United States Department of Agriculture

Progress Report

Title:	**Stop the rot: Combating onion bacterial diseases with pathogenomic tools and enhanced management strategies**

Sponsoring Agency	NIFA	**Project Status**	ACTIVE
Funding Source	Non Formula	**Reporting Frequency**	Annual
Accession No.	1020312	**Grants.gov No.**	
		Award No.	2019-51181-30013
Project No.	WNP03104	**Proposal No.**	2019-03171
Project Start Date	09/01/2019	**Project End Date**	08/31/2023
Reporting Period Start Date	09/01/2020	**Reporting Period End Date**	08/31/2021
Submitted By		**Date Submitted to NIFA**	

Program Code: SCRI

Program Name: Specialty Crop Research Initiative

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11/12/2021

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Non-Technical Summary

Onion bulb crops are grown on ~140,000 acres/year in the U.S. at a farm-gate value of $925M. Bacterial pathogens cause >$60M in losses annually to this industry. Losses can be particularly severe for stored bulbs as bacterial bulb rots typically only develop in storage, after all production costs have been incurred. Poor scientific understanding of the diversity and epidemiology of bacterial pathogens, and the lack of systemic bactericides limit industry capacity to mitigate these losses; this is in sharp contrast to the significant work that has been accomplished with fungal pathogens of onion. This 'Stop the Rot' project organizes 24 scientists in diverse disciplines across the U.S. to research the complete system (host, pathogen, and environment) of bacterial diseases of onion. The long-term goal is to support profitability and sustainability of onion production in the U.S. using a coordinated, national survey of bacterial pathogens affecting onion crops combined with a stakeholder-focused, systems approach to investigate how production practices, inoculum sources, and environmental conditions can be managed to develop effective, practical, economically-viable, and environmentally-sound strategies to limit losses to bacterial diseases.

The project has two primary objectives linked iteratively in a systems approach. The first objectives utilizes comparative genomics to identify genetic factors that enable some bacteria to cause diseases on onion, and to develop practical diagnostic tools as well as phenotypic resistance screening methods for bacterial pathogens of onion. A survey of onion bacterial diseases over three seasons in each of 12 states representing the seven primary regions of onion production in the U.S. will be used to understand the diversity of onion bacterial pathogens in the U.S., and to develop a National Onion Bacterial Strain Collection. Genomic assessment of this bacterial collection will enable us to understand the genetic basis of bacteria that can cause diseases of onion across the U.S. This, in turn, will be used to design rapid, accurate, and robust methods of detecting and identifying onion bacterial pathogens. The collection also will be used to develop methods of screening onion germplasm for resistance to bacterial pathogens. The screening methods can then be used in breeding programs to develop cultivars with greater resistance than currently available. The second objective focuses on onion bacterial disease management by examining

how irrigation practices, fertility practices, pesticide programs, cultural practices, post-harvest practices, and bacterial disease modeling can be managed to develop effective, practical management programs. A 12-person, nation-wide onion Stakeholder Advisory Panel worked with our team from 12 states to prioritize the objectives and develop approaches for this project. Broad, stakeholder-based evaluations of the research results over the duration of the project will ensure results are delivered to constituents and that solutions developed are viable economically and environmentally.

Accomplishments

Major goals of the project

The ability of growers to manage bacterial diseases of onion is limited compared to many fungal diseases because of unique epidemiological and management aspects of the bacterial pathogens, which cause >$60 million/year in damages to the U.S. onion industry. Losses can be particularly severe for stored onions as bacterial bulb rots typically develop after harvest, with losses ranging from 5-100% in individual fields. The cost of sorting symptomatic bulbs can result in rejection of entire fields if the incidence of rot exceeds 30%. There are effective tools to manage other factors that can cause storage losses (e.g., sprouting and fungal bulb rots), in contrast to bacterial rots. This project takes a systems approach encompassing the pathogen, host, environment, economics, and stakeholder priorities to address the need to manage onion bacterial diseases far more effectively. The goal of this project is to support long-term profitability and sustainability of onion production in the U.S. using a stakeholder-informed, systems approach by:

1. Undertaking a national survey of onion bacterial diseases;
2. Developing a National Onion Bacterial Strain Collection (NOBSC);
3. Using this resource for genotypic characterization of the pathogens to design rapid, accurate, and robust methods for detecting and identifying onion bacterial pathogens across the U.S.;
4. Developing methods of screening onion germplasm for resistance to these bacteria;
5. Integrating the diagnostic and detection tools into comprehensive integrated disease management research trials;
6. Generating predictive bacterial disease models across diverse regions of onion production in the U.S.; and
7. Implementing a broad, Stakeholder Advisory Panel (SAP)-informed dissemination plan to deliver results to constituents.

The goal utilizes a coordinated, national survey of bacterial pathogens affecting onion crops combined with a stakeholder-focused, systems approach to investigating how production practices, inoculum sources, and environmental conditions can be managed to develop effective, practical, economically-viable, and environmentally-sound strategies to limit losses to bacterial diseases. The project has two primary objectives linked iteratively in a systems approach:
A: Utilize comparative genomics to identify virulence factors and develop practical diagnostic tools, as well as phenotypic resistance screening methods for bacterial pathogens of onion (60% of the effort for this project). The four activities for this objective entail: **A1**) national onion bacterial disease surveys and development of a National Onion Bacterial Strain Collection (NOBSC), **A2**) onion bacterial pathogenomics, **A3**) development of onion bacterial pathogen detection tools, and **A4**) development of onion phenotyping (screening) protocols for reactions to bacterial pathogens.

B: Examine how key production practices, environmental factors, and inoculum sources that impact bacterial disease outbreaks can be managed to develop practical solutions that are viable environmentally and economically (40% of the project effort). This will entail six areas of investigation: **B1**) irrigation management, **B2**) fertility management, **B3**) pesticide programs, **B4**) cultural management, **B5**) post-harvest management, and **B6**) bacterial disease modeling.
Objective A addresses the SCRI focus area of "efforts to identify and address threats from pests and diseases" by clarifying for stakeholders nationwide, using standardized protocols, the diversity of onion bacterial pathogens causing losses in each of three growing seasons. The National Onion Bacterial Strain Collection resulting from this project will provide an invaluable resource for this and future projects on genetic studies of pathogens and associated bacteria in/on onions, e.g., whole genome sequencing to find virulence factors in order to develop robust molecular tools to detect and differentiate pathogenic vs. non-pathogenic bacteria. This will facilitate development of effective phenotypic methods of screening onion germplasm for reactions to diverse bacterial pathogens, alone and in combinations, which will facilitate future efforts to breed for resistance.
Objective B addresses the SCRI focus area efforts to identify and address threats from pests and diseases" as well as "efforts to improve production efficiency, handling and processing, productivity, and profitability". This coordinated, multi-state objective will determine how regional onion production practices, environmental conditions, and inoculum sources can be managed using practical, economically-viable, and environmentally-sound strategies to limit losses to bacterial rots in fields, storage, and shipping.

What was accomplished under these goals?

OBJECTIVE A: PATHOGENOMICS TOOLS

Accession No. 1020312 **Project No. WNP03104**

A1. Bacterial disease surveys, NOBSC

Onion foliage and bulbs were surveyed in southern states in 12/2020 to 06/2021, and in northern states from spring to fall 2021. To date, 84 bacterial genera have been identified from 2,594 isolates. Distribution and pathogenicity vary among regions. Few genera produce symptoms on onion. Prevalent genera include: Pantoea, Pseudomonas, Burkholderia, Enterobacter and Erwinia. The GA survey showed temporal progression in genera on foliage. A subset of 612 strains were sent to UGA for the NOBSC.

A2. Onion bacterial pathogenomics

To design molecular diagnostic tools for onion-pathogenic Pantoea strains, we targeted P. agglomerans. We showed that P. agglomerans strains use similar onion-virulence genes as P. ananatis, but strains with these genes are uncommon. Whole genome sequencing for 32 Pantoea strains showed a perfect correlation between red scale necrosis phenotype and presence of the HiVir operon. PCR primers were designed to detect the HiVir cluster in 3 Pantoea species, which appears necessary for infection. Strains without the alt gene, which imparts tolerance to sulfur compounds, do not colonize onion well.

Bacterial DNA extracted from symptomatic and asymptomatic onion bulbs from GA and WA revealed differences in bacterial communities between the types of bulbs, and between GA and WA bulbs. Genes involved in pathogenicity, detoxification and secretions systems were identified in the metagenome-assembled genomes of the known pathogens of center rot.

A3. Diagnostic tools

In Year 2 we:

- Verified the specificity and sensitivity of a real-time PCR assay for P. agglomerans;
- Developed a low-cost water trap to sample irrigation and rain water for onion pathogens;
- Demonstrated that a bulk soil DNA extraction method will need optimization for muck soils.

Public Pantoea species-specific PCR primers were evaluated and new PCR primers designed for P. agglomerans using the strains described above. HiVir operon sequences generated for 58 Pantoea strains are being used to design primers for HiVir+ onion-pathogenic strains of Pantoea. Genomic DNA was purified from 20 genomes sequenced for P. agglomerans, P. ananatis, and P. allii strains (15 HiVir+/RSN+ and 5 HiVir+/RSN-) to design diagnostic tools for onion-pathogenic strains.

A4. Phenotypic screening methods

Four cultivars (short-day [SD] dehydration, SD sweet, long-day [LD] dehydration, and LD sweet) were selected to represent what might be susceptible (sweet) vs. partially resistant (dehydration) to bacterial pathogens. Plants were inoculated with P. ananatis in a greenhouse with spray inoculation and cut leaf-tip methods at two inoculum concentrations. No consistent pattern emerged in disease severity among cultivars with either method or inoculum concentration. A cultivar field trial in WA was repeated in Season 2 using 3 cultivars from each of 4 maturity groups. The trial was irrigated with sprinklers instead of center-pivot to cease irrigation at the recommended time for each maturity group. Bulbs are in storage to be rated for bacterial rot in Feb. 2022.

OBJECTIVE B: ONION BACTERIAL DISEASE MANAGEMENT

Results of Year 1 trials were finalized once onion bulbs in storage were rated for bacterial storage rots. Field trials in Year 2 investigated the impacts of irrigation practices (WA, OR, GA trials), fertility practices (WA, NY, GA, OR, PA), cultural practices (WA, GA, NY), pesticide programs (CO, UT, WA, NY, GA, TX), and postharvest disinfection practices (WA) on development of onion bacterial diseases. In both years, field trials focused on practices and products currently used by onion growers to reflect stakeholder priorities and concerns. When feasible, trials involved inoculated and non-inoculated plots, using local bacterial strains to ensure adequate disease pressure for differentiating treatment effects. Some valuable results with potential application have been shared with stakeholders:

- Using drip vs. overhead irrigation reduced internal bacterial rot incidence without significant effects on marketable yield or size class distribution.
- Earlier termination of final irrigation reduced bacterial rot without affecting marketable bulb yield.
- Earlier termination of the final N application resulted in less bacterial rot in GA trials, but not in WA trials due to high residual N at the WA site.
- Copper-based bactericides significantly reduced internal bulb rot incidence in the 2020 GA trial. In the WA and NY 2020 trials, copper and other bactericide treatments had no effect on incidence or severity of bacterial bulb rot at harvest or after 5 months of storage.
- WA trials on undercutting, rolling tops, and the timing of topping showed no significant effects on marketable yield or bacterial bulb rots. GA trials evaluating harvesting methods showed significantly less bacterial bulb rot when using: a chain digger vs. a straight-blade undercutter; mechanical harvest vs. manual harvest; topping onion necks to a length of 3 or 5 inches vs. 1 inch. NY trials showed rolling tops had no effect on bacterial bulb rots, while quick artificial curing with forced heated air reduced bacterial bulb rots by 50%.
- WA trials indicated no benefit to applying ozone or hydrogen peroxide + peroxyacetic acid products to onion bulbs in storage for managing bacterial rots, probably because the products do not penetrate the dry wrapper scales.

RISK MODELING
The risk modeling team received crop and weather data for the 2017 production season from 5 onion fields planted to the same cultivar in the Columbia Basin of the Pacific Northwest. Excess late-season irrigation was hypothesized as a contributor to bacterial rots in these fields. Large amounts of field data are needed on plant water uptake and transpiration needs based on soil moisture status, weather, and plant growth stage, in order to extract signals from data and help identify hypotheses as the basis for risk-based decision-making. To this end, data was collected in NY trials by team members in Year 2.

ECONOMIC ASSESSMENTS
Surveys were delivered to onion growers, the project team and the SAP.

- Growers estimate that, on average over the past 5 years, >10% of the onion crop in their area was lost to bacterial diseases. Approx. 20% of growers reported losses of >50% of the onion crop in their area in the past five years due to bacterial diseases.
- Respondents consider the most effective management strategies are irrigation and water management, and post-harvest curing (forced air and heating).
- The average grower is very concerned about bacterial diseases, limited in their ability to identify the diseases or bulb rot pathogens, and feels only moderately equipped to manage them.
- Growers rely on information from University-based extension services to manage onion bacterial diseases.

OUTREACH AND EXTENSION
Year 1 outreach focused on increasing awareness of bacterial pathogens of onion, and sharing results of Season 1 field trials. Outreach has included (for a total of 3,897 direct contacts):

- Articles and quizzes in Onion World and Veg News, with wide circulation in the onion industry;
- Articles in newsletters of the National Onion Association, regional grower associations and extension services;
- Presentations at grower meetings and conferences;
- Release of 2 videos on diagnosing, understanding and managing bacterial bulb rots;
- Field days and tours in 7 states;
- A session on onion bulb rot at the NY Empire Producers Expo in Jan. 2021;
- Overhaul of the Alliumnet.com website serving members of the Allium research and extension community.

PROJECT MANAGEMENT
Coordination, communication and integration across the team and with SAP members have been supported by monthly videoconferences, bi-monthly conference calls, annual team and SAP meetings, and the adoption of the Project Charter. Work sessions included: streamlining work in regional laboratories; developing phenotypic screening methods; developing diagnostic tools.

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

STUDENTS
The grant has provided the opportunity for several graduate students and post-doctorates to work on different aspects of the project, as described below.

- Verushka Ibanez, MSc student at the University of Pretoria, South Africa. Dissertation title: Metagenome analysis of healthy and diseased onion bulbs from Georgia. Anticipated completion date: October 2021. Advisors: Teresa Coutinho, Pedro Lebre.
- Christopher Liakos, MSc student at the University of Pretoria, South Africa. Dissertation title: Metagenome analysis of healthy and diseased onion bulbs from Washington State. Anticipated completion date: October 2021. Advisors: Teresa Coutinho, Jacquie van der Waals, Pedro Lebre.
- Tessa Belo, MS student in the Soils & Water program at the Northwestern Washington Research and Extension Center of Washington State University. Advised by collaborator LaHue and co-advised by PD du Toit. Thesis topic: Impacts of irrigation and fertilization practices on bacterial diseases in onion bulb crops in the Columbia Basin. Expected completion date: Spring 2022.
- Antoinette Machado, MS student at Colorado State University in January 2020, supervised by co-PI Uchanski. Thesis topic: Evaluation of bactericides and plant defense inducers to manage center rot of onion in Colorado. Expected completion date: Spring 2022.
- Bed Prakash Bhatta, a PhD student at Texas A&M AgriLife Research - Uvalde is being trained on isolation, culture, and inoculation of bacteria under this project. Thesis topic: Breeding for anthracnose and Fusarium wilt resistance in watermelon.
- Ram Neupane, Plant Pathology PhD student at Pennsylvania State University (started Spring 2021). Thesis topic will be related to bacterial diseases of onion.
- Dr. Mei Zhao, postdoctorate in co-PD Dutta's program at the University of Georgia, is focusing on the bacterial survey, identification and characterization of bacterial isolates in GA, and assisting with greenhouse and field evaluations.

• Dr. Hossein Noorazar, postdoctorate at Washington State University, is focusing on data analysis and disease risk modeling efforts, supervised by co-collaborators Rajagopalan and Kalyanamaran. His part-time appointment on the project started in fall 2020. He initially focused on hypothesis extraction tools and exploring satellite imagery, and is now working with data provided by members of the team to identify factors that influence development of bacterial diseases.

• Dr. Navdeep Singh, postdoctoral research associate at Washington State University (started 16 Sep. 2020), primarily is working on other projects but is contributing to this work, particularly Objective B1, and will be engaged in this research until Fall 2022.

• Dr. Gi-Yoon (Gina) Shin, postdoctorate in co-PI Kvitko's lab at the University of Georgia, is assisting with the bacterial survey, investigating the pathogenomics of Pantoea spp. that are pathogenic and non-pathogenic on onion, and identifying target regions of the genomes for developing molecular diagnostic tools for onion bacterial pathogens.

• In Utah, five undergraduate students have been working on this project with collaborator Nischwitz (making media, culturing bacteria, storage evaluations, molecular identification of bacteria, conducting red scale necrosis tests, and helping with foliar and bulb assays). One student is writing an undergraduate research grant proposal for a side project identifying the gut bacteria of thrips in Utah and their potential as bacterial bulb rot pathogens.

• In New Mexico, two graduate students assisted with bacterial surveys in four autumn-sown onion fields.

• Shaun Stice, supervised by co-PI Kvitko, was a PhD student at the University of Georgia at the start of the project. He completed research on the genetic basis of P. ananatis virulence of onion as well as distribution of identified virulence loci among Pantoea isolates. He graduated in 2021.

• Dr. Gaurav Agarwal was a post-doctoral research associate under PI Dutta who assisted with the pathogenomics investigation of Pantoea spp.

PROFESSIONAL DEVELOPMENT

WSU team members (du Toit, Derie, and Waters) were hosts for training on 21-22 July 2021 for two team members from Texas A&M University (technician Khamal and PhD student Bhatta) to review methods for lab, greenhouse, and field trials on onion bacterial diseases.

Postdoctorate Shin (UGA) prepared a review session for the team on 30 June 2021 on using the SILVA database (in comparison with RDP and GenBank) for identifying bacterial isolates to genus-level based on 16S rDNA sequences. Following the session, the co-PIs agreed to switch from using RDP to SILVA as the primary database for identifying bacterial isolates to genus-level that are collected in each regional lab for the state surveys each season.

How have the results been disseminated to communities of interest?

In this reporting period, we have been actively reaching out to growers and stakeholders to: share new information and learning from the project as well as current scientific understanding of onion bacterial diseases and their management; understand growers' current state of knowledge about causes and management of bacterial diseases of onion, and identify priority concerns for growers and the onion industry as regards management of bacterial diseases. Information has been shared with growers and stakeholders through a range of communication channels, including the newly revamped Alliumnet website, research summaries, extension bulletins, articles in trade publications and presentations at growers' meetings and field days. Results were also shared at professional scientific meetings (American Phytopathological Society's Plant Health 2021 Online.

REACHING GROWERS AND ONION INDUSTRY STAKEHOLDERS:

• Outreach to growers and onion industry stakeholders, field representatives and extension staff has occurred through informal visits with growers during field surveys, grower meetings, field days and technical workshops.

• Our Stop the Rot video playlist https://www.youtube.com/playlist?list=PLajA3BBVyv1zf2obB16bNEdQPQeLW_XB_ now contains several videos aimed at growers and the onion industry (introduction to rot identification, in both English and Spanish; a longer video providing an overview of bacterial diseases in onion and current management options; a short video of the experimental equipment for our field trials of post-harvest disinfection treatments.) A short time-lapse video of the Red Scale Necrosis (RSN) assay has been added. We plan to include more short videos from field surveys and laboratory work during the next year. Refer to details above for statistics on number of views for each video and two awards given for the 14-minute video on the whole project produced by WSU.

• Outreach and dissemination of preliminary results to onion stakeholders and the industry has been conducted a number of informational articles in trade publications and extension newsletters (see list of publications) and websites/online alerts.

• Outreach to growers and industry stakeholders through the Stop the Rot Stakeholder Advisory Panel:

• Our 14-member Stakeholder Advisory Panel (SAP) brings a diverse range of expertise and experience to the project. The Panel includes growers, pathologists and onion breeders from onion growing regions across the US. Four new members joined the Panel in Year 2: Larry Duell of Gowan Seeds in Colorado; Margreet Asma, seed pathologists with Bejo Zaden in the Netherlands; David Burrell of National Onion Labs in Georgia; and Kalie Christensen of Gumz Farms in Wisconsin.

• Panel members are active in sharing information about the project to growers and onion industry stakeholders through

their own regional and national networks in 12 states, and in bringing insights and information into the project from their networks. Our second annual meeting with the SAP was held on February 5th, 2021 by videoconference. Several panel members have participated in team work sessions on phenotypic screening methods as subject matter experts. Panel members have actively participated in our monthly project team meetings by videoconference and they continue to receive project updates, notes from the monthly meetings and our internal project team newsletters.

REACHING ACADEMIC RESEARCHERS, EXTENSION PROFESSIONALS AND GRADUATE STUDENTS

- The project team now includes 24 research collaborators from 12 states, representing all seven major U.S. onion growing regions, as well as onion bacteriologist Prof. Teresa Coutinho from South Africa. During this year, Prof Steven Beer of Cornell and Drs. Paul Stodghill and Jo Ann Asselin of the USDA-ARS also joined the team as collaborators. We hold monthly team videoconferences to share preliminary results and experiences and discuss recent findings. These monthly meetings of 1.5 to 2 hours have facilitated research and Extension collaborations within the project. Almost all the project collaborators are also involved in extension services and education in their regions, which makes for efficient transfer of new information and research results from the team to extension professionals and thence to growers. Technicians and graduate students from each of the regional teams also join our monthly meetings and contribute actively to the discussions, providing feedback on methods and protocols and sharing their latest results with the full team in a collegial setting.
- Once final results of 2020 field trials became available in spring 2021 after evaluation of bulbs in storage, we compiled results from the first season's bacterial surveys and field trials into a summary of Year 1 research findings. The summary is available on Alliumnet.com https://alliumnet.com/wp-content/uploads/2021/09/StoptheRot_Year1_summaries.pdf.
- Preliminary results of the national bacterial survey were shared at the American Phytopathological Society annual meeting, 2021 Plant Health Online, and will be shared with relevant agencies in each participating state.
- The results of Year 1 field trials in participating states have been published in 12 peer-reviewed Plant Disease Management Reports (see list of publications).
- Five articles have been published in scientific journals (see list of publications).
- Four Research on Demand presentations were shared at Plant Health 2021 Online, the annual conference of the American Phytopathological Society.

What do you plan to do during the next reporting period to accomplish the goals?

OBJECTIVE A: PATHOGENOMICS TOOLS

A1. Bacterial surveys:

In the next reporting period, we plan to repeat the bacterial surveys in all the onion regions. In addition, directed surveys will be carried out using a real-time PCR assay being developed for Pantoea agglomerans strains virulent on onion, based on whole genome sequencing of bacterial strains collected in this survey and strains received from other collections. Regional labs will again send both pathogenic and non-pathogenic isolates of Pantoea to UGA, endeavoring to represent the diversity found in their regions. As strains of the other genera are characterized, each lab will send pathogenic and non-pathogenic strains that represent the diversity detected in the samples. Strains sent to UGA will be identified to species using MLSA schemes and tested for pathogenicity on onion. Metadata and results will be added to the NOBSC database and made available publicly on Alliumnet. During the next two years of the project, regional labs will also plan for long-term storage and back-up of their regional collections. The NOBSC will only include strains that are in the national collection.

A2. Pathogenomic analyses, virulence factors & bacterial community analyses

Additional strains of P. agglomerans will continue to be added to the NOBSC as Year 2 survey collections are finalized at each regional lab. A subset of those strains will be subjected to whole genome sequencing and examined to identify putative virulence factors associated with the ability to cause diseases on onion, as detailed above for strains evaluated in Year 2 to validate the virulence factors identified in the first 2 years of this project and for testing the molecular diagnostic tools being developed for onion-pathogenic strains of Pantoea spp. .. Results of the bacterial community analyses from asymptomatic and symptomatic bulbs collected from a field in each of GA and WA will be published in Year 3, when the two MS students working on this aspect of the project will graduate. The results will help assess potential interactions among the complex microflora associated with asymptomatic and symptomatic onion bulbs. Results from both students' work will be used to design additional microbial community analyses to increase our understanding of how non-pathogenic, pathogenic, and opportunistic bacteria and other microflora interact in onion bulbs.

A3. Molecular diagnostic tools

We will use the results of initial pilot studies in Year 2 to evaluate how to undertake directed surveys for onion bacterial pathogens using molecular diagnostic tools. This will entail collecting onion seed lots as well soil, debris, and water trap samples from fields at critical stages of onion production. Real-time PCR assays of DNA extracted from these materials, using the molecular diagnostic tools in development, should inform us of the relative importance of potential inoculum sources of onion bacterial pathogens in different regions of the US.

Results of the pathogenomics work in Years 1 and 2 revealed that it will be challenging to design a sensitive real-time PCR assay for Pantoea spp. pathogenic on onion that works across all onion production regions. We have detected substantial

regional variation in the pathogenicity of P. agglomerans strains collected across 12 states during the Season 1 and 2 surveys. For example, all the Idaho and Oregon strains of P. agglomerans tested to date were RSN-, and very few P. agglomerans strains collected in Washington have been RSN+, even when isolated from symptomatic plants or bulbs. This is in contrast to GA, where a majority of P. agglomerans strains collected were RSN+. Therefore, our approach in Year 3 will be to design an assay which detects the presence of the HiVir cluster and, potentially, other genes associated with virulence to onion, and then use a decision support system to identify the associated risk of that bacterial species being pathogenic on onion. The regional survey results will be important for developing a decision support system relevant to different onion production regions of the US.

A4. Phenotypic resistance screening methods

Based on results of the lab and greenhouse onion phenotyping protocols evaluated in Years 1 and 2, the focus of this project will shift to field evaluations. Dutta at UGA will screen commercial short-, medium-, and long-day cultivars as well as diverse Allium germplasm from the USDA NPGS for resistance to P. ananatis by treating the seed of these cultivars and inoculating plants in the field at various growth stages. Field phenotypic screening trials also will be completed in TX and WA in Season 3 with pathogens predominant in each state. Cultivars planted will reflect those suitable to each region as well as germplasm with diverse phenotypic reactions from Season 1 trials. Based on results of the WA Season 2 cultivar trial, modifications will be incorporated into the Year 3 cultivar field trial in WA.

OBJECTIVE B: ONION BACTERIAL DISEASE MANAGEMENT

Lab, greenhouse, and field trials will be continued in Year 3 to evaluate irrigation practices, fertility practices, pesticide programs, cultural practices, and postharvest bulb treatments for control of bacterial diseases of onion. Specific aspects investigated in each region will be prioritized based on regional practices and constraints, and on stakeholder priorities as well as results from Years 1 and 2 of the project. Results from Season 1 and 2 trials will be shared at the team and SAP meetings to refine research plans for Season 3, in an iterative approach guided by stakeholders to ensure translation of results into practical tools. Data from Stop the Rot field trials in Years 1 and 2 will be used to inform and validate disease risk models. Data on onion production practices, crop microclimate, and bacterial diseases will be sought to assess whether the risks of diseases can be modeled statistically, to predict if a grower should sell bulbs at harvest or can store bulbs with minimal risk of bacterial rots.

ECONOMIC ASSESSMENTS

The potential for adoption of management recommendations based on results of these trials will be assessed annually by the SAP and stakeholders with economist Colson. Economic analyses will be integrated into various trials based on inputs (expenses) and outputs (marketable yield). Work on this objective will include:

- Drafting an academic article reporting survey findings.
- Assisting the Extension team and grant leadership to incorporate findings from field trials into website and outreach materials.
- Working with team members as field trial data becomes available to translate findings into cost-benefit analyses.

OUTREACH AND EXTENSION

- Results from Year 2 work will be shared in research summaries; on Alliumnet.com; in project newsletters, trade publications, regional websites, and presentations at growers' meetings and field days.
- Plans are underway to develop more videos, "quick guides", highlights of annual project results, training and extension materials, and an interactive map of the USA with results of the bacterial disease surveys.
- The newly redesigned Alliumnet.com website will be maintained to provide a home for national onion research collaborations, including USDA projects, National Allium Research Conference information and proceedings, links to National Onion Association meetings and events, and the W-1008, W-2008, and W-3008 (and the future W-4008) onion multi-state project reports, activities, and meeting details.

PROJECT MANAGEMENT AND COORDINATION

The third annual project team meeting is scheduled for March 1, 2022 in Denver CO, and the Stakeholder Advisory Panel videoconference is scheduled for March 25, 2022.Regular monthly videoconferences for the full team, regular meetings for the co-PIs every two months, and weekly meetings of the PD and Project Manager will continue in Year 3.

Participants

Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	

Accession No. 1020312 **Project No. WNP03104**

Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.4	0	0.7	0.8	1.9000000000000001
Professional	0	0	0	0	0
Technical	2.3	0.7	0	0	3
Administrative	0.5	0	0	0	0.5
Other	4.6	0	0	0	4.6
Computed Total	7.8	0.7	0.7	0.8	10.0000000000000001

Student Count by Classification of Instructional Programs (CIP) Code

Undergraduate	Graduate	Post-Doctorate	CIP Code
	1		01.00 Agriculture, General.
	2		01.01 Agricultural Business and Management.
2	1	2	01.11 Plant Sciences.
	1		01.12 Soil Sciences.
		1	01.99 Agriculture, Agriculture Operations, and Related Sciences,
6	2		26.03 Botany/Plant Biology.
	1		26.05 Microbiological Sciences and Immunology.

Target Audience

Stakeholders involved in the U.S. onion industry are the primary audience for this project. This includes onion producers (farmers), packers, shippers, and associated stakeholders engaged in various capacities in onion production, distribution, and marketing, e.g., agronomists, crop consultants, farm managers, field workers; personnel associated with agricultural supply companies (fertilizer and pesticide dealers, irrigation supply companies, etc.), seed companies, and dealers; onion breeders (public and private); and onion storage and shipping/transport personnel and companies. Public and private research and extension specialists, undergraduate students, graduate students, and postdoctorates working with diverse aspects of onion production are also a target audience for this project.

The target audiences reached during this reporting period included:

- Onion growers, packers, processors and associated stakeholders in all 7 onion-growing regions of the US;
- Extension professionals in the 12 onion-growing states that are represented in this project;
- The project's Stakeholder Advisory Panel members represent onion farms, regional onion associations, and major vegetable seed companies (including onion breeders and plant pathologists) from across the U.S., with one international member. Panel members conducted further outreach to their own networks on behalf of the project. Panel members who are industry representatives include Greg Bird (President of the Michigan Onion Committee), Bob Ehn (California Garlic and Onion Research Advisory Board), Charles Hall (Executive Director of the Georgia Fruit and Vegetable Growers Association), Robert Sakata (President of the Colorado Fruit and Vegetable Growers Association).
- Four Masters students, two PhD students and four postdoctorates have worked on research directly related to the Stop the Rot project objectives in this reporting period. They are conducting their research in Washington State, Georgia, Texas, Pennsylvania, Colorado, and South Africa.

We used several channels to reach our target audience during this reporting period, including grower meetings and field days, conferences and workshops, the Alliumnet.com website, industry newsletters, trade publications and extension videos. Project team members also reached out directly to growers in each of the onion-growing regions to recruit them for participation in the bacterial field surveys.

Approximately 1,008 growers, agronomists and industry professionals were reached directly through presentations at conferences, workshops, field days and grower meetings in this reporting period. Many of the events were necessarily shifted away from in-person to virtual format due to COVID-19 constraints across the USA and in South Africa, which made it difficult to accurately count the number of people who were reached through presentations. Approximately 2,796 people have viewed our series of project videos.

Communication and outreach materials and specific activities are reported in detail in the list of products.

Products

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	YES

Citation

Agarwal Gaurav, Choudhary Divya, Stice Shaun P., Myers Brendon K., Gitaitis Ronald D., Venter Stephanus N., Kvitko Brian H., Dutta Bhabesh (2021). Pan-genome-wide analysis of Pantoea ananatis identified genes linked to pathogenicity in onion. Frontiers in Microbiology vol 12:2381 https://www.frontiersin.org/article/10.3389/fmicb.2021.684756

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	YES

Citation

Koirala, S., Zhao, M., Agarwal, G., Stice, S., Gitaitis, R., Kvitko, B., & Dutta, B. 2021. Identification of two novel pathovars of Pantoea stewartii subsp. indologenes affecting Allium sp. and millets. Phytopathology. 10.1094/PHYTO-11-20-0508-R. https://www.researchgate.net/publication/349422413_Identification_of_two_novel_pathovars_of_Pantoea_stewartii_subsp_indologenes_affecting_Allium_sp_and_millets

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	YES

Citation

Stumpf, S., Leach, L., Srinivasan, R., Coolong, T., Gitaitis, R., Dutta, B. Foliar chemical protection against Pantoea ananatis in onion is negated by thrips feeding. Phytopathology. 2021 Feb. 111(2):258-267. doi: 10.1094/PHYTO-05-20-0163-R. Epub 2021 Jan 13. PMID: 32748732. https://pubmed.ncbi.nlm.nih.gov/32748732/

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	YES

Citation

Stice, S., Shin, G.Y., Armas, S.D., Koirala, S., Galvan, G.A., Siri, M.I., Severns, P.M., Coutinho, T.A., Dutta, B., and Kvitko, B. 2021. The distribution of onion virulence gene clusters among Pantoea spp. Frontiers in Plant Science https://doi.org/10.3389/fmicb.2021.00184

Accession No. 1020312 **Project No. WNP03104**

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	YES

Citation

Zhao, M., Kvitko, B.H., Gitaitis, R.D., and Dutta, B. 2021. Bacterial streak and bulb rot of onion. Plant Health Instructor. DOI: 10.1094/PHI-E-2021-0421-01

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

da Silva, A., H.I. de Jesus, B. Dutta. 2021. Effects of irrigation method, nitrogen rate, and fertilizer application timing on bacterial diseases in Vidalia onion, Georgia 2020. Plant Disease Management Reports 15:V012.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

Dutta, B., Tyson, C., Edenfield, J., Williams, Z., Tanner, S., Shirley, A., Reeves, B., and Powell, S. 2020. Evaluation of onion growth stage directed chemical applications and thrips management program on center rot incidence in onion bulbs in Georgia, 2019. Plant Disease Management Reports 14:V091

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

Dutta, B., Tyson, C., Edenfield, J., Williams, Z., Tanner, S., Shirley, A., Reeves, B., and Powell, S. 2021. Evaluation of onion growth stage directed chemical applications and thrips management program on center rot incidence in onion bulbs in Georgia, 2020. Plant Disease Management Reports 15:V023. https://doi.org/10.1094/PDMR15

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

Dutta, B. and Tyson, C. 2021. Evaluation of harvesting methods on post-harvest incidence of center rot and sour skin in onion, Georgia 2020. Plant Disease Management Reports 15:V025. https://doi.org/10.1094/PDMR15

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

Dutta, B. and Tyson, C. 2021. Evaluation of digging methods on post-harvest incidence of center rot and sour skin in onion, Georgia 2020. Plant Disease Management Reports 15:V026. https://doi.org/10.1094/PDMR15

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

Dutta B. and Foster M.J. 2021. Evaluation of bactericides and plant defense inducers to manage center rot of onion in Georgia, 2020. Plant Disease Management Reports 15:V027. https://doi.org/10.1094/PDMR15

Accession No. 1020312 **Project No. WNP03104**

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

du Toit, L.J., Derie, M.L., Gundersen, B., Waters, T.D., and Darner, J. 2021. Efficacy of bactericides for management of bacterial leaf blight and bulb rots in an onion crop in Pasco, WA, 2020. Plant Disease Management Reports 15:V107. https://doi.org/10.1094/PDMR15

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

du Toit, L.J., Derie, M. L., and Gundersen, B. 2021. Efficacy of disinfectants applied to onion bulbs in storage for control of bacterial bulb rots, Pasco, WA, 2020-2021. Plant Disease Management Reports 15:V102. https://doi.org/10.1094/PDMR15

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

du Toit, L.J., Derie, M. L., and Gundersen, B. 2021. Efficacy of late-season cultural practices on bacterial leaf blight and bulb rots in an onion bulb crop in Pasco, WA, 2020. Plant Disease Management Reports 15:V100. https://doi.org/10.1094/PDMR15

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

Belo, T., du Toit, L., Waters, T., Derie, M., and LaHue, G. 2021. Effects of irrigation frequency and final irrigation timing on onion bacterial diseases in the Columbia Basin of Washington, 2020. Plant Disease Management Reports 15:V109 https://doi.org/10.1094/PDMR15

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

Hoepting, C.A., Caldwell, S. K., and van der Heide, E. R. 2021. Evaluation of selected pesticides for control of bacterial bulb rot in onion, 2020. Plant Disease Management Reports 15:V108 https://doi.org/10.1094/PDMR15

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2021	NO

Citation

Hoepting, C.A., Caldwell, S. K., and van der Heide, E. R. 2021. Variety evaluation and effect of copper bactericide on bacterial bulb rot in onion, 2020. Plant Disease Management Reports 15:V107. https://doi.org/10.1094/PDMR15

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2020	YES

Citation

Dutta, B., and Gitaitis, R. 2020. Bacterial diseases of onion in Georgia. University of Georgia Cooperative Extension Bulletin B1534.

Accession No. 1020312 **Project No. WNP03104**

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2020	YES

Citation

Hoepting, C.A. 2020. Stop the Rot: New York part of ambitious multi-state, multi-disciplinary, multi-year, multi-million-dollar project to combat bacterial diseases of onion. Cornell Cooperative Extension Cornell Vegetable Program extension newsletter, Veg Edge August 5, 2020: 16(18), pp 6-7. https://rvpadmin.cce.cornell.edu/pdf/veg_edge/pdf198_pdf.pdf

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2021	YES

Citation

Hoepting, C.A. 2021. Pesticides for onion bulb rot – Do they Work? Featuring 2020 field trial results. Cornell Cooperative Extension Cornell Vegetable Program extension newsletter, Veg Edge July 21, 2021: 17(15), pp 6-7.

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2021	YES

Citation

Hoepting, C.A. 2021. The rot race: Harvest practices to avoid bacterial bulb rot in onion. Cornell Cooperative Extension Cornell Vegetable Program extension newsletter, Veg Edge July 28, 2021: 17(16), pp 8-9.

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2020	YES

Citation

Stop the Rot newsletter #1 in September 2020, which was also posted to the project page on Alliumnet.com https://bugwoodcloud.org/mura/alliumnet/assets/File/Newsletters/StopRotteamNewsletterSept2020(final).pdf

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2020	YES

Citation

LaHue, G.T., Waters, T., and du Toit, L., 2020. Revisiting nitrogen management recommendations in the context of onion bacterial diseases. Progress report for Columbia Basin Onion Research Committee. Submitted 31 October 2020.

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2020	YES

Citation

du Toit, L.J., Waters, T., Derie, M., and Darner, J. 2020. Battling onion bacterial diseases with bactericides. Onion World, December 2020:6-11. https://issuu.com/columbiamediagroup/docs/onion_world_december_2020

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2020	YES

Citation

Thompson, C. 2020. Organic Vidalia Onion Industry: Challenges in sour skin management. Vegetable and Specialty Crop News, 16 December 2020 (edits provided by B. Dutta)

Accession No. 1020312 **Project No. WNP03104**

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2021	YES

Citation

Dutta, B., and Gitaitis, R.D. 2021. Disease quiz II. Onion World, February 2021:13, 20.

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2021	YES

Citation

MacKay, H.M. 2021. Stop the Rot! Researchers finish first year of field, lab work in 4-year project. Update in January 2021 newsletter of the National Onion Association.

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2021	YES

Citation

Dutta, B. 2021. Onion disease management in Georgia. Vegetable and Specialty Crop News, February 2021.

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2021	YES

Citation

Nischwitz, C. 2021. Bacterial Bulb Rot Diseases in Utah, Utah Pests Quarterly Newsletter, Vol. 15, Spring 2021. https://extension.usu.edu/pests/files/up-newsletter/2021/UtahPestsNews-spring21.pdf

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2021	YES

Citation

MacKay, H., and du Toit, L. 2021. Progress report. Stop the Rot: A national collaboration to research, combat bacterial diseases of onion. Onion World, May/June 2021:12-14. https://issuu.com/columbiamediagroup/docs/onion_world_may_june_2021/12

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2021	YES

Citation

du Toit, L., and Waters, T. 2021. To disinfect or not? Can postharvest applications of disinfectants reduce bacterial bulb rots in storage? Onion World, July/August 2021:6-9.

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2021	YES

Citation

Stop the Rot Collated Summaries of Year 1 Technical Reports. Compilation of the final Year 1 topic summaries, August 2021. https://alliumnet.com/wp-content/uploads/2021/09/StoptheRot_Year1_summaries.pdf

Accession No. 1020312 **Project No. WNP03104**

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2021	YES

Citation

Wohleb, C.H., Waters, T.W., du Toit, L.J., and LaHue, G. 2021. Washington State University Extension Onion Alert, 1 September 2021. https://mailchi.mp/wsu/wsu-onion-alert-sept1-2021-1305808?e=72ba613792

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2020	YES

Citation

Dutta, Bhabesh (2020). 'Stop the Rot' Research Project on Bacterial Diseases of Onion. Invited presentation at the Annual Meeting of the National Onion Association, August 11, 2020 (virtual meeting). (28 participants)

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2020	YES

Citation

November 19, 2020. WA, OR, ID Pacific Northwest Vegetable Grower's (virtual) Annual Convention. Stop the Rot SCRI project update (Lindsey du Toit). 185 participants

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2020	YES

Citation

December 4, 2020. OR, WA Hermiston Farm Fair. Understanding and managing Iris yellow spot virus and onion bacterial rots in Columbia Basin onion production (Lindsey du Toit). 213 participants

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2020	YES

Citation

December 9, 2020. CO. Eastern Colorado Crop Production (virtual) Conference. Stop the rot project update (Antoinette Machado). 59 participants

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2021	YES

Citation

January 6, 2021. GA Southeast Fruit and Vegetable (virtual) Conference. Onion disease update (Bhabesh Dutta). 45 participants

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2020	YES

Citation

Collaborator (Subas Malla) participated in South Texas Onion Committee meetings on June 12, 2020 and October 13, 2020. He highlighted and updated TX findings of the project to the Committee members. There were around 20 attendees at each meeting.

Accession No. 1020312 **Project No. WNP03104**

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2021	YES

Citation

MacKay et al. 2021. "Combating onion bacterial diseases with pathogenomics tools and enhanced management strategies: Research objectives and preliminary results." Research on Demand presentation for American Phytopathological Society's annual meeting, Plant Health 2021 Online, 2-6 August 2021. (All topic leads are co-authors).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2021	YES

Citation

January 14, 2021. NY. Empire State Producers Expo – Onion Bulb Rot Session. (Lindsey du Toit, Bhabesh Dutta, Brian Kvitko, Christy Hoepting). 86 participants.
-Rotten Onions 101 Part I – What kind of rot you got
-Rotten Onions 101 Part II – The many ways onions rot
-Stop the Rot: Rot-free onions in the making – Overcoming challenges to identify varietal tolerance to bulb rot.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2021	YES

Citation

Brendon Myers, Gaurav Agarwal, Ronald D. Gitaitis, Brian H. Kvitko and Bhabesh Dutta. 2021. Pan-genomic approaches to predicting virulence factors in Pantoea ananatis affecting Allium porrum and Allium fistulosum. Research on Demand presentation for American Phytopathological Society's annual meeting, Plant Health 2021 Online, 2-6 August 2021.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2021	YES

Citation

Santosh Koirala, Mei Zhao, Gaurav Agarwal, Ron Gitaitis, Shaun Stice, Brian Kvitko, and Bhabesh Dutta. 2021. Identification of two novel pathovars of Pantoea stewartii subsp. indologenes affecting Allium sp. and millets. Research on Demand presentation for American Phytopathological Society's annual meeting, Plant Health 2021 Online, 2-6 August 2021.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2021	YES

Citation

Gaurav Agarwal, Santosh Koirala, Brian H. Kvitko and Bhabesh Dutta. 2021. Pan-genome of novel Pantoea stewartii subsp. indologenes identifies genes involved in onion pathogenicity and lateral gene transfer. Research on Demand presentation for American Phytopathological Society's annual meeting, Plant Health 2021 Online, 2-6 August 2021.

Other Products

Product Type

Survey Instruments

Description

A survey was developed and delivered to project team members and the stakeholder advisory panel to: (a) assess the quality and value of the "Stop the Rot" annual meeting in February 2021; (b) gather opinions and suggestions for management of information, documents, and other elements of the grant project; and (c) help plan future meetings. Results from the survey were communicated to the grant leadership and factored into team planning and activities.

Product Type

Survey Instruments

Description

A baseline grower survey was distributed May through December 2020, to assess onion growers': (a) experience with onion losses due to bacterial diseases and rots of onions, (b) strategies used to reduce losses, and (c) perceptions of the efficacy of management practices that potentially could reduce losses. The survey was distributed to growers and onion stakeholders through project team members, SAP members and online through the National Onion Association and other industry communication channels. 68 responses were received. Results were reported to the project annual meeting in February 2021.

Product Type

Audio or Video

Description

Combating onion bacterial diseases with pathogenomics tools and enhanced management strategies. 3 min. Antoinette Machado and Brandon Sandoval. Part of virtual field day. Posted Sep. 1, 2020. (146 views) https://youtu.be/ZrLcRcmN03k

Product Type

Audio or Video

Description

Combating bacterial diseases of onion: How do we stop the rot? 14.41 min. Lindsey du Toit, Heather MacKay, Darrell Kilgore, and Matthew Ziegler. Posted Dec. 15, 2020 (1,932 views). https://youtu.be/VLpZqCrQuPw (1,613 views). Also posted on Gartebau Profit trade magazine website in German. Translated into Serbian and presented at virtual grower meeting in Serbia, Jan. 18, 2020. The video won two awards: 1) a Gold from the Hermes Awards, and 2) an Honorable Mention from the DotComm awards.

Product Type

Audio or Video

Description

Evaluation of post-harvest treatments on onion. 5.29 min. Tim Waters and Lindsey du Toit. Posted Dec. 18, 2020. (114 views) https://youtu.be/VSExAvjJAT8

Product Type

Audio or Video

Description

Red Scale Necrosis Assay time-lapse video. 1 min. Claudia Nischwitz. https://youtu.be/WOcdDrTmaEA Posted Jan. 20, 2021. (32 views)

Product Type
Other

Description
The Alliumnet.com website has been redesigned and updated, based on inputs received from a User Reference group established for this purpose. www.alliumnet.com The site contains information resources for researchers, extension staff, and producers on the management of onion pests and diseases, links to current and recently completed research projects funded by federal and other grants, information on upcoming research meetings and conferences, and archives of previous research meetings. The Alliumnet website is developed, maintained, and hosted by the Southern IPM Center and the Center for Invasive Species and Ecosystem Health at the University of Georgia.

Product Type
Protocols

Description
"Stop the Rot Project Standardized Bacterial Survey Protocols for Season 2" is a set of standardized protocols for field collection and processing of onion samples, isolation and identification of bacterial strains, testing of pathogenicity and virulence of bacterial strains. The protocols are accompanied by electronic data entry forms to facilitate the preparation of a centralized project database on onion bacterial isolates. These standardized approaches and methods for sampling, isolating, and testing virulence on onion are the foundation for improving our understanding of onion bacterial pathogens in different regions of production across the USA, over the three field seasons of surveying/sampling during this project.

Product Type
Protocols

Description
"Stop the Rot Project Charter: Collaborative Agreement. The Project Charter was developed by the project manager and co-PIs, and all project team members were asked to read and acknowledge its contents. The charter is guiding our work together as a team over the duration of the project. Topics covered include principles for collaboration, project team organization and management, internal review and accountability, data management and confidentiality, authorship and acknowledgement, and conflicts of interest. https://alliumnet.com/wp-content/uploads/2021/11/Stop-the-Rot-project-charter-final-Rev2-hm20211019.pdf

Product Type
Databases

Description
Bacterial survey data submitted by each of the 12 participating state teams have been compiled into a centralized database for use by the project team. Crop and environment data were recorded for each location and onion sample when the samples were collected. Field data are connected to each strain through unique, anonymized sample and field codes. The database will be used to populate a public searchable platform for the National Onion Bacterial Strain Collection.

Product Type
Physical Collections

Description
National Onion Bacterial Strain Collection (NOBSC): Selected strains from stored collections maintained at Colorado State University (Uchanski), Washington State University (du Toit), Pennsylvania State University (Gugino), University of Georgia (Dutta), University of Pretoria (Coutinho), and Cornell University (Beer) were sent to co-PI Kvitko's lab at UGA to initiate building the National Onion Bacterial Strain Collection. Bacterial strains collected from the fields and storage facilities surveyed in 12 states each of three seasons will be added to the NOBSC over the duration of this project. At the end of Year 2, the collection contained 612 curated strains.

Product Type

Physical Collections

Description

Regional onion bacterial strain collections: Each of the regional labs for this project has stored all the bacterial isolates collected during surveys in the state(s) in that region. This includes a regional lab at each of: 1) Washington State University (du Toit) for isolates from Washington and California; 2) University of Idaho (Woodhall) for isolates from Idaho and Oregon; 3) Utah State University (Nischwitz) to store isolates from Utah, Colorado, and New Mexico; 4) Texas A&M University (Malla) for isolates collected from Texas; 5) Pennsylvania State University (Gugino) for isolates collected from Pennsylvania and New York; and 6) University of Georgia (Dutta) for isolates collected in Georgia. A subset of the strains stored and characterized at each regional lab is being used to populate the NOBSC to represent the diversity of bacterial strains associated with onion crops across the USA. Each regional lab, therefore, also serves as a backup repository for part of the NOBSC collection.

Product Type

Educational Aids or Curricula

Description

Dutta, B., and Gitaitis, R. 2020. Bacterial diseases of onion in Georgia. University of Georgia Cooperative Extension Bulletin B1534.

Product Type

Other

Description

ID-OR February 2, 2021. Idaho Eastern Oregon Onion Crop Protection Meeting. Project update (James Woodhall, Stuart Reitz). (150 participants)

Product Type

Other

Description

UT: Utah Onion Association meeting was held virtually on February 9, 2021 with 44 attendees. An update on Stop the rot was given by Claudia Nischwitz.

Product Type

Other

Description

CO: Hosted a virtual field day and posted the videos, including a Stop the Rot specific one, to our website during this reporting period: https://specialtycrops.agsci.colostate.edu/ https://youtu.be/ZrLcRcmN03k (146 views)

Product Type

Other

Description

WA: WSU team members (Lindsey du Toit, Michael Derie, Tim Waters) provided training on 21-22 July 2021 for two team members from Texas A&M University (Manzeal Khamal and Bed Bhatta) on methods for Stop the Rot lab, greenhouse, and field trials.

Product Type

Other

Description

NY: Onion variety rot trial tour, Elba, NY, Christy Hoepting, August 10, 2021 (10 participants).

Changes/Problems

Bacterial surveys: managing workloads in the regional labs

Two meetings were held in December 2020 (virtual) where the regional labs reviewed Season 1 work on bacterial surveys and planned for Season 2 surveys. The workload in Season 1 associated with processing onion survey samples proved to be very challenging for the labs, but the Season 2 survey program was greatly streamlined by reducing the number of visits to each survey location as well as the number of strains to be isolated from each sample collected in the field or in storage. Postdoctorate Shin (UGA) prepared a review session for the team on 30 June 2021 on using the SILVA database (in comparison with RDP and GenBank) for identifying bacterial isolates to genus-level based on 16S rDNA sequences. Following the session, the co-PIs agreed to switch from using RDP to SILVA as the preferred database for identifying bacterial isolates to genus-level that are collected in each regional lab for the state surveys each season.

Risk-based modeling: access to commercial production data

Access to production data from growers and packers has been very limited as stakeholders are reluctant to share proprietary crop information in a highly competitive market. In addition, growers do not always have detailed environmental data records, particularly for soil moisture status which we hypothesize is one of the most important risk factors in bacterial disease development. This has affected the ability of the team to develop and evaluate bacterial disease risk models. To resolve this, we plan to focus on collecting more data from our project field trials in Year 3, including deploying additional sensors to monitor environmental conditions in the trial plots.

Weather-related problems

Winter storms in Texas in January 2021 led to severe commercial onion crop losses and curtailed the bacterial survey in commercial fields for Year 2. Excessive heat and drought in Utah in summer of 2021 also caused significant commercial crop losses there, but bacterial surveys were still conducted. In UT research trials, onion plants stopped growing just as the crops were at bulb initiation due to extreme heat, and by harvest the plants were still very stunted. Final results are not available yet.

Cancellation of LabGuru licenses

During Year 2, we decided to centralize and simplify the compilation of regional bacterial survey data, using an Excel spreadsheet instead of proprietary software from LabGuru. We did not renew the LabGuru licenses for Year 2 so the funds ($3,800) were instead allocated to support additional staff time for redesign of the Alliumnet website.

COVID19 impacts

- The lack of in-person SAP meetings has led to a shortfall in reported cost-share funding, since almost all SAP members had committed their travel and time to attend team meetings as part of their match. We plan to replace this with the value of strains donated to the project from pre-existing collections held by several team members and collaborators - these strains allowed us to move ahead with preliminary pathogenomics work while the Season 1 bacterial surveys were completed.
- In WA, restrictions regarding travel and hotel stays increased in-state travel expenses considerably (no sharing of hotel rooms, more cars needed to travel between Mount Vernon and Pasco each time. In addition, a positive COVID-19 incident in the WA program in Nov. 2020 resulted in that individual being on medical leave for 3 weeks, which added to the workload of others in the program working on this project, with delayed ability to make as much progress on the survey work (bacterial strain identifications) as planned.
- In PA, the University mandated COVID-19 restrictions delayed processing of the Year 1 bacterial survey samples from PA and NY. In addition, the inability to extract DNA for sequencing and conduct pathogenicity testing prior to putting the isolates into long-term storage significantly increased the labor and material costs required for this portion of the survey. However, as restrictions are beginning to lift and more people can access the lab, PA team members are finalizing the genera identifications of select isolates as well as running the onion pathogenicity tests. PA and other states anticipate there will be more opportunities for Extension and outreach in Season 3.
- At the University of Pretoria, the research undertaken in Year 2 was largely computer-based so both MSc students and their advisors either worked from home or from the lab (there was a rotational scheme in place because of COVID restrictions so the University of Pretoria lab is limited in the number of staff/students that can be present in the lab each day).
- Due to COVID-based restrictions, Postdoctorate Shin was not able to secure a visa to allow her to start her position with the Kvitko group at UGA in September 2021. A temporary position was created for her in the lab of collaborator Coutinho at the University of Pretoria until early 2021, when she was granted a visa to travel to Georgia. Dr. Shin contributed to project objectives from South Africa by genotyping and phenotyping South African Pantoea strains, which will ultimately add diversity and robustness to our analysis, and she conducted bioinformatics analysis. Shin also conducted a meta-analysis of published and unpublished Pantoea species-specific PCR primer sets, which will simplify Pantoea species identifications.
- Many of the states involved in this project could not hold regular in-person meetings and other events such as field days for sharing results or demonstrating field trials from this project. If COVID constraints continue to ease up over the next year, we anticipate more in-person events will take place for all team members.

Other changes

• New SAP members joining in this period included: Larry Duell (CO), Margreet Asma (Netherlands), Kalie Christensen (WI), David Burrell (GA). Russell Hamlin and Kerrick Bauman stepped down from the SAP due to time constraints.

• New team members joining in Year 2 included: Paul Stodghill and Jo Ann Asselin of the USDA-ARS in Ithaca, NY. Their work is not funded through Stop the Rot but their current research is highly complementary to this project, so they are collaborating with the Stop the Rot team on the pathogenomics objective.