results from year 1 will be integrated into a multi-year data set. A webinar on organic seed production delivered through eOrganic will train farmers and seed companies and share preliminary project results. A project poster will be developed to present at agricultural conferences with the target of presenting at X events. Field evaluations of the USDA spinach accessions are underway at the Uvalde and Fayetteville farms. By conducting a second-year trial in both locations, we aim to address potential limitations and improve the value of the

phenotypic dataset generated by this project. The preliminary dataset generated from these evaluations will be combined with the dataset from the Uvalde trial and used for further analysis and reporting. The phenotype data collected from the multi-location and multi-year trials will be utilized in subsequent genomic analyses and identification of trait-associated markers, as well as improving genomic selection models and breeding and selection of high-yielding nutrient-denser improved cultivars fitting the organic production system. These genetic analyses will help us identify markers linked to specific traits and enhance our genomic selection models.

Impacts  
What was accomplished under these goals?  
Objective 1 - Enhancing the nitrogen use efficiency (NUE) of organic spinach  
Objective 1.1 Exploring the natural variation for NUE and associated traits. Around 300 USDA spinach germplasm panels and four check varieties were planted at the Organic Farm in Uvalde, TX (Dec 2022) and Fayetteville, AK farm (Feb, 2023) in an augmented design. Uvalde Farm: 277 Spinach accessions from the USDA National Germplasm Repository and 4 commercial checks. 30 plants of each accession were grown in 2 rows in a 4 ft plot with a plant-to-plant distance of 2.5 inches following augmented design with repeated checks, namely, Acadia F1, Corvair F1, Space F1, Tundra F1 at locations (Uvalde, TX) in 2022-23. Chlorophyll content (MC-100 chlorophyll concentration meter; Apogee), stomatal conductance (gsw), photosynthetic electron transport rate (ETR), and PSII actual photochemical quantum yield (PhiPS2) using LI-600 Porometer/Fluorometer. Individual per plant basis fresh and dry-biomass were recorded at physiological maturity by pooling 3 plants per accession. The data analysis confirmed a normal distribution for all the traits relevant to organic productivity in the selected germplasm. Several significantly associated markers and candidate genes were identified for the selected traits. SNP markers will facilitate the breeding of organic spinach varieties with enhanced productivity. Fayetteville farm: The four check varieties were randomly replicated twice within each block. Each block consisted of 22 plots, and approximately 20 seeds of each accession were planted in each plot to ensure around 10 plants per accession for phenotyping. The trial was planted later than usual, in late February of this year, due to poor weather conditions, and the project will start later in 2022. Typically, our trials are conducted in late September or early October, but we had to adjust our schedule this year due to heavy snowfall in January and February. The campus facilities were closed during this time, and the fields were covered in snow. To obtain soil nutrient parameters, we collected samples from the field before planting at 30-60 cm depth. The samples will be examined for factors such as total nitrogen (N), free nitrates, and other macro- and micronutrients. We used MultispeQ (<https://www.photosynq.com/>) to measure the relative chlorophyll content of all plants by estimating chlorophyll fluorescence parameters. All plants were harvested at physiological maturity to collect fresh and dry biomass to understand spinach's nitrogen use efficiency under an organic production system. However, we did not evaluate the nutrient content composition, which we plan to perform during our second trial performed in Fall 2023, detailed below. Pre-sowing soil analysis was performed to evaluate the nutrient content of the soil. Around 300 USDA spinach germplasm panels were planted at the Fayetteville farm in October 2023, along with four check varieties replicated twice in each block in an augmented design. The plants are growing well so far, and we plan to measure the chlorophyll content of all plants and the fresh and dry weight biomass. We will homogenize the dried samples of each accession to estimate the total Kjeldahl N (TKN) concentrations using the Kjeldahl method and the levels of free nitrates (NO<sub>3</sub>, NH<sub>4</sub>, total N) at PD-Joshi's lab. The mineral contents of 14 elements will be determined, including Ca, Fe, Zn, Mg, Mn, N, P, K, Cu, I, Se, Cr, Cl, and Mo. Further, targeted metabolome profiling will be performed to estimate the nutraceutical traits for free amino acids, oxalic acids, Vitamin C/ Ascorbic acid, Vitamin A (Retinol), and Vitamin E (alpha-tocopherol).

Objective 1.2 Genome-wide association study (GWAS) and genomic prediction (GP) of NUE and associated traits in organic systems Using the traits collected from the accessions screen at Uvalde, TX, principal component analysis (PCA) and genetic diversity were analyzed with GAPIT 3 (Wang and Zhang, 2021) by setting PCA = 2 to 10 and NJ tree = 2 to 10. Phylogenetic trees were drawn by using the neighbor-joining (NJ) method. Variation in the spinach population was apparent for all the measured traits, which showed normal distributions. 88,288 SNPs distributed on 6 chromosomes were used to perform GWAS for organic productivity and photosynthetic traits after filtering and keeping MAF > 2%, Missing < 9%, and Het < 15%. Significant SNPs and candidate genes identified under the organic production system for the selected traits would help unlock the resource use efficiency and develop spinach varieties adapted to organic production.

Objective 3 - Enhancing the seed production and availability of organic spinach: At the Organic Seed Alliance Research farm in Chimacum, WA, researchers evaluated a single replicate trial with 3 repeated check cultivars of more than 200 accessions from the Germplasm Resources Information Network (GRIN) collection for phenotypic traits related to seed production. Evaluation of each plot for included assessment of the timing of bolting, the ratio of pollen and seed-producing plant types, and per plot seed yield. All plots were evaluated for field traits in Yr 1, are currently dried, and will be processed for seed yield and quality in the winter of 2023. Seed types varied across accessions with pointy and round seed shapes. Photo documentation captured overall plant stature per plot and seed samples from each accession collected. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Exploiting the Natural Variation in Spinach Germplasm to Enhance Organic Productivity. D Thompson, M Moreno, M Ramezani, A Shi, V Joshi - 2023 ASHS Annual Conference, 2023 Type: Websites Status: Published Year Published: 2023 Citation: Formiga, A., V. Joshi, M. Colley. 2023. Challenges in the

market for organic spinach seed. eOrganic article. Available at <https://eorganic.org/node/35758> Progress 09/01/22 to 08/31/23 Outputs Target Audience:Field day conducted at AgriLife Research and Extension Center in Uvalde May 11, 2023 attended by over 60 participants representing growers, processors, shippers, packers and general public interested in organic production. Changes/Problems:Finding a suitable post-doctoral candidate has been difficult. Further, the candidate hiring was substantially delayed due to visa processing delays. The new hire is likely to join Joshi Lab in mid-July 2023. We anticipate the delay won't slow down the project plan but if needed, we may request a no-cost extension at the end of 3rd year. Weather problems delayed planting at the Arkansas location. We are re-sowing the experiments in order to generate quality data. The sowing will be performed again in the fall of 2023. What opportunities for training and professional development has the project provided?University of Arkansas: Dr. Gehendra Bhattarai, a postdoctoral researcher at the University of Arkansas, led this project. Under the supervision of Dr. Ainong Shi (Co-PD) and Dr. Vijay Joshi (PD), Dr. Bhattarai played a crucial role in executing project tasks. Dr. Haizheng Xiong, a research associate in Dr. Shi's lab, was responsible for field management and contributed to the project. As a senior researcher in Dr. Shi's lab, Dr. Bhattarai provided guidance and mentorship to a Ph.D. student (Ibtisam Alatawi) and two MS students (Terasha Phiri and Kenani Chiniwa) in completing their research projects and gained valuable experiences through their involvement in the lab's ongoing activities. The graduate students gained practical experience by participating in the lab's ongoing activities. They helped in field trial activities and data collection for this project to enhance spinach's nitrogen use efficiency (NUE) and nutrient components. Texas A&M AgriLife Research: A Technician, Dalton Thompson, post-doc Mazi Ramazani, and an undergraduate student, Matte Moreno, were trained on several project-related activities research activities. Two new students (MS and Ph.D.) will join Joshi's lab later in the year, participate in the ongoing research activities, and be trained on organic production practices at the AgriLife Research Center. A field day organized at the AgriLife Research Center (May 2023) allowed producers and the general public to learn about the project. As a part of the 4-H STEM club organized by PD Joshi, students will be allowed to get familiar with project activities and learn about organic production and practices. Organic Seed Alliance (OSA): OSA staff holds skills and experience in organic spinach seed production, including authorship of the Guide - Principles and Practices of Organic Spinach Seed Production by Program Director Micaela Colley. OSA's research farm manager, Kayla Ierlan, and field research assistant, Johanna Willingham, bring spinach seed production experience but are gaining professional development in the project through knowledge exchange with project partners and respective research on spinach nutrition and pathology as well as first-hand knowledge of the diversity of spinach phenotypes from GRIN. OSA's annual interns will benefit from learning seed production practices and the research methodology. How have the results been disseminated to communities of interest?Project outreach will continue in year two with annual field days showcasing the project and online communications through eOrganic, the OSA website, and social media. In year 2, a webinar on organic. Spinach seed production will advance seed growers' knowledge of production practices, including horticultural management, isolation, disease and pest management, and harvest and cleaning practices. This project will expand the number of organic producers with skills and knowledge in spinach seed production and seed companies' knowledge of relative seed yields of spinach varieties and breeding lines. The long-term impact will be to expand organic spinach seed availability. eOrganic will provide peer-and National Organic Program compliance review and editorial support for at least three publicly available articles by project members, which will be published on the eOrganic website and announced in our newsletter and social media. We will also conduct at least three webinars and evaluate them for quality and utility. In addition, eOrganic has been preparing a report on the organic spinach seed market for the project website. What do you plan to do during the next reporting period to accomplish the goals?Texas A&M AgriLife Research: Joshi lab will undertake heat stress evaluations and speed breeding research under controlled environments in 2023-24, as described in the work plan in the proposal. The hiring of a postdoctoral research associate has been significantly delayed due to visa approval and processing times. The position has been offered to a candidate expected to arrive at Joshi's lab beginning July 2023. The new hire will focus on objective 2, to characterize the role of the microbiome in nutrient uptake for sustainable spinach production. Samples will be collected to analyze selective rhizosphere (soil) and endospheric (root) microflora acclimatized to commercial organic spinach at multiple locations. The field evaluation of organic spinach will be performed in 2023-24 using the previously established processes (described before). University of Arkansas: Dr. Bhattarai (post-doc in Shi's lab) planted over 300 accessions from the USDA germplasm panel at the Fayetteville farm. Our objective is to evaluate these accessions for various important physiological traits. We will conduct the trial again next year to obtain additional data. The preliminary dataset generated from this evaluation will be combined with the dataset from the Uvalde trial and used for further analysis and reporting. Our plan for the next year is to plant our trial during the fall season. By conducting a second-year trial in both locations, we aim to address potential limitations and improve the value of the phenotypic dataset generated by this project. As a part of our project, we will use the phenotype data collected from the multi-location and multi-year trials to perform genomic analysis. This analysis will help us identify markers linked to specific traits and enhance our genomic selection models. This USDA NIFA Organic Research and Extension Initiative-funded research project will lay a foundation to develop spinach varieties for organic

producers and consumers with an improved nutrient acquisition, improve our understanding of how spinach leaf and root-associated microbiomes interact with organic fields dedicated to spinach production, and create a breeding model to develop varieties with higher organic seed productivity. Organic Seed Alliance (OSA). OSA will continue to replicate the seed yield and quality trial annually. We will refine horticultural and evaluation protocols based on year one outcomes, though no major modifications are anticipated. Entries were all sourced from GRIN with enough seed for multi-year trials. Entries not germinating in the first year will be dropped in year 2. Impacts

What was accomplished under these goals? Objective 1: Enhancing the nitrogen use efficiency of organic spinach: Recognizing suitable genetics with higher nitrogen use efficiencies for low-input organic spinach production. Seeds of 278 diverse spinach genotypes consisting of obtained from the USDA Germplasm repository (GRIN) were used for field evaluation at the certified organic field at the Texas A&M AgriLife Research and Extension Center in Uvalde on Nov, 2022. Pre-sowing soil analysis was performed to evaluate the organic matter, total nitrogen, and other micro-elemental analyses. Each genotype/accession was grown in a field with 18 rows (each 230 ft), each with 42 sub-plots. Each sub-plot (4 ft) had two rows accommodating 30 plants. Each check within a row was randomized in the augmented design using four checks (Acadia F1 OG, Corviar F1 OG, Space F1 OG, and Tundra F1 OG). Additionally, six commercial varieties (Regiment F1, Renegade F1, Minkar, Nembus, Kiowa, Frontier) were also included in the design. The pests and diseases were controlled manually and OMRI-approved products. At physiological maturity, plants were harvested to collect biomass, chlorophyll content, stomatal conductance (gsw), photosynthetic electron transport rate (ETR), and PSII actual photochemical quantum yield (PhiPS2). Variation in the spinach population was apparent for all the measured traits, which showed nearly perfect normal distributions. Around 300 USDA spinach germplasm panels and four check varieties were planted at the Fayetteville, AK farm in an augmented design across 14 blocks. Within each block, the four check varieties were randomly replicated twice. Each block consisted of 22 plots, and approximately 20 seeds of each accession were planted in each plot to ensure around ten plants per genotype for subsequent data measurement. Our trial was planned later than usual, in late February of this year, due to poor weather conditions and the project starting later in 2022. We used MultispeQ (<https://www.photosynq.com/>) to measure the relative chlorophyll content of all plants by estimating chlorophyll fluorescence parameters. The measurements for fresh weight and dry weight are ongoing. Objective 3. Enhancing seed production and availability of organic spinach: Assessing selected spinach germplasm for enhanced seed production potential using seed traits through genome wide-association analysis and genomic prediction. Organic Seed Alliance (OSA) (Micaela Colley's Lab): The first project year focused on establishing project protocols, communications, and partnerships and launching the first year of field research. Project outreach included establishing a project website with eOrganic, distributing a project press release, and a blog post in Organic Seed Alliance's (OSA) summer newsletter. At OSA, the field trials of 270 varieties were planted in the greenhouse in mid-March and transplanted into the field in late April. Accessions varied in bolting response and seed morphology. Of all seed, accessions documented the varied seed phenotypes. Trial protocols are in place to evaluate the timing of flowering, the ratio of pollen and seed-producing plants in the populations, the timing of seed maturation/ harvest, and seed yield (total weight and 1000 seed weights). Plans are underway to feature the project in a fall field day at the OSA research farm in Chimacum, WA. Objective 4: Developing an outreach plan to provide relevant information to stakeholders in the organic spinach industry: Establishing a platform in partnership with eOrganic and Organic Seed Alliance to share project progress and outputs with stakeholders. eOrganic (Alice Formiga Lab, Oregon State University) created a website for this project which describes the objectives and collaborators, photos of trials and the different types of spinach, and project publications. Additional resources and webinar recordings will be added to this website as the project progresses. The website is available at <https://eorganic.org/spinach>. Publications Type: Conference Papers and Presentations Status: Submitted Year Published: 2023 Citation: "Exploiting the Natural Variation in Spinach Germplasm to Enhance Organic Productivity," for the 2023 Annual Conference of the American Society for Horticultural Science (ASHS), has been accepted for a POSTER presentation. <https://ashs.confex.com/ashs/2023/meetingapp.cgi/Paper/40044>

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# Genomically Optimized Organic Dairy (good): Genome Selection Against Uterine Diseases to Improve Fertility and Longevity in Cattle

Accession No.	1029005
Project No.	WNP00899
Agency	NIFA WN.PI
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2022-51300-38058
Proposal No.	2022-04085
Start Date	01 SEP 2022
Term Date	31 AUG 2026
Grant Amount	\$1,500,000
Grant Year	2022
Investigator(s)	Jiang, Z.; Maquivar, MA, .
Performing Institution	WASHINGTON STATE UNIVERSITY, 240 FRENCH ADMINISTRATION BLDG, PULLMAN, WASHINGTON 99164-0001

## NON-TECHNICAL SUMMARY

The prohibited use of antibiotics on organic dairy cattle challenges farm biosecurity and disease prevention, for example, resulting in high incidence of metritis and endometritis, low fertility and high culling rate. Here we propose to use genomic sciences and biotechnologies to help organic dairies systematically overcome these challenges and thus enhance market competition, technology modernization and operational sustainability. Therefore, it is imperative for us to establish, develop and maintain a nationally and internationally recognized GOOD (genomically optimized organic dairy) program and thus advance, promote and sustain global GOOD systems for generations to come. We will collaborate with thirty organic dairy farms for collection of blood, uterine swabs and endometrial cell samples to measure DNA variants, RNA dynamics and phenotypic variations. A novel genome wide association study approach will be established to examine allele frequency flows at the genome level, gene networks at the transcriptome level, and uterine disease progression at the phenome level. We will reach out to our advisory board members, organic industry advocates and broad communities for advice, help and support to strengthen our organic dairy program at Washington State University. Additionally, we will help the organic dairy farms participating in the project establish their capability in isolation of DNA for genotyping, extraction of RNA for diagnosis of uterine disease and determination of pathogens for cure strategy. We will present our innovation and discoveries at scientific conferences and freely release the laboratory protocols, training procedures and educational programs developed in the proposed research. We believe that our GOOD program represents a unique strategy to help organic dairy production in the United States of America to realize the projection of revenue increase to \$18.9 billion annually by the year 2026. Promotion of organic dairy and organic animal production will benefit the environment, animal well-being and human health. Our GOOD program will prepare next generation scientists and technical personnel to move organic dairy farming to the next level.

## OBJECTIVES

Many American consumers perceive that organic products are friendlier to the environment, animal well-being and human health. The prohibited use of antibiotics on cattle, however, challenges farm biosecurity and disease

prevention. For example, lack of antibiotic treatments makes clinical endometritis and puerperal metritis among the most prevalent reproductive disorders in organic dairies. The overall goal of this project is, therefore, to develop a GOOD (genomically optimized organic dairy) program and use genome sciences and biotechnology to overcome these challenges. Briefly, our GOOD initiative is based on a new USDA blueprint for Animal Genome Research - "Genome to phenome (G2P): improving animal health, production and well-being." Specific Objective #1: Develop a G2P bridge mapping approach as our research core to advance the GOOD program. We will integrate DNA/RNA variants and performance variation information into a multi-omics pipeline and develop a G2P bridge mapping method to identify cows that are resistant to reproductive diseases, such as metritis and endometritis. Specific Objective #2: Develop a G2P focused education/extension program as our dissemination core to promote the GOOD program. We will add G2P sciences to the current organic programs for improved education and extension, thus adding tools and educating the next generation of dairy farmers to advance organic dairies world-wide using novel knowledge and technology. Overall, we will use various platforms to disseminate our GOOD progress as quickly as possible. Locally, we will call advisory board members, stakeholders and students to work together on development of training programs and presentation materials. Regionally, we will support organic dairies and train them to use genomic tools for maximized herd efficiency. Nationally, we will organize annual workshops to promote our GOOD program. Globally, we will hold a GOOD conference to disseminate our results worldwide.

## APPROACH

For Specific Objective 1, we will collaborate with Aurora Organic Dairy and Clover Sonoma to develop our G2P bridge mapping approach. As both companies own a total of 30 organic dairy farms, we will be able to collect samples from small-, medium- and large-scale organic dairies. In particular, all these companies possess Jerseys, Holsteins and their crossbreds. For phenome information, we will collect physiological, pathological, reproductive and productive phenotypes. Transcriptome profiling will involve endometrial cells for construction of whole transcriptome termini site sequencing libraries to capture alternative polyadenylation events. The blood samples collected from cows will be shipped to the Neogen Genomics (GeneSeek) service laboratory for DNA extraction and then 1x Bovine SkimSeek sequencing. With these datasets, we will compare two methods: G2P bridgeless and bridge mapping of quantitative traits loci for both metritis or endometritis subjective scores. No doubt, our findings will be important to all organic and potentially conventional dairy systems. Therefore, we will seek help and support from the Council on Dairy Cattle Breeding (CDCB, <https://www.uscdcb.com/>). Hopefully our G2P assay would be integrated in their genetic and genomic evaluation programs. For Specific Objective 2, We will establish a university-industry partnership model to jointly develop an applied G2P concept with a "knowledge-in/technology-out" strategy for mutual benefits. Our industry team will bring a broad phenome program from farms to the classrooms by 1) giving guest lectures in classes to expose our students to the strengths and challenges of organic dairies in comparison to conventional production and 2) guiding our undergraduate research in developing research priorities and strategies to address emerging phenotypic issues facing organic dairies. On the other hand, our university team will transfer transcriptome and genome techniques from laboratory to farms to monitor uterine disease severity and use genotypes for selection of cows resistant to metritis and endometritis. At the end, advisory board members, industry personnel and university researchers will work together to organize workshops, symposiums or seminars to disseminate information to organic dairy producers; edit a special issue in a scientific journal and present our results at various conferences to further promote our GOOD program. Efforts. We will provide students in either classes or clubs with hands-on training and experiential opportunities in the organic dairy industry, collecting phenotypic traits data and allowing them to learn about real-world scenarios to solve complex situations with emphasis on organic animal and veterinary medicine production. We will present our work in the seminar series in the Animal Sciences Department and at the Center for Reproductive Biology. We will use different social media to disseminate our work to a broad audience. In addition, we will organize workshops on the GOOD program. We may also edit special issues in peer-reviewed journals. Evaluation. For genome variants, each will have a unique ID number, chromosome, genome coordinates, wild type allele sequence(s) (based on the reference genome), mutated allele sequence(s), gene symbol, gene biotypes (at least five types), genome region classification, variant types (single nucleotide polymorphism or insertion/deletions), allele frequencies per locus and genotype per individual. For APA events, each site will have a unique ID number, chromosome, sense/antisense strand, genome coordinates, gene symbol, gene biotypes, transcript ID, genome locations, 3'-end genomic features, polyA signals and the raw/normalized counts for each sample. For trait information, we will estimate repeatability among different times and test time points to improve accuracy. For the student-centered learning outcomes, we will evaluate if participating students will be able to: 1) identify and discuss in a meaningful way issues related to organic dairy production systems; 2) describe and integrate different concepts (e.g., nutrition, reproduction, genetics, personnel, etc.) relevant to the organic production system; 3) critically assess the dairy production systems using records to promote and improve the overall performance and profitability of the farm without neglecting animal

welfare and the environment; 4) engage in problem solving using a teamwork approach with stakeholders, dairy farmers, professionals, workers, and managers and 5) potentially identify job opportunities to develop a technical or professional career within the dairy industry. Progress 09/01/23 to 08/31/24 Outputs Target Audience: Genome research, education and service community; Animal breeding and genetics companies; Organic and conventional dairy producers, processors and retailers; Veterinarians. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? The award has provided training opportunities for two PhD students, one Master's degree student and five undergraduate students. The training includes laboratory and farm safety, animal care and use, DNA and RNA extraction, WTTs-seq library construction, and sample/data collection, analysis and storage, for example. How have the results been disseminated to communities of interest? Results have been regularly disseminated to the USDA/NIFA National Program Leaders, the USDA National Organic Program funded project directors and/or participants, the US bovine FAANG group members, WSU Animal Biology and Biomedicine group members and graduate and undergraduate students who take the Perspectives in Biotechnology class at Washington State University in Fall semesters. What do you plan to do during the next reporting period to accomplish the goals? We will continue our collection of samples from Aurora Organic Dairy in Colorado, Clover Sonoma in California and potential expansion to other states and accumulation of our datasets in genome, transcriptome, metabolome, microbiome and phenome. We will work on data analysis and prepare manuscripts for publication. We will report to our advisory board members and national program leaders and solicit their advice for further improvement of the study design, public engagement and education/extension activities. Impacts What was accomplished under these goals? In year 2 of the funded research, we focused on collecting both endometrial cells and blood from organic dairy cows at  $7 \pm 2$  days post-partum and again at  $21 \pm 2$  days post-partum. The sampling involved both Holstein and Holstein x Jersey cows at two time periods: cool season (November - December) and warm season (May - July). In addition, we collected health-related phenotypes from each cow at sampling by measuring 1) body temperature; 2) mucus scores (uterine discharge score: 0 - clear, 1 - flakes of purulent exudate, 2 -  $>50\%$  purulent exudate and 3 - hemorrhagic discharge mixed with purulent exudate), 3) reproductive ultrasound measurements (uterine, right ovary, left ovary and cervix) and 4) body condition scores. Because we tried to pair healthy-sick cows for sampling on the same day, our datasets cannot be used to examine seasonal effects on metritis and endometritis in organic dairies. However, cows sampled in the cool season had higher body condition scores (2.75 vs. 2.22, respectively;  $P < 0.0001$ ), but lower body temperatures ( $101.1$  vs  $101.7$ °F, respectively;  $P < 0.01$ ) compared to those sampled in the warm season. Uterine swabs were sent to Washington Animal Disease Diagnosis Laboratory for identification of pathogens, such as mixed bacteria, *Escherichia coli* and *Trueperella pyogenes*. Interestingly, the pathogen profiles are hard to distinguish between healthy and unhealthy cows. A method for isolation of both RNA and DNA from the same sample was successfully established. An initial set of DNA samples were sent to GeneSeek for low genome coverage sequencing for genotyping. RNA isolation and construction of WTTs-seq (whole transcriptome termini site sequencing) libraries are underway. As planned, Dr. Maquivar prepared "Organic Animal Production" training materials to educate the public, college students and extension specialists, for example. The current topics include: 1) Introduction to Organic Animal Production, 2) National Organic Program; 3) Nutritional management in Organic Systems, 4) Animal health management in organic animal production systems; 5) Animal welfare assessment in Organic Animal Production systems, 6) Organic Animal Production systems and Economics, 7) Organic food marketing, 8) Organic dairy production, 9) Organic meat production, 10) Organic poultry production and 11) Other organic animal products. In collaboration with USAID Farmer to Farmer Program, Dr. Maquivar developed a training program to dairy producers in the south region of Madagascar in the town of Toliara. The program included 1) Dairy cow husbandry best practices, 2) Assessment of health and disease prevention of dairy cows (including administering veterinary medicines and detection and treatment of metabolic, infectious and reproductive diseases), 3) Production of silage and hay and utilization of forage resources, 4) Reproductive management and 5) Appropriate equipment for milking and milk transportation. As a result, producers got a training certificate in basic dairy cattle management and they continue to improve the management practices. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Maquivar M. Genetic and clinical aspects of uterine diseases in dairy cattle. Instituto Tecnológico de Costa Rica. Santa Clara, Alajuela, Costa Rica. Curso internacional de reproducción bovina. October 2023. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Maquivar M. 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breeder cows. National Autonomous University of Mexico. School of Veterinary Medicine. Mexico City, Mexico. November 2024 Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Stotts MJ, Zhang Y, Michal JJ, Velez J, Hans B, Maquivar M, Jiang Z. Genomically Optimized Organic Dairy (GOOD): Genome Selection against Uterine Diseases to Improve Fertility and Longevity in Cattle. USDA National Institute of Food and Agriculture 2024 Organic Agriculture Annual Project Director Meeting. 04/24 04/25, 2024 Orlando, Florida. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Jiang Z. Invited Plenary Speaker. Research Frontiers in Animal Phenomics. International Conference on Genetics and Genomics Research Fronts in Animals and Poultry, December 9 10, 2023. Shandong Agricultural University, China. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Jiang Z. Invited Seminar. Trust your gut or trust your RNA? Department of Animal Science, University of Maryland. 10/18/2024. Baltimore, MD. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Jiang Z. Guest Speaker. Want to be healthy? Look to RNA for solutions! Center for Birth Defects Research, University of Maryland School of Medicine. 10/17/2024. Baltimore, MD. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Jiang Z. Invited Seminar. You do what RNA does. Biotechnology Faculty, University of Ljubljana. 06/24/2024. Ljubljana, Slovenia. Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Jiang Z. Invited Seminar. Go abroad and learn more broadly. Animal Science Spring Seminar Series. February 7, 2024. Washington State University. 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Cannabis Sativa targets mediobasal hypothalamic neurons to stimulate appetite. *Sci Rep*. 2023;13(1):22970. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Maquivar M. NCBA - USRSB (U.S Roundtable For Sustainable Beef) General Assembly Meeting Collaborating for Continuous Improvement May 2023 Boise, Idaho Title of the talk: Impact Of Hispanic Employees On The Sustainability Of Livestock Production Systems In The United States Of America: BQA example. Progress 09/01/22 to 08/31/23 Outputs Target Audience: Genome research, education and service community; Animal breeding and genetics companies; Organic and conventional dairy producers, processors and retailers; Veterinarians. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? The award has provided training opportunities for one PhD student, one visiting PhD student, one Master's degree student and three undergraduate students. The training includes laboratory safety, animal care and use, RNA extraction, APA profiling library construction, data analysis and SOP training, for example. How have the results been disseminated to communities of interest? Results have been regularly disseminated to the US bovine FAANG group members, WSU Animal Biology and Biomedicine group members and graduate and undergraduate students who take the Perspectives in Biotechnology class at Washington State University. The information was also shared with broad international communities through a selected talk at the Ruminant Genetics Workshop, 39th International Society for Animal Genetics Conference, Cape Town, South Africa and an invited talk at the Central Dogma of Phenomics Workshop, the American Society of Animal Science Annual meeting 2023, Albuquerque, NM. What do you plan to do during the next reporting period to accomplish the goals? We have a plan to collect samples from Aurora Organic Dairy in Colorado and Clover Sonoma in California and accumulate our datasets in genome, transcriptome, metabolome, microbiome and phenome. We will continue fine-tuning of new statistical models and novel pipelines for data analyses. We will develop cost-effective tools for our industry partners to pursue diagnosis of both metritis and endometritis on farm. We will organize a symposium on organic dairy and facilitate knowledge and technology transfer from research to application. Impacts What was accomplished under these goals? We started this funded research project by completing a preliminary study. We have strong evidence to show that alternative polyadenylation (APA) profiles can be used to generate an endometritis progression panel for exploring the bridges between genome and the disease. Especially, pathogens force the host to execute more up-regulated APA sites. In comparison to healthy cows, unhealthy cows tend to use more intronic, but less distal APA sites. Four genes: CD59 molecule, Fc fragment of IgG receptor IIa, lymphocyte antigen 75 and plasminogen may serve as initial contacts or combats with pathogens on

cell surfaces, followed by activation of nuclear receptor subfamily 1 group H member 4 to regulate AXL receptor tyrosine kinase, FGR proto-oncogene, Src family tyrosine kinase, HCK proto-oncogene, Src family tyrosine kinase and integrin subunit beta 2 for anti-inflammation. In addition, we also observed that microRNA genes: MIR21 and MIR30A might be a good pair of antagonistic biomarkers for diagnosis of either inflammation or anti-inflammation in the uterus. During this transition stage from a small-scale study to large-scale research, we focused on development of our protocols, procedures and processes before heading to farms for sampling. Specifically, it is our priority to efficiently use samples collected from each animal and thus maximize data collection in genome, transcriptome and phenome to develop the bridge maps for both metritis and endometritis in organic cows. For each cow, we will have pedigree information, production records and reproduction performance plus ultrasonic measurements collected as phenome. Blood samples will be used to produce metabolome and genome datasets, while uterine samples are expected to have both microbiome and transcriptome profiles. The standard operation procedures (SOPs) have been drafted for collection of these datasets for the funded project. In fact, we have been designing a database to include all of these datasets plus sample management systems, including sample storage location, sample processing method and sample analyses results. We started to look at potential challenges associated with big data analysis. Currently, we use the principal component analysis (PCA) to build a disease progression panel. For our funded project, we will collect materials from 1,000 cows. As such, we see potential challenges to pursue PCA analysis on such a large dataset. Instead, we are exploring novel statistical models based on phylogenetic tree construction to overcome the challenge. A key component of transcriptome analysis is to enrich functional pathways. However, understanding the relationships among hundreds or thousands of pathways remains a challenging task. We are developing clustering approaches to make pathway classifications more meaningful to our funded research. In order to apply APA profiles in diagnosis of metritis and endometritis, we are looking into simplified approaches to do so and save costs for organic industries. Using our Department's newsletter, we have released our funded project information to the public as one of our outreach activities. Basically, our GOOD (genomically optimized organic dairy) project will engage an advisory panel of scientists, industry partners and organic dairy producers to understand gene networks underlying uterine disease and thus develop novel tools to identify cows that are resistant to both metritis and endometritis for genetic improvement. No doubt, we have been closely working with our project partners - Aurora Organic Dairy in Colorado and Clover Sonoma in California to develop protocols, procedures and processes for collection of genome, metabolome, microbiome, transcriptome and phenome datasets. We are ready now to start the large-scale sampling process. Publications Type: Journal Articles Status: Published Year Published: 2023 Citation: Stotts MJ, Zhang YZ, Zhang SW, Michal JJ, Velez JS, Hans B, Maquivar M, Jiang Z. 2023. Alternative polyadenylation events in epithelial cells sense endometritis progression in dairy cows. *J. Integr. Agric.* 2023;22(6):18201832. Type: Journal Articles Status: Published Year Published: 2023 Citation: Wu XL, Ding X, Zhao Y, Miles AM, Brito LF, Heringstad B, Zhao S, Jiang Z. Editorial: Lactation genomics and phenomics in farm animals: Where are we at? *Front Genet.* 2023;14:1173595. Type: Journal Articles Status: Published Year Published: 2023 Citation: Carrion SA, Michal JJ, Jiang Z. Alternative Transcripts diversify Genome Function for Phenome Relevance to Health and Diseases. *Genes.* 2023;14(11):2051. Type: Journal Articles Status: Published Year Published: 2023 Citation: Carrion SA, Michal JJ, Jiang Z. Imprinted genes, genomics conservation, transcriptomic dynamics and phenomic significance in health and diseases. *Int J Biol Sci.* 2023;19(10):3128-3142. Type: Journal Articles Status: Published Year Published: 2023 Citation: Mikec V, Horvat S, Wang H, Michal J, Kunej T, Jiang Z. Differential alternative polyadenylation response to high-fat diet between polygenic obese and healthy lean mice. *Biochem Biophys Res Commun.* 2023;666:83-91. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Jiang Z. Invited Seminar. Genome Science and Healthy Longevity. Annual Advanced Science Seminar Series, Rugao Health Council. 04/18/2023. Rugao City, China. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Jiang Z. Invited Seminar. The Central Dogma of Phenomics. College of Animal Sciences and Biotechnology, Nanjing Agricultural University. 04/23/2023. Nanjing, China. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Jiang Z. Selected Speaker. Functional Mapping of Alternative Polyadenylation in Cattle. Ruminant Genetics and Genomics Workshop, 39th International Society for Animal Genetics Conference. Cape Town, South Africa. July 2-7, 2023. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Jiang Z. Invited Seminar. Central dogma of phenomics:  $P = G + R + E + G \times R \times E$ ? Workshop on the central dogma of phenomics. 2023 American Society of Animal Science Annual Meeting. July 16 July 20, 2023. Albuquerque, NM, USA Type: Conference Papers and Presentations Status: Accepted Year Published: 2023 Citation: Jiang Z. Invited Plenary Speaker. Research Frontiers in Animal Phenomics. International Conference on Genetics and Genomics Research Fronts in Animals and Poultry, December 9 10, 2023. Shandong Agricultural University, China Type: Other Status: Published Year Published: 2023 Citation: Michal JJ. GOOD: Genetically optimized organic dairy. Animal Sciences Department News Letter. <https://ansci.wsu.edu/2023/05/04/good-genetically-optimized-organic-dairy/> Type: Other Status: Published Year Published: 2023 Citation: Zaragoza A. 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## Corn/endophyte Partnerships for Organic Farmers.

<b>Accession No.</b>	1028979
<b>Project No.</b>	WISW-2022-04037
<b>Agency</b>	NIFA WISW\
<b>Project Type</b>	OTHER GRANTS
<b>Project Status</b>	NEW
<b>Contract / Grant No.</b>	2022-51300-38057
<b>Proposal No.</b>	2022-04037
<b>Start Date</b>	01 SEP 2022
<b>Term Date</b>	31 AUG 2025
<b>Grant Amount</b>	\$1,498,705
<b>Grant Year</b>	2022
<b>Investigator(s)</b>	Goldstein, W.; White, JA, F..
<b>Performing Institution</b>	MANDAAMIN INSTITUTE INC, 7194 MADAUS ST, LAKE GENEVA, WISCONSIN 531473616

### NON-TECHNICAL SUMMARY

This project builds on decades of research targeting a new type of corn that utilizes partnerships with seed-borne, bacterial endophytes to create environmentally-friendly, nutritious corn that is better adapted to organic farming. The project will develop organic breeding methods, evaluate economic benefits, do on-farm R&D, and breed non-commodity corn varieties suited for organic agriculture. It will engage organic farmers and companies, addressing their needs for: a) yield competitive hybrids with greater nutrient density (methionine and minerals), b) adapted inbreds for organic conditions (vigor, weed problems, nitrogen limitations), and c) reduced manure inputs and nitrate pollution. The work includes breeding, laboratory and on-farm research by relevant expertise. Outcomes will be vigorous inbreds and hybrids that foster endophyte partners. Such cultivars a) produce competitive yields while reducing fertilizer inputs and excess nitrate soil residues; b) increase nutritional density in grain and silage leading to higher feeding and sales value. Breeding improved field and sweet corn cultivars will be coupled with gaining new information on a) how composition of endophytic communities relates to their functional services of rhizophagy, nutrient uptake and nitrogen fixation; b) how these partnerships function in different inbreds/hybrids/environmental conditions and with different seed drying practices; c) how to test seed for benefits and durability of endophytic partnerships, d) how to optimize the use of soil quality tests, e) how to manage organic manures for optimal endophytic corn performance, soil quality, and environmental benefits, and f) the composition of grain for alternative diets and markets based on this non-commodity corn.

### OBJECTIVES

Our long-term goal is developing climate friendly, high quality, non-commodity corn that utilize partnerships with the microbiome to be more nutrient dense and better adapted to organic soil and weed conditions. Corn is the most grown organic row crop. Organic seed producers and farmers need our non-commodity, robust cultivars, which are, a) productive under organic conditions with weed competition and low available N; and b) provide higher nutritional value grain and thereby higher feeding and financial value. These traits are partially based on plant/microbial partnerships. Connections between corn breeds, soil and manure management and quality, seed borne endophyte communities, rhizophagy, N fixation, mineral uptake efficiency, nutritional value are important. Understanding them will help organic seed companies and farmers. The Mandaamin program has selected for plants that possess specific partnerships with seed-borne endophytes, however there are gaps in our knowledge.

Combinations of plants and microbes which partner in rhizophagy cycles produce somewhat differentiated results. Progress was made breeding such plant partnerships but it is not yet clear what the endophytes are, what they do in different parts of the plants, how stable their communities are from year to year, and how management affects them. It appears that the Mandaamin hybrids are obtaining N from microbial biomass and necromass, and not exclusively from nitrate and ammonium, but we do not know how much. The enhanced uptake of macro and micro nutrients and nitrogen appears paired. Fresh manure application to the Institute's C4-6-based hybrids decreases yield and nutrient uptake but increases those parameters for the commercial hybrid check or for Mandaamin's NG10-based hybrids. Furthermore, N<sub>2</sub> fixation (which lowered δ<sup>15</sup>N in grain) can be substantial but it appears to be specific to certain conditions and hybrids. Based on experience and the literature our key hypotheses are that: 1) development of optimal Mandaamin field and sweet corn hybrids can replace direct application of fresh manures, reduce soil nitrate residues, and increase root biomass relative to conventional hybrids; 2) Mandaamin hybrids can obtain N and minerals more effectively due to a healthier, more strongly branched rooting system in the topsoil, coupled with their consumption of whole microorganisms and the uptake of microbial protein/necromass; 3) fresh organic manures interfere with rhizophagy in C4-6 based hybrids due to bacterial interference with the seed-borne community; 4) soil organic N/soil protein buildup (with green manures, and animal manures) will enhance the productivity of the Mandaamin hybrids; 5) microbes living in foliar tissues of the Mandaamin hybrids, and especially in trichomes, can fix N<sub>2</sub>; 6) hybrids differ in their regulation of rhizophagy and N<sub>2</sub> fixation through how they interact with bacteria but these capacities can be optimized through selection; 7) environments, manuring and breeding/seed production practices can positively or negatively affect composition of the next generation of seed borne endophytes. In addition to these hypotheses, organic farmers and seed companies want to know how to manage endophytes in our hybrids. They wanted to understand how manure, direction of crossing when making hybrids, harvest date and seed drying date and conditions affect endophyte function. To achieve our objectives we will: Breed and select field and sweet corn varieties for endophytes and N efficiency/fixation. Characterize the cultivars for endophytes, agronomic value and nutritional density. Test hypotheses linking seed microbiota, soil and manure quality characteristics, plant/microbial partnerships, root growth and health, grain production and nutrient density. Characterize the populations of seed borne endophytes, their functional capacity, and their durability when plants are grown on different sites with and without manure. Develop understanding of and screening protocols for selecting for plant/endophyte partnership performance in different cultivars, including assessing rhizophagy and N<sub>2</sub> fixation. Address questions posed by farmers and seed companies about managing endophytes.

## APPROACH

Research methods. Towards developing practical screening methods, in WI seedling roots from breeding lines or hybrids will be grown in sterile media and assessed for microbial colonization of root cells and movement of bacteria from root hairs, and superoxide production by plants and nitric oxide production by endophytes. Seedlings grown in containers on N-limited soil media will also be assessed for chlorophyll content, and tissues will be assessed microscopically for microbial components and metabolites. Seedling assays will also test the effects of harvest timing and drying on rhizophagy and N efficiency. Relevant inbreds will be harvested at weekly intervals from before black layer to 15 % moisture in the field. Seed will be dried to approximately 12 and 7% moisture. Seedling roots from this seed will then be assessed for rhizophagy using sterile media. Seedlings will also be grown in N-limited media and foliar trichomes and chlorophyll scores will be examined. Effects of direction of crossing will also be tested. Hybrid seedlings should demonstrate to what degree the maternal phenotype of microbial partnership dominates. Field corn breeding. Diverse, relevant breeding families will be sequentially self-pollinated while under selection in summer nurseries in WI and winter nurseries. Hand pollination occurs in WI on approximately 2,000 rows selected from the best breeding families. 200 rows comprising mostly of later maturing breeding families or hybrids will be grown each summer in Central IL under organic conditions, inoculated for a palette of foliar disease, hand pollinated, and selected for disease resistance and agronomic traits. Early yield testing: In WI replicated yield trials on two sites for two years precede strip trials. Yield, grain moisture at harvest, and plant intactness at harvest (lodging) are criteria. Plot size is mostly 2 or 4 rows wide x approximately 20 feet long, with two or three replications per site. Hybrid testing begins at the S3 level of inbreeding. Local yield trials test up to 1,000 different hybrid combinations with one site considered to be low soil nutrient availability, and another medium or high. Feedback from yield trials determines selection. Successful inbreds will be bulked and released for further testing or direct commercialization after the S6 to S8 stage. Projected outputs are 4-8 finished hybrids for strip testing each year of the project based on performance. Mandaamin Institute will rent local organic fields to establish isolations for producing hybrid seed allowing sufficient seed for trials with cooperating farmers and seed companies. A clustered set of ten inbreds and ten hybrids will be planted in replicate 4 row plots on an organically managed, moderately N limited site in Wisconsin each year of the project across manured and not manured strips. The inbreds will be selected from N efficient families. Two conventionally bred, ex-PVP inbreds and the hybrid between them will be chosen as control treatments. These inbreds and hybrids will also be tested

in the other Mandaamin, Rutgers and Johnston-Monje trials. Seed from selected inbred lines will be evaluated for root hair, bacterial load, and N efficiency before planting. Soils samples will be analyzed by Cornell University's Soil Health Lab. Soil protein samples will be analyzed for  $\delta^{15}\text{N}$ . In September residual nitrate in the top two feet of soil will be determined. Roots, stover, and grain will be harvested at the end of the season, weighed, dried, and analyzed for N isotope ratios. The inbreds and hybrids will be scored for relative vigor, plant height, number of leaves, hairiness of leaf sheaths and leaves, flowering date, anthesis-silking interval, and grain yield and moisture. Root monoliths will be excavated and number of root tips and branches, root length and necrosis will be determined to estimate root length and health. Roots, stover (stalks, tassels, leaves), and grain will also be harvested, dried, and weighed at harvest to determine dry matter partitioning and mineral content, total N and mineral uptake and natural abundance estimates of  $\text{N}_2$  fixation. Grain samples will be evaluated using NIR spectroscopy to predict protein, oil, starch, density, and methionine, cysteine, and lysine content in whole grain. Ten samples each year will be analyzed for carotenoids using HPLC. Data will be gathered, variances and means will be calculated and compared using spreadsheets and JMP statistical programs. Models will be constructed to account for variation in crop performance associated with soil and pre-plant scoring parameters and microbial assays from Rutgers and Max Planck Institute. Research on plant/microbiome N-fixation partnerships and rhizophagy by Rutgers University and David Johnston-Monje. Seed of the same set of 20 inbreds and hybrids tested by the Mandaamin Institute, will be examined at both institutions. At Rutgers the nitrogen-transfer activity of endophytes in roots, stems, cob leaves, leaf sheaths (including trichomes and other non-photosynthetic cells) will be evaluated using light and confocal microscopy. Enriched  $^{15}\text{N}$  atmospheres will quantify N fixation and in which organs it occurs. Isolated bacteria (Monje lab) will be tagged with the mCherry gene, then inoculated in corn. Bacteria will be visualized within corn cells in roots, leaf sheaths, leaf blades, stems and husk leaves. Corn seedlings will be inoculated with isolated N-fixing bacterial endophytes--then assessed for  $^{15}\text{N}$  absorption in inoculated and non-inoculated plants to confirm that bacteria isolated were responsible for  $\text{N}_2$ -fixation. Rhizophagy, roots will be scored as mentioned above. Experiments will suppress plant rhizophagy and compare results with unsuppressed plants for nutrient absorption. David Monje will study seed borne, nitrogen fixing microbiomes and whether there are contrasting patterns as they colonize the organs and rhizosphere of the plant. In the first year, plants will be grown in sterile sand fertigated with N free solution. In the second year 6 inbreds will be grown on an agricultural soil as: a) seed from the same lot that was tested in Colombia the first year; b) seed that was grown from the same lot in Mandaamin Institute trials on a un-manured plot in WI; and c) seed from that same inbred grown on a manured plot side by side with b. Plants will be sequentially sampled to check for transmission of bacteria to the next generation. Harvested plants will be separated into rhizosphere, root, shoot, and seed. DNA will be isolated, the bacterial 16S will be amplified and sequenced to profile the taxonomy of endophyte bacteria. Cultured endophytes will be screened for N on N free media, Nif genes will be amplified used to identify bacteria. Candidate strains will be inoculated into sterile plants and evaluated for their ability to restore the N fixation trait. On-farm research. For varietal trials, 5 hybrids will be planted out in two or three replicated randomized blocks on each 4 farms. Variety x manuring trials will be carried out on 6 farm sites each year. The best- Mandaamin hybrid will be compared with a commercial hybrid. Farmers will grow these cultivars in strips plots across an organic manure treatment, with three replicates on each farm. Manuring practices may include with and without cover crops, timing of planting after green manures, type and placement of animal manure or compost in the crop rotation before growing corn. Mandaamin Institute will collect and analyze soil, plant, and yield samples as described in the attached SARE report. We will document specific practices on each farm, and work with them to describe and estimate their costs and net profitability. Soils, roots, stover, and grain from the project will gathered, and analyzed as mentioned above. Progress 09/01/22 to 08/31/23 Outputs Target Audience: Organic farmers, organic seed company employees, researchers concerned with endophytes and with corn, intern, staff of the Mandaamin Institute, graduate student. Changes/Problems: Feedback from farmers, seed companies, and scientists is that 1) we should focus more effort on proving whether or not nitrogen fixation is occurring in the Mandaamin plants and put less emphasis on other details of the research; 2) we should produce seed of our best inbreds and hybrids for larger scale testing. As described above, we took steps to bring our inbreds and produce hybrids to address the needs of on-farm testing. However, it did not prove possible to begin testing with farmers in 2023 due to the lack of hybrid seed. Our cooperating company, Foundation Direct Seed, sold out its seed stocks of our hybrids and there was little to work with. They also ceased producing organic seed. Thus we focussed on producing hybrid seed ourselves in 2023 for trials with farmers and manuring in 2024. Another obstacle was that David Johnston Monje, our microbiology cooperator, and a re-known World expert on corn endophytes, lost his position at Valley University in Colombia. We were able to move forward with him on an abbreviated plan and actions to identify microbial partners in 19 different inbreds and to start in analysis of nif genes. However, we had to curtail his participation in clarifying manuring effects. On the other hand, new microscopic and fatty acid data from last year's work suggest that the Mandaamin plants are fostering the accumulation of a much larger internal biome consisting in more gram negative bacteria, fungi, and protozoa; b) that considerable bacterial necromass is accumulating in specific tissues of the Mandaamin maize; and c) that isotope partitioning may be occurring in different tissues of the

maize. All this makes interpretation of natural abundance studies more complex. Dr. White will continue his studies next year with  $^{15}\text{N}$  enriched atmospheres. However, we are presently seeking alternative ways of satisfying our plan for analyzing microbial communities and nitrogen fixation with another microbiologist; possibly by quantifying nitrogenase production for Mandaamin and conventional varieties. What opportunities for training and professional development has the project provided? A technician, a student intern, and a graduate student learned about maize endophytes and about microscopy technique pertinent to examination of field and pot grown maize plants for examining endophytic relationships with bacteria. This included the use of histological stains. The new technician and the intern learned maize breeding philosophy and methods including selection, pollination, harvesting, seed treatment, learning to read phenotypes, etc. Two of the scientists on the project learned more about where and how endophytic colonization is occurring in different maize cultivars, including Mandaamin and conventional inbreds. Ward labs and those scientists learned about utilizing fatty acid analysis to quantify endophytes in maize leaves. How have the results been disseminated to communities of interest? Outreach: This first consisted in a field day at the Hughes Farm near Janesville in September showing corn grown organically without fertilization in two 20 acre blocks next to conventional corn. The Field day occurred in conjunction with Marbleseed and the University of Wisconsin organic advisory services and approximately 90 people, mostly farmers were present. The Mandaamin hybrid looked good and mineral analysis and chlorophyll measurements showed it had been more efficient at obtaining N than commercial hybrids grown in neighboring blocks. Consequently, the PD wrote an article on nitrogen fixing corn for the Organic Broadcaster which was subsequently published in ACRES USA, thereby reaching a wide spectrum of organic farmers. This was followed up by 1) a poster showing our results at the Marbleseed Conference in LaCrosse in February which was displayed at the conference in front of the Foundation Seed Direct booth in the commercial section; and 2) an interview with the PD which appeared in MadAgriculture, a magazine for sustainable farming on N efficiency and endophytes. Attendance at the Marbleseed conference allowed for interaction with farmers about endophyte containing corn and nitrogen. Discussions were held with feed and seed company personnel. The PD outlined the results of the breeding program with endophytes obtained so far in a publication by the Journal for Agriculture, Food Systems, and Community Development. The article is called: "The evolution of a partnership-based breeding program for organic corn." Other outreach during this period consisted in several scientific publications written by James White and Walter Goldstein which documented results with endophytes gained from research on the corn. The titles of these publications is documented in this report in preceding sections. The research covered endophytes found in our corn seedlings and their transfer to other cultivars as well as general implications for understanding host/endophyte relationships. What do you plan to do during the next reporting period to accomplish the goals? Progress was made breeding for plant partnerships, determining what the endophytes are, what they do in different parts of the plants. Results show that the Mandaamin inbreds are fostering microbial biomass and microscopy showed microbial necromass especially in convoluted cell possessing tissues in the epidermis and in seed bracts. We assume plants are generating N from microbial biomass and necromass, and not exclusively from soil provided nitrate and ammonium. We intend to focus on clarifying fixation and where it is occurring through  $^{15}\text{N}$  lab studies and studies of nif gene expression. We intend to learn more about how manuring affects microbial performance in plants. We are planning to conduct several studies in future experiments as follows. Mandaamin institute will continue breeding and testing activities on small plots to focus emphasis on the most commercially competitive, N efficient, nutritious cultivars as in the first year of work. Seed of the most promising inbreds and hybrids will be produced. In addition we will distribute seed of hybrids to cooperating organic farmers and work with them to test them with conventional checks on their farms. This will hopefully include some studies with and without application of animal manures. Effects of manuring will be quantified by yields and mineral uptake, and tested with microscopic and isotopic examination of tissues. The results of this work should help us to interface with companies and farmers to start associative testing efforts on a larger scale. Objectives of the White lab include: We will use isotopic nitrogen tracking to identify locations in plants where nitrogen is being fixed and accumulating. We will evaluate internal colonization of chloroplasts by endophytic bacteria and its role in the 'stay green' phenomenon through use of confocal microscopy. We will isolate specific microbes from the corn endophyte community and test the effects of individual microbes on replicating nitrogen fixation in plants--and on chloroplast durability and the 'stay green' effect. We will explore/document microbial effects on genetic variability of the corn genome. Endophytic bacteria within pollen mother cells secrete ethylene and nitrate. These microbe-produced substances are known to trigger chromosome replication in plants--and during replication transposons and other genetic elements may increase variation in the pollen genomes--resulting in greater genetic variability in the highly microbial corn selections. Impacts What was accomplished under these goals? Report from Walter Goldstein (Mandaamin Institute). 1) Characterizing endophytic colonization in cultivars: We studied colonization of seedlings by seed-borne endophytes by germinating seed in Petrie dishes, sectioning their tissues, and examining them with microscopy under supervision from Dr. White. In the seedling tissues bacteria appeared to be released from nuclei in excreted vesicles, which confirmed results published by Dr. White's team at Rutgers. In multiple cases we observed bacteria apparently penetrating into nuclear sheaths and exiting them. We developed a seedling test for

examining and measuring root hair length which was associated with microbial colonization/hormonal production. This included a survey of root hair production by numerous Mandaamin inbreds and conventional inbreds. Mandaamin inbreds (especially the most N efficient inbreds) consistently had more root hairs than conventional inbreds and repeated trials with Mandaamin inbred C4-6 showed they expanded the active diameter of roots by approximately one third. Microbial activity and diversity seemed most apparent intracellularly in root cap cells which break off from the root apex and produce inoculated mucoidal substances that lubricate root growth and create soil-root bonds. Bacteria were observed intracellularly, in periplasmic spaces, and streaming intracellularly and between cells. We surveyed numerous field-grown Mandaamin and conventional inbreds for colonization by microbes. Results were recorded in films and photographs. For Mandaamin inbreds, nitrate-secreting bacteria are living in vascular tissues, in the profuse hairs in our cultivars, in epidermal cells in leaves and husks, on the surfaces of chloroplasts, in seed bracts, and in reproductive tissues such as cob bracts, silks, pollen, and embryos. Trichomes, convoluted epidermal cells in epidermis, areas surrounding chloroplasts, and the seed bracts of the cultivars harbored colonies of bacteria that stained for nitrate production. Differences in colonization of chloroplasts between Mandaamin and conventional inbreds were especially profound. The most N efficient Mandaamin inbreds showed colonization of both bundle sheath and mesophyll chloroplasts. Different inbreds were rated for the percent of chloroplasts colonized by bacteria based on multiple films of leaf structure. Inbreds bred under conventional conditions generally had very low bacterial colonization while the most N efficient inbreds had scores between one third to three fourths of chloroplasts colonized.

2) Multiplying pertinent inbreds and producing hybrids: A new organically managed winter nursery was established in Chile with 660 rows. Inspection there showed that evolution and selection are causing convergent phenotypes in Mandaamin breeding families from widely different genetic backgrounds. Mandaamin inbreds tend to possess profuse root hairs and branched rooting systems in the topsoil, pilose leaves and leaf sheaths, and thick, folded and buckled leaves with high chlorophyll contents. Hybrid seed produced in Chile and in Wisconsin was grown out in replicated yield trials in the East Troy area on 9 acres of plots. Plot weediness was qualitatively rated. Hybrid production and seed multiplication took place on 2.5 acres on the Zinniker organic/biodynamic farm and 1 acre on the Goldstein organic farm. On these farms multiple hybrids were produced with two different N efficient/N fixing male testers. Furthermore, we advanced inbreds through hand pollination on a 2.5 acre nursery on the Zinniker farm. A set of N efficient Mandaamin inbreds and conventional inbreds from adjacent rows in that nursery were utilized for comparisons. Mandaamin inbreds had greater root and grain production, greater microbial biomass in leaves, and more tassel branching. Leaf and root samples were tested for microbial biomass in conjunction with Ward Labs. Root samples were frozen for further investigative work.

3) Testing new knowledge by selecting plants for N efficiency. We grew a set of N efficient inbreds on a field that had been cropped two years with corn without fertilizer. We visually selected plants for N efficiency based on new insights gained on linkages between plant phenotype and bacterial colonization. Young plants that were identified to have the N efficient/bacterial colonization phenotype were marked before flowering and later they were intermated. In the Fall all plants were measured and flowering date, chlorophyll content in leaves, and brace root scores were recorded. The plants that were visually chosen to be N efficient had earlier flowering dates, higher chlorophyll scores in September, and greater brace root scores than the other plants.

Report from David Johnston Monje on identifying endophytes. Nineteen N efficient maize inbreds were grown to seedlings in pots. 256 bacterial endophytes were extracted from leaves and roots of plants that were grown in sterile soil. DNA was extracted from shoot and root tissues of 63 plants grown on sterile soil, plus 63 plants grown on normal soil. DNA was extracted from 21 sterilely grown root tips and from another 21 root epidermal peels. Subsequently 16S rDNA was amplified from all the bacterial stains and will soon be sent in for sequencing so that we can know the taxonomy of the isolates. The isolates are being tested for nifH with PCR on the isolates. Testing the primers on the first 96 strains, got 0 hits, so we are working to optimize the reaction to work better (a positive control did work). NifH PCRs will be run on all the bacterial isolates once the PCR reaction is optimized; 16S and nifH PCRs will be run on all the DNA extracts, and the resulting samples will be sent for sequence analysis.

Report from James White (Rutgers) on differences between N efficient and conventional inbreds: Leaf epidermal cells and trichomes, as well as root hairs were larger in highly microbial colonized corn cultivars (e.g., Mandaamin selection C4-6), compared to commercial cultivars. This increased cell growth was attributed to hormonal effects of endophytic bacterium-produced ethylene and nitrate within plant cells that serve as plant growth stimulants. We determined that C4-6 showed abundant bacteria within leaves, and that these bacteria were evident in the vascular bundle sheath cells around and within the bundle sheath chloroplasts. Chloroplasts in C4-6 tended to remain green and functioned better under conditions of low nitrogen application compared to commercial cultivars. In commercial cultivars chloroplasts tended to degrade in older leaves under low nitrogen conditions resulting in chlorosis in older leaves as nutrients are removed from older leaves to fuel growth in younger leaves. Preservation of chloroplasts in C4-6 resulted in more consistent photosynthesis. Through collaboration with Ward Labs, Inc, fatty acid analysis of Mandaamin and commercial cultivars showed that microbial-rich Mandaamin inbreds contained significantly more bacterial biomass and it was predominantly gram-negative. We developed methods to move endophytic bacteria from highly microbial plants into plants lacking microbes. Leaves were triturated to extract endophytic microbes.

Seedlings germinating in the microbial extracts absorbed the bacteria into their tissues as they germinated. We demonstrated that bacteria from C4-6 were capable of nitrogen-fixation in the test seedlings of lettuce, sorghum and creeping bluegrass based on use of histochemical stains to detect nitrate in seedling tissues. Transference of the endophytic bacteria from C4-6 into creeping bluegrass (*Poa reptans*) resulted in the 'stay green' feature seen in C4-6 and it was also associated with bacterial colonization of chloroplasts in leaves. We hypothesize that nitrogen delivery to the chloroplasts by endophytic bacteria protracts survival of functioning chloroplasts.

Publications Type: Journal Articles Status: Published Year Published: 2023 Citation: X. Chang, B. Young, N. Vaccaro, R. Strickland, W. Goldstein, L. Struwe, & J.F. White. 2023. Endophyte symbiosis: evolutionary development, and impacts of plant agriculture. *Grass Research* 3, Article number: 18 (2023).  
<https://www.maxapress.com/article/doi/10.48130/GR-2023-0018>. Type: Journal Articles Status: Published Year Published: 2023 Citation: Goldstein, W.A. The evolution of a partnership-based breeding program for organic corn. 2023. Refereed journal article accepted for fall publication in the *Journal of Agriculture, Food Systems, and Community Development*. <https://doi.org/10.5304/jafscd.2023.131.011> Type: Book Chapters Status: Awaiting Publication Year Published: 2023 Citation: J.F. White and W.A. Goldstein. The rhizophagy cycle in roots and nitrogen-transfer endosymbiosis in plant hairs are fundamental processes for sustainable plant cultivation. Book title: *Microbioma: One-Health: Dal Suolo la Benessere Umano* Type: Websites Status: Published Year Published: 2023 Citation: Walter Goldstein, Juan E. Andrade Laborde, Pierre Meyer, Ece Gulkirpik. M. Toc. 2023. Testing the quality of corn that has been selected for organic poultry. *eOrganic publications*.  
<https://eorganic.org/node/35728> Type: Journal Articles Status: Published Year Published: 2022 Citation: W. Goldstein. 2022. article titled: Nitrogen Fixing Corn. *Organic Broadcaster*, Sept 20, 2022  
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