

# Combating Onion Bacterial Diseases with Pathogenomics Tools and Enhanced Management Strategies



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More information, reports and research results at <https://alliumnet.com/stop-the-rot/>

## About the Project

'Stop the Rot' is a USDA NIFA SCRI-funded project (2019-2024) investigating the host, pathogens, and environmental factors influencing bacterial diseases of onion. The project has two primary research objectives:

1. Understand the diversity of bacterial pathogens of onion across onion growing regions of the USA, and the genomics of these pathogens;
2. Through research trials, identify onion production practices, environmental factors, and inoculum sources that impact bacterial diseases, and use this knowledge to develop effective, economically-viable disease management strategies as well as predict the risk of losses to these diseases.

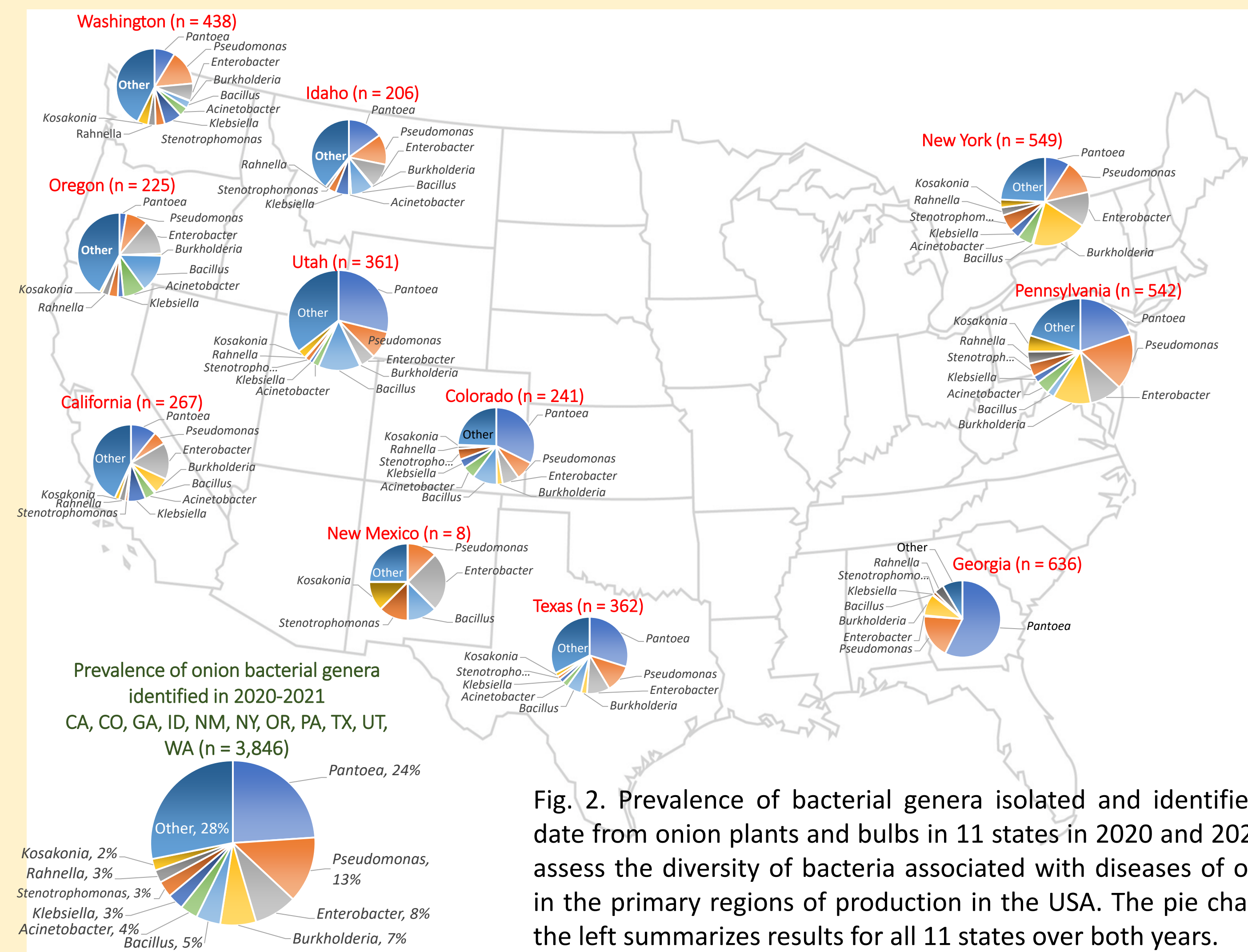


Fig. 2. Prevalence of bacterial genera isolated and identified to date from onion plants and bulbs in 11 states in 2020 and 2021 to assess the diversity of bacteria associated with diseases of onion in the primary regions of production in the USA. The pie chart to the left summarizes results for all 11 states over both years.

## Regional Surveys

- To provide regional characterization of onion bacterial pathogens, team members from 11 states in the USA surveyed symptomatic onion foliage and bulbs in 2020, 2021, and 2022 from 174 locations.
- Based on the 2020 and 2021 surveys, 116 bacterial genera have been isolated to date from >3,500 samples of symptomatic onion foliage and bulbs.
- The distribution and pathogenicity of bacterial genera varied across the USA (Fig. 2).
- The five most prevalent genera in the 2020-2021 surveys were: *Pantoea* (921 strains to date), *Pseudomonas* (501), *Burkholderia* (271), *Enterobacter* (325) and *Bacillus* (184).
- Identification of additional isolates from the three years of surveys are in progress.

## Pathogenicity Testing

Although the survey revealed >116 bacterial genera associated with symptomatic onions, strains of very few genera caused symptoms when tested for pathogenicity on onion using a red scale necrosis assay. Pathogenicity of strains varied among regions and between the 2020 and 2021 seasons (Fig. 3). A subset of the strains is also being tested for pathogenicity to onion using foliar and bulb assays.

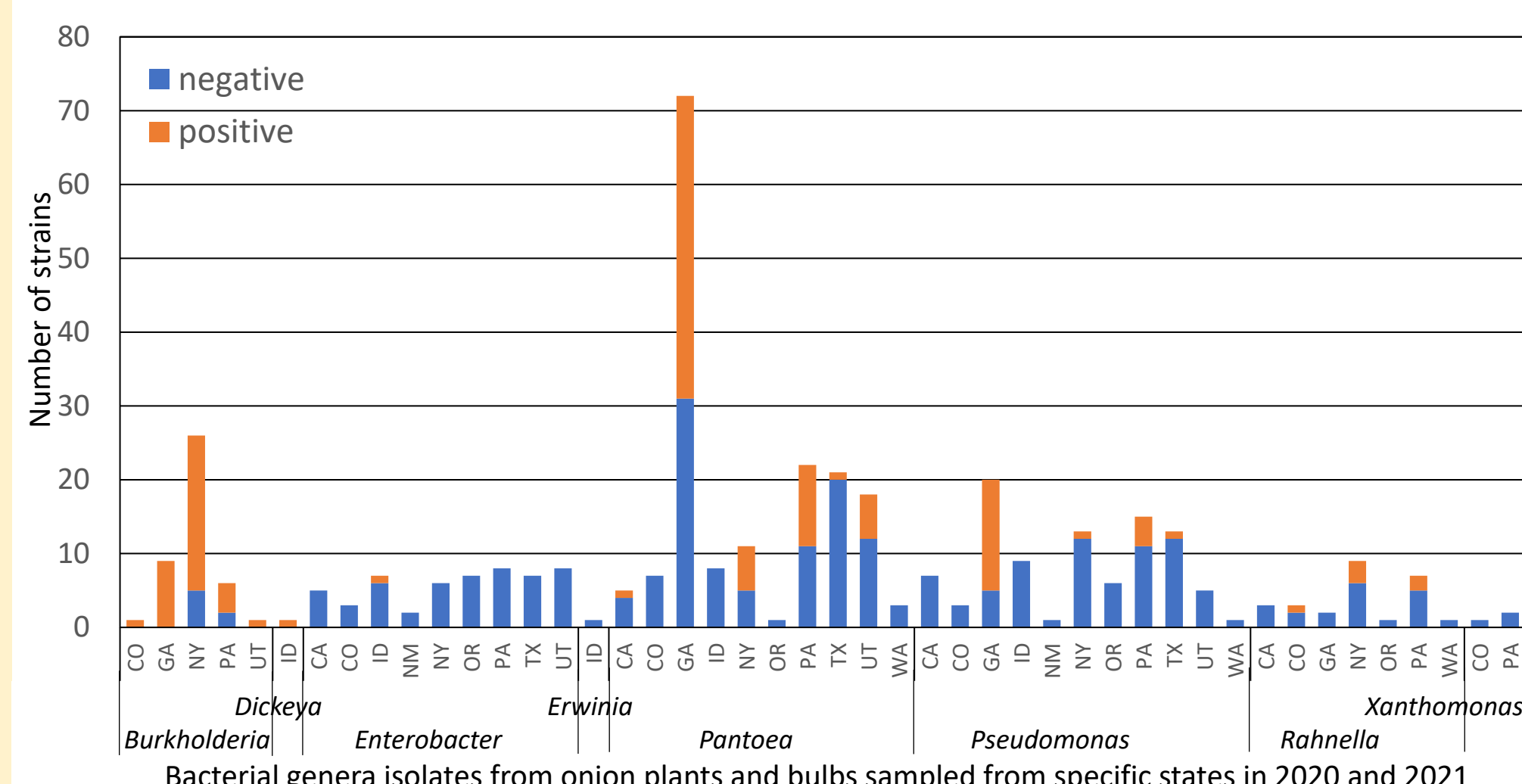


Fig. 3: Pathogenicity to onion of bacterial strains isolated from onion plants and bulbs across the USA in 2020 and 2021, that have been deposited in the National Onion Bacterial Strain Collection (NOBSC) at the Univ. of Georgia. Pathogenicity was determined using a red scale necrosis assay. The NOBSC currently houses 1,376 strains from onion.

## Pathogenomics and Microbiomes

The initial pathogenomic focus of this project is on *Pantoea agglomerans*, a ubiquitous bacterium in onion crops, to identify genes associated with pathogenicity to onion and facilitate developing diagnostic tools to differentiate pathogenic vs. non-pathogenic strains.

### Virulence Mechanisms

The red scale necrosis (RSN) assay for pathogenicity of *P. agglomerans* strains to onion correlated significantly with presence of the **HiVir gene cluster** that encodes a biosynthetic pathway for the phytotoxin pantophos. HiVir is also a critical onion pathogenicity factor in *P. ananatis* strains.

### DNA-based Diagnostic Tools

We are designing species-specific **real-time PCR** and **loop mediated isothermal amplification (LAMP)** assays for key bacterial pathogens of onion based on genetic markers for pathogenicity to onion.

### Copper Tolerance Genes

Copper (*cop*) tolerance gene clusters were identified in ~50% of the onion-associated *P. agglomerans* genomes, usually alongside resistance genes for other metals (arsenic and silver) as well as genes conferring tolerance to sulfur compounds (*alt*) in onion bulbs (Fig. 4). The *cop* genes may explain the limited efficacy of copper bactericides in field trials. Similar *cop* genes were not identified in genome sequences of *P. ananatis* strains from onion, suggesting potential differences in copper resistance mechanisms among *Pantoea* species pathogenic to onion.

### Onion Bulb Microbiomes

Comparison of total bacterial DNA extracted from symptomatic vs. asymptomatic onion bulbs from a field in each of Washington and Georgia showed diverse bacterial communities in symptomatic onions, including known pathogens of onion, and differences in bacterial communities in symptomatic vs. asymptomatic bulbs as well as in bulbs from the two states.



Fig. 4. Type 1, 2, and 3 copper tolerance gene clusters found in strains of *Pantoea agglomerans* infecting onion.

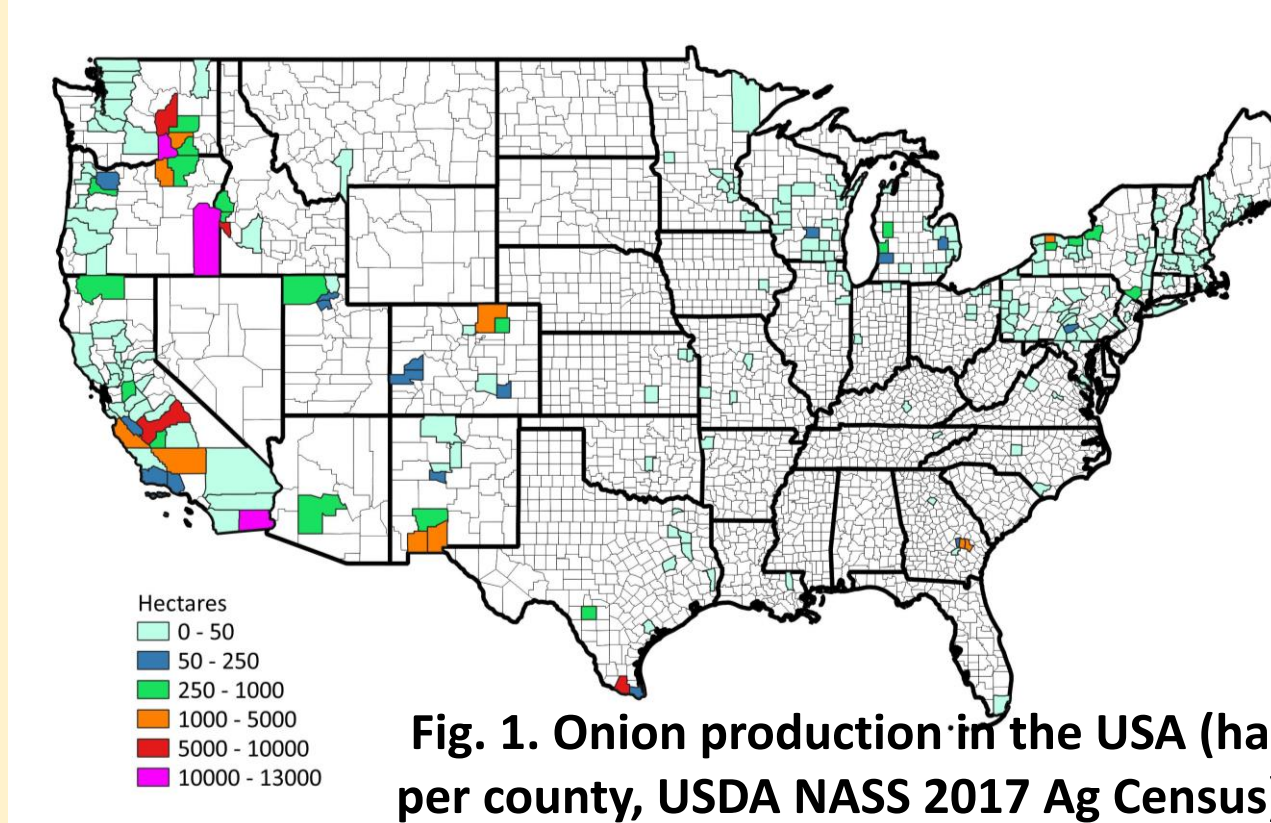


Fig. 1. Onion production in the USA (ha per county, USDA NASS 2017 Ag Census)

## Onion bacterial diseases in the USA

Primary states for onion production in the USA are California, Colorado, Georgia, New Mexico, New York, Oregon, Texas, and Washington (~56,600 ha, Fig. 1). The USA also produces >20% of the world's supply of onion seed. Bacterial diseases of onion affect all regions of production and cause significant losses. Bacterial bulb rots typically develop in storage, after all production and harvest costs have been incurred. Species of more than a dozen bacterial genera are pathogenic on onion, but the virulence mechanisms and interactions of these pathogens with onion plants and the environment are not well understood for most of these pathogens.

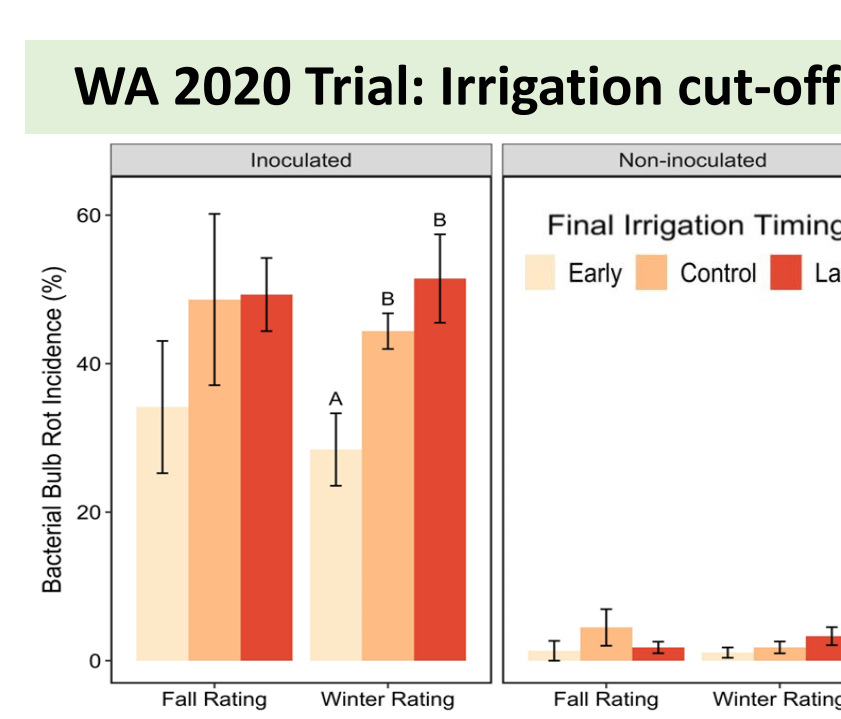
## Enhanced Management Strategies for Onion Bacterial Diseases

Field trials across multiple states are focused on the impacts of various management practices on development of onion bacterial diseases. The trials are evaluating production practices and products to address stakeholder priorities and concerns. For detailed results, see <https://alliumnet.com/stop-the-rot-publications-and-resources>.

### Irrigation

Moisture in onion crops through the growing season has a significant influence on bacterial diseases of onion.

**Late termination of irrigation increased bulb rot:** In semi-arid central Washington, terminating irrigation late (50-100% 'tops down') increased bacterial bulb rot compared to topping early (5% tops down).

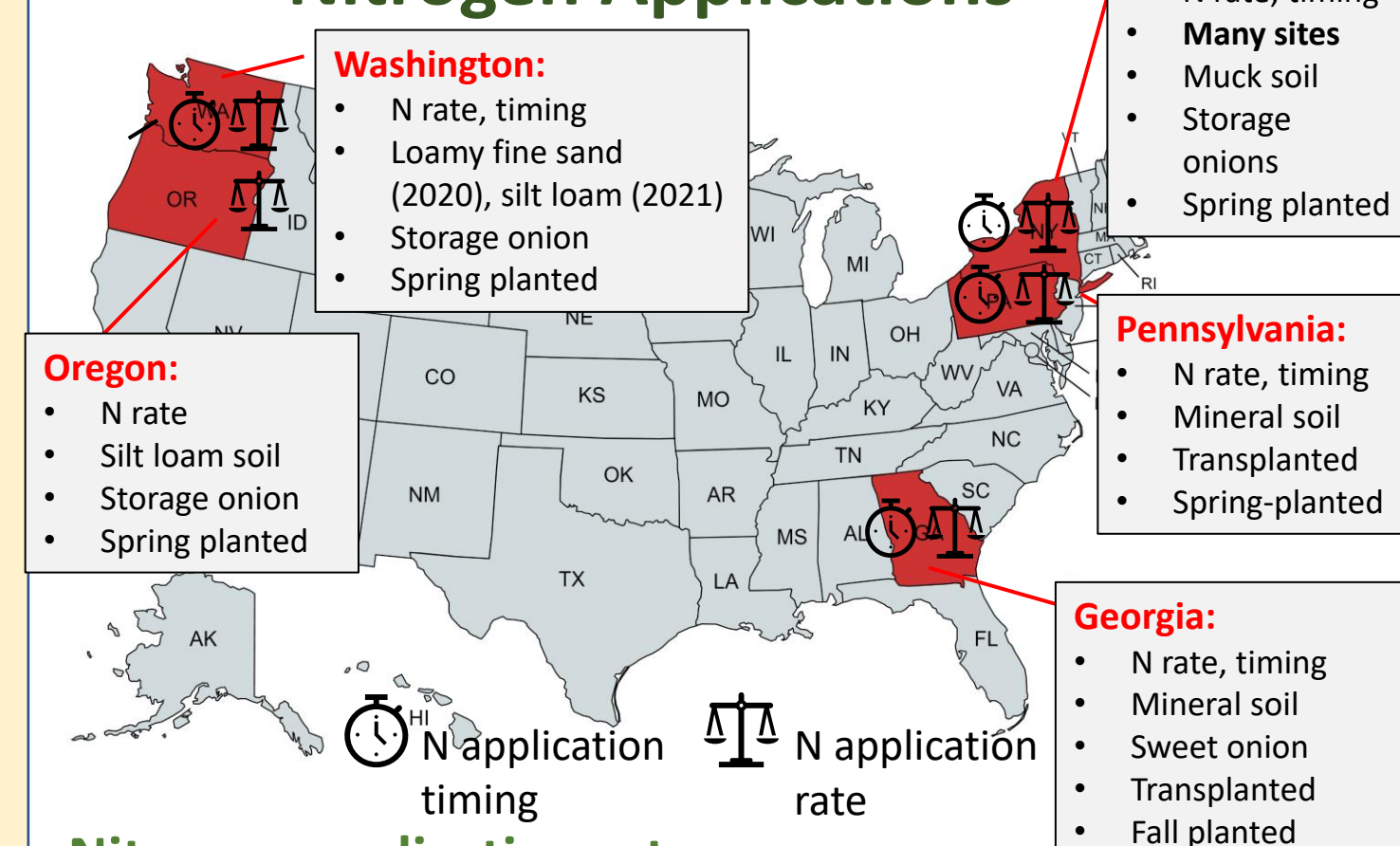


### Drip irrigation reduced bulb rot:

In California, drip irrigation reduced bacterial bulb rot by 97% compared to sprinkler irrigation.

CA 2021 Trial: Irrigation method			
Irrigation method	Bacterial leaf blight (AUDPC)	Bulb yield (t/A)	Bacterial bulb rot (%)
Sprinkler	339 a	48.5 b	22.3 a
Drip	96 b	59.1 a	0.7 b

### Nitrogen Applications



- Nitrogen application rates:**
- No effect on bacterial bulb rot (GA, NY, OR, PA, WA).
- Nitrogen application timing:**
- GA: Applying N after bulb initiation increased bacterial bulb rot.
  - NY: No effect of N timing on bulb rot on muck soil.
  - WA: Applying final N 0, 1, or 2 weeks after bulb initiation did not affect bacterial bulb rot.

## Cultural Practices

### 1. Type of Mechanical Harvester

**Georgia trials:** Harvesting bulbs with a TopAir chain digger decreased the incidence of bacterial bulb rot by 56-88% compared to a TopAir straight-blade undercutter. The latter harvester caused more soil disturbance and bulb wounds.

Method of digging bulbs	2020	2021	2022
Chain digger	3.5 b	9.0 b	1.3 b
Straight-blade undercutter	10.2 a	20.5 a	10.7 a

### 2. Mechanical vs. Manual Harvest

**Georgia trials:** Mechanical harvest of onion bulbs decreased the incidence of bacterial bulb rot by 50-90% compared to manual harvest. Neck length after topping was much shorter with manual harvest.

Mechanical vs. manual harvest	2020	2021	2022
Mechanical (TopAir chain digger)	2.2 b	4.5 b	3.0 b
Manual	10.5 a	14.5 a	12.5 a

### 3. Neck Length after Topping Bulbs

**Georgia trials:** Leaving longer necks (5.0-12.5 cm) after topping sweet onion bulbs decreased the incidence of bacterial bulb rot by ~80% compared to topping necks short (0-2.5 cm long). Topping was done manually when necks were still green.

2021 trial: Neck length	Bacterial bulb rot incidence (%)	2022 trial: Neck length	Bacterial bulb rot (%)
12.5	4.5 y	7.5	10.0 b
7.5	4.0 y	5.0	11.5 b
2.5	19.0 z	2.5	18.0 a
		0	19.5 a

### 4. Timing of Topping Bulbs

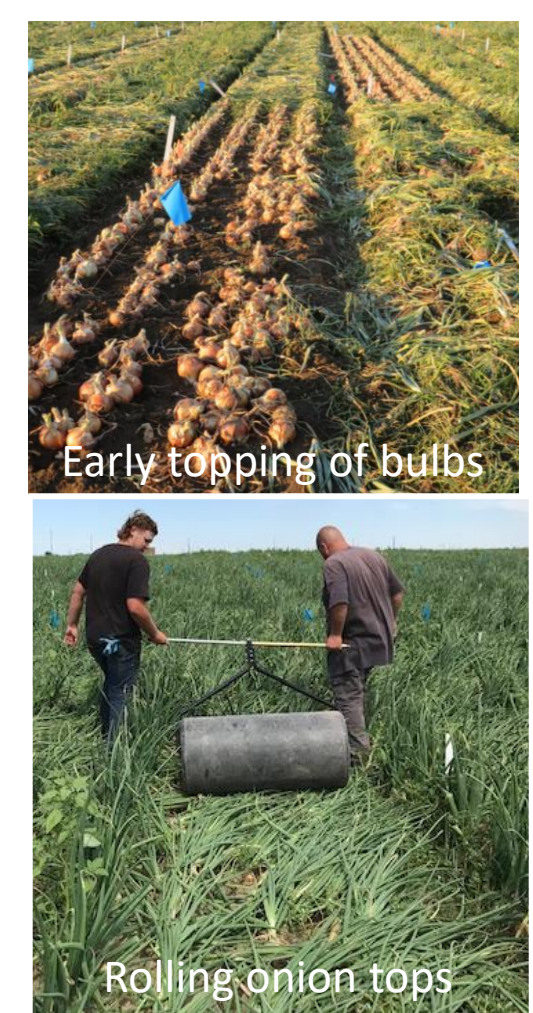
**Washington trials:** Topping bulbs early (<50% tops down) increased the incidence of bacterial bulb rot by 27-34% compared to topping at 100% tops down in 2021 and 2022 trials.

### 5. Timing of Undercutting Bulbs

**Washington trials:** Early undercutting of onion bulbs (~50% tops down) decreased bacterial bulb rot incidence by 12% in a 2022 trial.

### 6. Rolling the Tops of Bulbs

**Washington and New York trials:** Rolling tops while still green increased bacterial leaf blight incidence by 29% in a Washington trial in 2022 but had no effect on the incidence of bacterial bulb rot in Washington and New York trials in 2020, 2021, or 2022.



## Chemical Control

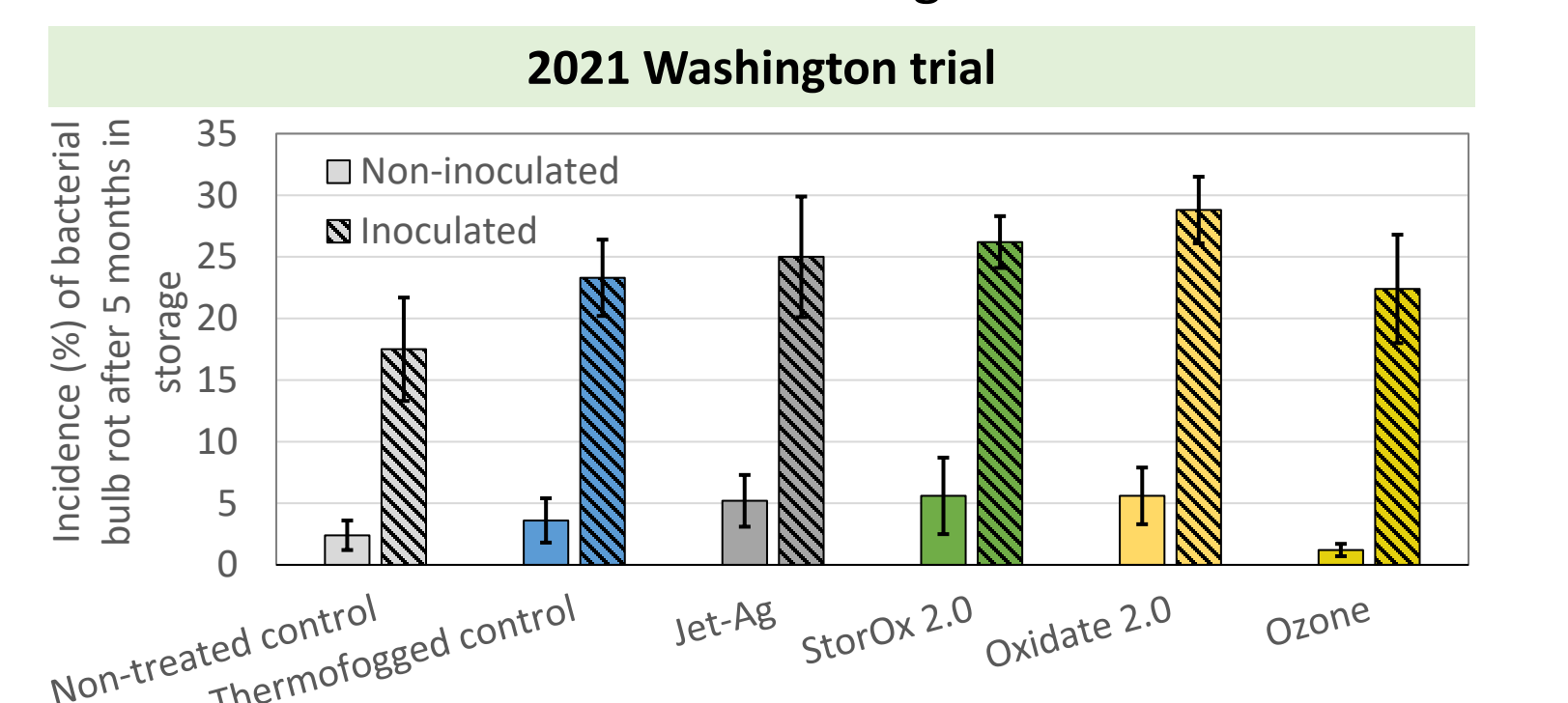
### Foliar Applications of Bactericides

Foliar applications of copper bactericides and LifeGard (*Bacillus mycooides*) provided good control of bacterial bulb rot in winter sweet onion production in Georgia trials, but very poor control in summer production areas in western states. In western states, bacterial infections usually start in the necks rather than the leaves. Also, 50% of *Pantoea agglomerans* strains from the survey of onion crops in western states had copper tolerance genes, unlike strains from Georgia.



### Postharvest Application of Disinfectants

In 2020 and 2021 trials in Washington and a 2022 trial in Colorado, application of ozone or hydrogen peroxide + peroxyacetic acid products to onion bulbs after harvest did not control bacterial bulb rot in storage.



## Acknowledgements

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