THESIS

EVALUATION OF BACTERICIDES AND PLANT DEFENSE INDUCERS IN THE PRESENCE AND ABSENCE OF ONION PATHOGENS *PANTOEA* SPP. AND *BURKHOLDERIA GLADIOLI* IN COLORADO

Submitted by

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ABSTRACT

EVALUATION OF BACTERICIDES AND PLANT DEFENSE INDUCERS IN THE PRESENCE AND ABSENCE OF ONION PATHOGENS *PANTOEA* SPP. AND *BURKHOLDERIA GLADIOLI* IN COLORADO

Bacterial diseases can cause significant annual crop losses if left untreated. U.S. onion growers combat bacterial disease pressure through a variety of management practices, including crop rotation, irrigation management, and proper cold storage postharvest. In 2019, twelve national research institutions formed a collaborative project called "Stop the Rot" (StR) to develop diagnostic identification tools and research cultural practices across different onion growing regions to mitigate onion bacterial rots. As part of that effort, CSU researchers conducted two years of field trials in 2020 and 2021 to compare the efficacy of commercially available bactericides to prevent bacterial rot on three different onion cultivars (Avalon, Snowball, Vaquero). Artificial bacterial inoculation was prepared using *Pantoea* spp. and *Burkholderia gladioli* isolates. The pesticide products included the following types: traditional copper bactericides (Kocide and ManKocide), sanitizers (Oxidate 5.0), biological microorganisms (BlightBan A506), and plant defense inducing productions (Actigard and Lifegard). At harvest in year one, the incidence of bacterial rot was not significantly impacted by bactericide treatment since rot was not present in 2020. In 2021, for 'Vaquero' onion, the Kocide treatment had significantly more rot compared to other treatments and the control after cold storage. For 'Snowball', the BlightBanA506 treatment had a significantly- higher rot percentage than the ManKocide treatment. These results will help Colorado onion growers accurately assess

production risks and select bactericides that meet their needs on the farm. Ongoing research on bactericide efficacy, combined with collaborative results from colleagues in Georgia, New York, Utah, and Washington, will help domestic producers “stop the rot” in their onion crops.

ACKNOWLEDGEMENTS

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DEDICATION

To those who have emotionally, spiritually, and mentally guided me;

The female scientists, who encouraged me to keep going;

The farmers who, day after day find solutions to uncompromising challenges;

My Portuguese immigrant parents, Joe and Teresa Machado, and grandparents, Francisa Avila, and Joe and Odilia Machado, who risked and sacrificed their whole selves for the opportunities I have today, I am forever grateful and honored to be your legacy,

This research thesis is dedicated to you!

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CHAPTER 1 Introduction

Plant pathogenic bacteria can cause significant losses in production agriculture. For onions (*Allium cepa*), bacterial diseases and bulb rots have the potential to cause devastating economic and production losses. This threat is particularly large for onion growers due to limitations in detection, management strategies, and bacterial epidemiological nuances. Onions are often listed in the top four most consumed vegetables annually and have a long history of medicinal, nutritional, and culinary importance (Griffiths et al., 2002; National Onion Association, n.d.; USDA, 2020). An estimated total of 134,700 acres of onions were planted throughout the United States in 2020; an increase of 2% from the previous year. Nationally, the crop value of onions in 2020 was an estimated $878 million (United States Department of Agriculture & National Agricultural Statistics Service, 2020). Specifically in Colorado, an average of 2,860 acres has been harvested annually from the last three years (USDA & NASS, 2021). Onions are one of the top vegetables produced in Colorado contributing $28 million in production value in 2020 (USDA & NASS, 2021).

Colorado has a semi-arid climate which is ideal for growing onions. By weight, Colorado grows and ships an estimated 260 million pounds of onions annually across the U.S. and internationally (Addison & Larson, n.d.). Farmers in Colorado primarily produce dry bulb storage onions. This means the onions are grown throughout the summer, harvested in the fall, placed into storage for two to six months, and then sold throughout winter, or when the market prices are best (Schwartz, 2013). It is during this storage period that onion bacterial rots can develop and cause deterioration within individual bulbs making them unmarketable and therefore must be culled.

Bacterial diseases are very disruptive to the national storage onion industry. An estimated $60 million in losses can be attributed to onion bacterial diseases each year (SCRI Grant Proposal No. 2019-51181-30013(REESI,USDA, 2019)). The American Phytopathological Society (APS) has identified over 15 genera and species of bacteria that cause a variety of onion and garlic bacterial diseases (Schwartz et al., 2015). Many of these bacteria species persist on plant material, such as leaves bulbs, weeds, and seeds. They are dispersed through water, seeds, infected plant parts, and insects (Schwartz, 2013). Many bacteria generally live epiphytically on a plant; it is not until they physically enter a host plant that they begin to cause disease. Bacteria can only enter an onion plant through natural openings such as lower senescing leaves and wounded leaves (e.g., insect feeding, hail, mechanical equipment, etc.). Additionally, splashing water, over-head irrigation, and rain can all move bacteria into the neck opening of the onion bulb (Swett et al., 2019).

Onion bacterial pathogen prevalence varies greatly throughout production regions in the United States based on climate, management practices, pest pressures, etc. In an effort to better understand bacterial pathogens, 12 research institutions throughout the country, including Colorado State University (CSU), joined an initiative focusing on five primary genera; *Burkholderia, Enterobacter, Pantoea, Pseudomonas,* and *Xanthomonas.* The project was called "Stop the Rot" (StR) and its stated mission is "to combat onion bacterial disease with pathogenetic tools and enhanced management strategies". All these genera can cause significant disease and yield losses throughout growing regions in the United States. In addition, all five genera are known to occur in Colorado's production fields. However, onions can also host complex microbial communities inside the bulbs (Yurgel et al., 2018), so it can be presumed that often more than one species of bacteria are present within an individual bulb. By determining

when, how, and which bacteria cause infection, growers can better manage the development of disease and prevent the loss of marketable bulbs. The scope of this project was to evaluate different chemistries and modes of action of currently available bacteria-control products currently available on the market and understand yield and rot impacts.

All five of these bulb-rotting pathogens are gram-negative, rod-shaped bacteria that rely on natural openings to gain entry into the plant. The bacteria enter the onion, either through wounded leaves or the neck opening, begin to establish, and can then cause infection. Infected plants often show few symptoms in the case of mild infection. In high pathogen pressure environments, common symptoms may include some or all of the following, depending on the species causing disease: 1. water-soaked lesions streaking down the length of the leaf blade, 2. partial wilt and dieback of young center leaves, 3. yellowing and die-back of outer leaves, 4. white, tan and/or brown lenticular lesions with water-soaked margins, 5. outer scales appearing watery and collapsed (Schwartz & Gent, 2011; Schwartz & Mohan, 2016; Swett et al., 2019). The risk is that some of these symptoms often go unnoticed and/or untreated.

Specifically, *Burkholderia gladioli* pv. *alliicola* (syn. *Pseudomonas gladioli* pv. *alliicola)*, and *Pantoea ananatis* are both gram-negative, rod-shaped bacteria that can cause bulb rotting diseases. *Burkholderia gladioli* causes the disease called slippery skin. Generally, there are no leaf or external symptoms at harvest except for softening of the neck tissue. As the onion remains in storage, the rot progresses from the top of the infected scales downward. Once it reaches the basal plate, the pathogen can spread to adjacent scales. Infected scales can be visible when the bulb is cut vertically (from neck to basal plate) and will look soft, water-soaked, and may have a yellow or yellow-brown discoloration (Schwartz & Mohan, 2016). *Pantoea ananatis* is the causal agent for the disease center rot. Foliar symptoms may appear on young center leaves

of the plant. Additionally, small water-soaked lesions extending the length of the leaf develop downward toward the neck resulting in bulb infection. Rotten scales near the center of the bulb may turn soft and watery with a faint yellow appearance. In severe cases, a bacterial ooze may develop and the foliage can tear away from the bulb (Schwartz & Mohan, 2016).

Harvest crews are often trained not to pack decaying bulbs or those that have necks that appear to be soft and uncured as this is a symptom of bacterial bulb disease. The concern is that many bulbs do not show these symptoms at harvest since they manifest internally. Most bacterial bulb diseases develop visible symptoms only after several months in storage, and after destructive (i.e. cutting bulbs) sampling. This can be a devastating discovery when a grower is relying on selling this stored crop and realizes the onions are not marketable and must be culled. Therefore, onion producers need to be proactive in the preventative management of onion bacterial diseases. Some mitigation strategies can be very simple, whereas others may require extensive resources.

Traditional management strategies for bacterial pathogens vary greatly throughout different growing regions. Many growers and agronomists plan their strategies based on the disease triangle concept. The disease triangle focuses on three factors: a conducive environment, a susceptible host plant, and a virulent pathogen. All three of these factors contribute to the pathogen's success in causing disease (Francl, 2001). Modifying the environment, the plant, and/or managing the pathogen are all ways to prevent a successful bacterial infection. The environment can be altered through some of the following: irrigation practices that limit overhead water distribution, irrigation timing to mitigate excess humidity in the canopy, and effective pest management to avoid excess damage to plant leaves. The environment in storage spaces post-harvest is also critical for pathogen prevention. Storage sheds and coolers should

remain between 35-41°F to prevent most microbial growth. There are currently no resistant cultivars commercially available. However, the use of certified pathogen-free seed, proper plant nutrition, and chemical spray applications of plant defense inducers are methods of reducing a pathogen's success by altering the plant/host. Lastly, the pathogen itself can be the primary focus for disease prevention. Bacterial pathogens are almost always present in natural field settings so diminishing their ability to cause disease is critical. This can be done through crop rotation, not placing cull piles in or near the field, and removing infected bulbs and plants from the field. Additionally, many growers and field managers use spray applications of bactericidal and/or sanitizing products to reduce the pathogen load during the growing season (Schwartz, 2013; Schwartz & Mohan, 2016). Onion producers have developed strategic long term field and storage management plans to best defend against onion bacterial pathogens. However, day-to-day field management often continues to rely heavily on complex, costly spray programs to prevent bacterial diseases. Investment cost, timing, climate and efficacy of preventative spray programs further complicate the issue, leaving producers wondering if they are even worthwhile.

Dating back to the mid-1800s with the use of the Bordeaux mixture, copper-based pesticides have been trusted to manage bacterial and fungal diseases (Klittich, 2008). Although the formulation has changed and evolved throughout the years, "copper-based products have broad-spectrum activity against microorganisms due to [the metal's] interaction with nucleic acids, interference with energy transport, and distribution of enzyme activity and integrity of cell membranes" (Pscheidt, 2021). Copper in the form of copper hydroxide has become the main active ingredient in commercially available bactericides marketed for onions in the field. Enhanced control of bacterial disease has been reported when the fungicides maneb or mancozeb have been mixed together with a copper hydroxide because it produces a copper carbamate

(Pscheidt, 2021). However, maneb and mancozeb both have ethylene bisdithiocarbamate (EBDC) as an active ingredient and may be phased out as reports continue to indicate it is a multipotent carcinogenic agent (Belpoggi et al., 2002; Houeto et al., 1995). Furthermore, several cases of plant pathogenic bacteria, including pseudomonads, xanthomonads, and *Erwinia*, have been reported worldwide to have achieved resistance to copper-based products. When these species develop this type of resistance, they continue to multiply without being affected by copper treatments at standard concentrations (Lamichhane et al., 2018; Scheck & Pscheidt, 1998). The general solution is to increase the concentration of the product to reach pathogen control. However, prolonged use of copper-based products and over-label use have led to accumulations of copper in surface horizons of agricultural lands (Lamichhane et al., 2018). Copper residues in the soil have the potential to be toxic to the soil microbiome which can ultimately affect soil structure, stability, and the mobilization of pesticides and other pollutants (Kent & Triplett, 2002). The long-term effects of copper on soil health are continually being evaluated. Restrictions and recommended reduction of copped-based pesticide use has been implemented throughout agricultural regions of the world and may impact current onion bacterial management practices. Therefore, it is imperative to determine alternative field strategies for onion bacterial disease management that minimize environmental impacts and reduces the likelihood of pathogen resistance. “Alternative” type products, such as, crop sanitizers, plant defense inducing chemicals, and antagonistic microorganisms have all shown promising results for various crops and bacterial infections either on their own or incorporated into an existing spray program (Galal, 2017; Gent & Schwartz, 2005; Lang et al., 2007; Obradovic et al., 2004; Stumpf et al., 2021).

Crop sanitizers in the form of peroxyacetic acid (PAA) have been highly successful in postharvest control of bacterial soft rots in cucumber, eggplant, okra, pepper, potato, tomato, and squash (Galal, 2017). Peroxyacetic acid is an oxidizing surface disinfectant and there is hope that it might show success in the field as well. These products work as a disinfectant by deteriorating the cell's membrane and oxidizing the inner cell structures of the bacteria present; ultimately making it a nonspecific biocide (EPA, 2012). PAA is widely used by commercial growers in eastern Colorado, but no independent, peer-reviewed research has been conducted to determine its efficacy in preventing and/or treating internal onion bulb rot in the state.

Alternatively, products that induce systemic acquired resistance (SAR) do not have antimicrobial properties but instead stimulate the plant's defenses. SAR is a heightened state of defense that the plant activates in the presence of damaged tissue at the site of infection. The SAR pathway is regulated through a series of mobile signals, accumulation of the plant growth regulator (PGR) salicylic acid, and secretion of proteins. When a foliar application is made, Acibezolar-S-methyl (ASM), a structural analog of salicylic acid, can cause the plant to activate SAR (Bargabus-Larson & Jacobensen, 2007; Fu & Dong, 2013; Kunkel & Brooks, 2002). The derived molecule, ASM, is the active ingredient in the commercial product ActigardWG (Syngenta Crop Protection Inc., Greensboro, North Carolina). Successful management of center rot, caused by *Pantoea ananatis* in Georgia, and leaf blight, caused by *Xanthomonas axonopodis* pv. *allii* in Colorado has been shown by incorporating the use of SAR inducer products in established crop protection spray programs (Gent & Schwartz, 2005; Lang et al., 2007; Stumpf et al., 2021). However, some studies have shown the possibility of decreased yield in fruiting vegetables, i.e. peppers (Romero et al., 2001). Gent and Schwartz et al. (2005) also showed that

onions can suffer a yield penalty if SAR activator products are applied at two and a half times the labeled rate and in the absence of a virulent pathogen.

There are different types of plant defense inducing/SAR products on the market. For example, LifeGardWG from Certis USA (Columbia, Maryland) uses a strain of *Bacillus mycoides*, as the primary active ingredient (Certis USA, 2018). Activation of SAR is achieved in a similar way without needing the accumulation of salicylic acid (Bargabus-Larson & Jacobensen, 2007). However, LifegardWG is not labeled for use against onion bacterial bulb rot. Yet, comparing the efficacy of this SAR inducer product to other bactericide modes of action can help determine the feasibility of incorporation into disease management systems.

Lastly, the addition of biological microorganisms (also known as antagonistic pathogens, bacteriophages, and/or biological control agents) have shown success in out-competing pathogenic bacteria. Biological control of pathogenic bacteria has had commercial success in the fruit tree industry when combating fire blight, caused by *Erwinia amylovora* (Stockwell et al., 2016). While researching antagonistic properties of over 70 epiphytic microorganisms, Long et al., (2003) observed 40 isolates that had *in vitro* antagonistic properties against plant pathogenic bacteria. Of these 40, a large portion of them were classified as members of the genus *Bacillus* and fluorescent Pseuedomonads (Long et al., 2003). Additional research in Colorado demonstrated biological control of Xanthomonas leaf blight at comparable levels to copper-based products when using a mixture of *Pantoea agglomerans* and *Pseudomonas flourescens* (Gent & Schwartz, 2005). In concluding their work, the authors identified the need for further research to be conducted to evaluate *Pantoea agglomerans* and *Pseudomonas flourescens* against other bacterial pathogens, such as those that cause center rot and sour skin. In an effort to fill the

gaps in existing research, this study compares four types of commercially available bactericide products on onions grown in a field environment in Colorado.

The objectives of this study were to evaluate how field applications of copper-based bactericides, plant sanitizers, SAR inducers, and biological control agents impact onion production in the presence and absence of bulb rot pathogens *Pantoea* spp. and *Burkholderia gladioli*. Based on previous literature, it was hypothesized that "alternative" product types will be as effective as copper-based products at managing disease caused by onion pathogens *Pantoea* spp. and *Burkholderia gladioli* compared to the water-treated control. Investigating new management strategies for bacterial pathogens are necessary for mitigating crop losses, minimizing long-term environmental impacts, and reducing input costs. Furthermore, it is important to prepare the industry as future restrictions on copper applications continue to expand and resistance spreads.

CHAPTER 2 Materials and Methods

2.1 Onion Stand Establishment

Field plots were established during the 2020 and 2021 growing seasons in Fort Collins, CO at Colorado State University's (CSU) Agricultural Research Development and Education Center South (ARDEC S.)(40.610012, -104.993979; elevation: 1523m). Prior to bed shaping, soil samples were collected and analyzed at the CSU Soil, Water and Plant Testing Laboratory on 2 March 2020, and a recommendation was given to add phosphorous before planting. Super phosphate fertilizer (11:52:0) at a rate of 39.2 kg/ha was incorporated 12 March 2020. Soil fertility testing conducted on 11 March 2021 by American Agricultural Laboratory Inc. in Cook, Nebraska, determined no need for any soil amendments for the 2021 season. Regional agricultural practices for the production of onions including bed shaping, seeding, irrigation, and pest management were followed for the establishment of the onion stand (Schwartz, 2013).

Onion cultivars Avalon (Gowan Seed, Chular, CA) and Vaquero (Nunhems, Haelen, Netherlands) were direct sown using raw and pelletized seed, respectively. In 2020, seeds were planted into 76.2 cm wide beds with two rows of onions per bed. The initial planting occurred 9 April 2020 at a depth of 7.6 cm followed by a replanting on 30 April, 2020 at a depth of 3.8 cm due to poor stand. Two hand thinning events were required early in the season before bulb initiation to achieve a final spacing of 7.6 - 10.2 cm between individual plants. Eight beds of onions were planted in total in 2020 (Figure 2.1). In 2021, onion cultivars Avalon and Vaquero were direct seeded on 29 March. Seeds were sown into 76.2 cm beds with three rows of onions per bed. In addition, two rows of white onion cultivar Snowball sets were transplanted on 10

June 2021. Eight beds of onions were planted in total, three ‘Vaquero’, three ‘Avalon’ (Figure 2.2) and two ‘Snowball’ (Figure 2.3).

After planting, sprinkler irrigation was used for the initial watering. In April, May, and June of 2020, 0.48cm, 5.05cm, and 3.25cm of natural precipitation fell, respectively. Spring of 2021 was wetter with April and May receiving 3.56cm and 8.05cm of natural precipitation, respectively (Colorado State University, CoAgMET, 2020-2021). In 2020 surface drip irrigation was installed using Irritc P1 Ulta 5/8” drip tape with a flow rate of 0.33 gallons per hour (gph) at 10 pounds per square inch (psi) (Irritec USA Inc. Fresno, CA). From April through October plots were irrigated for an average of three (3) hours once a week. 2021 surface drip irrigation was installed using Toro drip tape with a flow rate of 0.27 gph at 10 psi (The Toro Company, El Cajon, CA). From May through October plots were irrigated for an average of three (3) hours once a week.

Weed control was achieved by hand pulling combined with an application of Sonalan HFP (Gowan Company, Yuma, AZ) on 21 July 2020. Prowl H2O (BASF Ag Products, Ludwigshafen, Germany) at 1.5 pt./acre and Roundup PowerMax (Bayer CropSciences, Monheim am Rhein, Germany) at 22 fl. oz/acre was applied on 24 April and 9 July 2021 only between the rows. One hail event occurred on 8 June 2020 and caused minimal damage to the foliage of the onion crop. Wildfire smoke was notable in the late summer and fall of 2020. Aside from a historically high amount of precipitation in spring, no hail events or abnormal weather phenomena occurred during the 2021 growing year.

		Buffer							Buffer							Buffer							Buffer						
		Buffer							Buffer							Buffer							Buffer						
Inoculated	Vaquero	6	1	3	7	2	4	5	7	3	4	1	5	2	6	4	3	1	7	6	5	2	1	4	3	5	6	7	2
Inoculated	Vaquero	6	1	3	7	2	4	5	7	3	4	1	5	2	6	4	3	1	7	6	5	2	1	4	3	5	6	7	2
Inoculated	Avalon	6	1	3	7	2	4	5	7	3	4	1	5	2	6	4	3	1	7	6	5	2	1	4	3	5	6	7	2
Inoculated	Avalon	6	1	3	7	2	4	5	7	3	4	1	5	2	6	4	3	1	7	6	5	2	1	4	3	5	6	7	2
Buffer		Buffer							Buffer							Buffer							Buffer						
Uninoculated	Vaquero	1	7	5	2	3	4	6	5	6	3	7	2	4	1	6	7	4	2	5	3	1	3	7	4	2	1	5	6
Uninoculated	Vaquero	1	7	5	2	3	4	6	5	6	3	7	2	4	1	6	7	4	2	5	3	1	3	7	4	2	1	5	6
Uninoculated	Avalon	1	7	5	2	3	4	6	5	6	3	7	2	4	1	6	7	4	2	5	3	1	3	7	4	2	1	5	6
Uninoculated	Avalon	1	7	5	2	3	4	6	5	6	3	7	2	4	1	6	7	4	2	5	3	1	3	7	4	2	1	5	6
Buffer									Buffer							Buffer							Buffer						
		BLOCK I							BLOCK II							BLOCK III							BLOCK IV						

#	*Treatment*
1	Actigard
2	BlightBan
3	Lifegard WG
4	ManKocide
5	Kocide 3000
6	Oxidate
7	Control

North

Figure 2.1. 2020 field layout at ARDEC South in Fort Collins, CO for cv. Avalon and Vaquero. Field length was a total of 85.34 meters (286ft) east to west. Block size 21.28 meters long by 3.04 meters wide per cultivar. Plot size was 3.04 meters long by 1.52 meters wide. Barley was planted in the buffer row and maintained at 0.5 meters tall by mowing. Inoculated beds were located on north side of field and are represented in yellow. Numbers indicate the treatment randomly assigned to individual plots.

Uninoculated	Avalon	4	2	5	6	3	1		4	6	3	5	1	2	2	5	4	3	1	6
Uninoculated	Vaquero	4	2	5	6	3	1		4	6	3	5	1	2	2	5	4	3	1	6
Buffer		Buffer						Buffer	Buffer						Buffer					
Inoculated	Avalon	6	4	3	2	5	1		3	5	1	6	4	2	3	6	1	2	5	4
Inoculated	Avalon	6	4	3	2	5	1		3	5	1	6	4	2	3	6	1	2	5	4
Inoculated	Vaquero	6	4	3	2	5	1		3	5	1	6	4	2	3	6	1	2	5	4
Inoculated	Vaquero	6	4	3	2	5	1		3	5	1	6	4	2	3	6	1	2	5	4
		Buffer						Buffer	Buffer						Buffer					
		BLOCK III						DEAD ZONE	BLOCK II						BLOCK I					

#	*Treatment*
1	Actigard
2	Lifegard WG
3	ManKocide
4	Oxidate
5	Kocide 3000
6	Control

North

Figure 2.2. 2021 field layout at ARDEC South in Fort Collins, CO for cv. Avalon and Vaquero. Workable field length was 65.83meters (216ft). "Dead Zone" signifies the area of the field with significant seedling damping off. Inoculated plot size was 3.66 meters long by 1.52 meters wide. Uninoculated plot size was 3.66m by 0.76m. Barley was planted in the buffer row and maintained at 0.5 meters tall by mowing. Inoculated beds were located on south side of field and are represented in yellow. Numbers of each plot indicate the treatment randomly assigned to that plot.

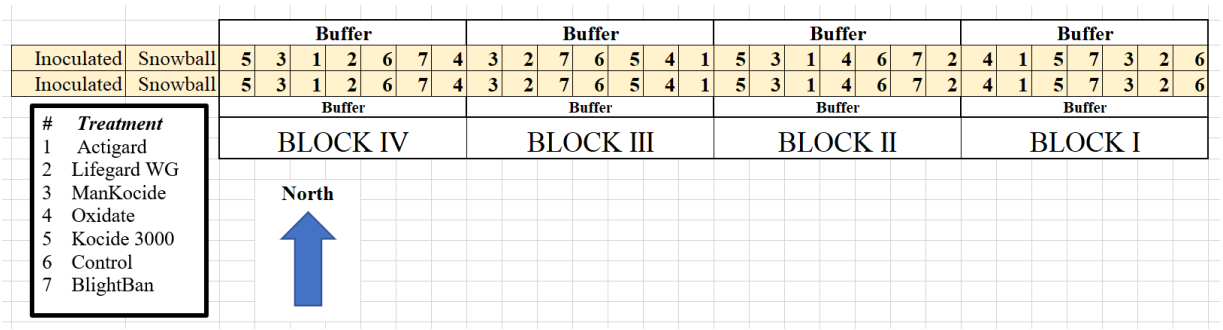

Figure 2.3. 2021 Field layout at ARDEC South in Fort Collins, CO for cv. Snowball. Workable field length was 85.34meters (280ft). Plot size was 3.05 meters long by 1.52 meters wide. Barley was planted in the buffer row and maintained at 0.5 meters by mowing. Inoculated beds are represented in yellow. There were no non-inoculated plots for this cultivar. Numbers of each plot indicate treatment randomly assigned to that plot.

2.2 Experiment Design/Field Design

2.2a Season 1, 2020

The field experiment was arranged as a randomized complete block design (RCBD) with bactericide treatments in split-split plots. The first split was for inoculation status (i.e., inoculated or uninoculated). The second split was for cultivar (Vaquero and Avalon). Because analysis for cultivars was done separately, the design becomes a classic split-plot "missing single randomization" due to the way the plots were seeded. From north to south, two beds of 'Vaquero' and then two rows of Avalon were planted and randomly selected as the inoculated portion of the field. Between the two portions of the field, a single bed of barley was planted to act as a buffer between inoculated and non-inoculated plots. Additionally, two buffer rows of barley were planted adjacent to the north most beds, one bed on the south most side (Figure 2.1). Barley was maintained at 0.5 meters tall by mowing throughout the growing season. West to east, the total field length was 85.34 meters long. To accommodate seven treatments (including a control), each individual plot was 3.04 meters long by 1.52 meters wide. Each plot consisted of two beds with two lines of onions per bed. The treatments were replicated four times down the

length of the entire field. No alley was allocated between plots; a physical barrier was used during treatment applications to avoid drift to/from adjacent plots (Figure 2.4).

Figure 2.4. Use of physical barrier between treatments during application to avoid product drift.

2.2b. Season 2, 2021

The field experiment was arranged as a randomized complete block design (RCBD) with split plots for inoculated and uninoculated in both cultivars ‘Vaquero’ and ‘Avalon’. Again, the design was a classic split-plot “missing a single randomization layer”. All of the ‘Snowball’ rows were inoculated (i.e. there were no uninoculated ‘Snowball’ bulbs).

From north to south, one bed of ‘Avalon’ and then one row of ‘Vaquero’ were planted and selected as the non-inoculated portion of the field. The second portion of the field was two beds of ‘Avalon’, directly followed by two rows of ‘Vaquero’. This became the inoculated portion of the field (Figure 2.2 and Figure 2.3). Between the two portions of the field, a single bed of barley was planted to act as a buffer dividing inoculated and non-inoculated plots. Directly south of the Vaquero beds was another row of barley followed by the two rows of the Snowball cultivar that were planted later in the season.

Based on stand development the total workable field length was 65.83 meters (216 ft) long west to east. For the 'Avalon' and 'Vaquero' portion of the field, six treatments, including a control, were randomized in each replication. The treatments were replicated three times down the length of the entire field. Each of the six treatments was an individual plot and the dimensions were 3.66m in length and 1.52m wide. For the Snowball cultivar, the entire 85.34m field length was used. The field was divided into four replications with seven treatments randomly assigned within a replication. Plot dimensions were 3.05m by 1.52m for each treatment. In all cultivars, a six-inch buffer was allocated between plots at the time of harvest. A large physical barrier was used during treatment applications to avoid drift to/from adjacent plots (Figure 2.4).

2.3 Pathogen Inoculation

2.3a *Pantoea ananatis* and *Pantoea agglomerans*, 2020

The north four beds of each plot were inoculated with the bacterial species *Pantoea ananatis* and *Pantoea agglomerans* on 8, 25 August 2020, respectively, 111 days and 128 days after the average planting date.

Bacterial strains were selected based on red and yellow onion scale assay (Stice et al., 2018). Two bacterial strains from historic plant pathogenic bacterial collections at Colorado State University were selected to inoculate field plots. Based on origin and available metadata, the strains were believed to be virulent.

Bacterial inoculum was prepared similar to Lang (2007), by streaking loops full of bacteria onto nutrient agar and incubating culture plates at 28°C for 72 hr. Cells were harvested from plates by flooding with sterile water and gently scraping the plates with a small, flame-sterilized spatula. A buffer solution was prepared from water. The cell suspension was adjusted

to 10^8 CFU/ml using a spectrophotometer (Optical Density$_{600}$=0.3). Application of inoculum was done in the evenings with a CO_2 pressurized backpack sprayer equipped with a 10ft boom and applied at a concentration of 10^8 CFU/ml (total mixed bacteria) at 40gpa and 20psi. Inoculum was applied to designated rows of cultivars Avalon and Vaquero, four rows total. The south four beds of each plot were sprayed with water to represent a non-inoculated control treatment.

2.3b *Burkholderia gladioli,* 2021

During the growing season, two inoculation events occurred. The first bacterial inoculation was done 108 days after initial planting on 15 July 2021. The plants were approximately 4-6 inches tall and at the 7-8 leaf stage. A second inoculation occurred approximately 2 weeks later at 123 days after initial planting on 30 July 2021.

Bacterial strains used to inoculate field plots were originally collected from Colorado commercial onion production fields from 1990s to the early 2000s. (Leach & Otto, personal communication). Pathogenicity of the four *Burkholderia gladioli* strains used in 2021 field inoculations was confirmed using the red onion scale necrosis assay (modified from Schroeder et al., 2010) to assess the pathogenicity of bacterial isolates to onion bulb scales. Two additional protocols were used to determine bacterial isolate pathogenicity to onion bulbs (Appendix 1) and leaves (data not presented). These pathogenicity evaluations were completed during the winter and spring of 2021 to determine the most virulent bacterial strains from the Colorado State University collection. Protocols used to confirm pathogenicity were approved and standardized for the entire Stop the Rot collaborative project. An assay to assess pathogenicity of bacterial stains on whole onion bulbs was adapted from Schroeder et al. (2010) 2008-2009 season protocols (Schroeder et al., 2010). Leaf assay for pathogenicity of bacterial strains was developed by Dr. Bhabesh Dutta from the University of Georgia Athens (unpublished). The

bacterial culture was prepared the same way as described above. Two leaves of onion seedlings at the five or more-leaf stage were cut approximately 5cm below the leaf tip using surface-sterilized scissors. Using a micropipette, two 10µl drops of bacterial suspension were placed diagonally on the cut surface of each of the cut leaves. Control seedlings were inoculated with sterile water in the same fashion. Seedlings were evaluated 5-days post-inoculation for the presence or absence of necrotic tissue >5cm in diameter.

Application of inoculum was done with a CO_2 pressurized backpack sprayer equipped with a 10ft boom and applied at a concentration of 10^8 cfu/ml (total mixed bacteria) at 40gpa and 20psi. In total, six beds of onions (two of each cultivar: Vaquero, Avalon, and Snowball) were inoculated with the mixture of four *Burkholderia gladioli* bacterial isolates. Bacterial inoculum was prepared by following approved methods from Stop the Rot collaborative project. In summary, the four *Burkholderia gladioli* bacterial pathogens were grown separately overnight in 750ml of nutrient broth at 28°C on a gyratory shaker set at 150-200 rpm. The optical density (600nm) (~10^8 cfu/ml) of the overnight bacterial suspensions was then measured. A bulk suspension combining all four bacterial isolates was then adjusted to a concentration of 10^5cfu/ml in 0.0125M phosphate buffer plus 0.01% Tween 20. This concentrated suspension was then transported to the field site on ice in the dark. Once at the field site, the concentrated inoculum was further diluted to 10^8 cfu/ml using the 0.0125M phosphate buffer plus 0.01% Tween 20. A one-hour overhead sprinkler irrigation occurred after every inoculation event to enhance the potential of successful infection. The non-inoculated beds (one of cv. Vaquero, one of cv. Avalon) located at the north most side of the field were sprayed with only water to serve a control treatment using the same protocol.

2.4 Bactericide Treatment and Timing

Each treatment product was applied to the crop five times at a 7-day interval, on 31 July, 7, 14, 21, and 28 August, 2020. The same application interval was followed in 2021 on 7, 14, 21, 28 July, and 4 August, 2021. Product Actigard was not applied on the 5th interval due to reaching the season maximum labelled rate. Treatments were applied with a CO_2 pressurized backpack sprayer calibrated to deliver 40 gallons per acre at 35 psi using TeeJet XR 11004 VS spray nozzles (TeeJet, Springfield, IL). The surfactant Activator 90 (Loveland Products, Inc., Loveland, CO) was used with each product at 32 oz/100 gal water (0.25% volume to volume ratio) as per label rate. Control plots were treated with water and surfactant. Application rates remained the same, but the total spray volume applied differed from the first season to the next (Table 2.1).

Table 2.1. Product trade name, product type, active ingredient, treatment application rates and spray volumes per year for onion bactericide trials in Colorado for 2020 and 2021 growing season.

Product Name	Product Type	Active Ingredient	Application Rate	2020 Spray Volume	2021 Spray Volume
Actigard 50WG	SAR Inducer	Acibenzolar-S-Methyl (50% by weight)	1 oz/Acre	60-70 Gal/Acre	40 Gal/Acre
BlightBan A506	Biological	*Pseudomonas fluorescens* A506 71% by weight 1 billion (1 x 10^10) CFU/gram	200 grams/Acre (28.35 grams =1oz)	60-70 Gal/Acre	40 Gal/Acre
LifeGard WG	SAR Inducer	*Bacillus mycoides* isolate 30 billion (3 x 10^10) viable spores/g of product	4.5oz/100 gal (28.35 grams=1oz)	60-70 Gal/Acre	40 Gal/Acre
ManKocide	Copper	Mancozeb (15% by weight) + Copper Hydroxide (46.1% by weight)	2.25 lbs/Acre	60-70 Gal/Acre	40 Gal/Acre
Kocide 3000	Copper	Copper Hydroxide (46.1% by weight)	1.5 lb/Acre	60-70 Gal/Acre	40 Gal/Acre
Oxidate 5.0	Sanitizer	Hydrogen Dioxide (27.1%) + Peroxyacetic Acid (2.0%)	Curative rate "1:256 (0.39% v/v"	60-70 Gal/Acre	40 Gal/Acre
Control	NA	NA (water)	NA	60-70 Gal/Acre	40 Gal/Acre
		Total of Treatments = 7 (including water control treatment)			

[1]All applications included the non-ionic surfactant Activator 90 (Loveland Produce, Inc.) at the maximum label rate of 0.25% volume/volume.
[2] BlightBan A506 used only on cultivar 'Snowball' 2021 season.

2.5 Harvest Details

Commercial harvesting practices for Colorado onion producers were followed (Schwartz, 2013). In brief, onion plants were undercut and remaining tops were rolled down 12-15 days before the onions were collected into bags. No undercutting was done in 2020 season, only tops rolled down, due to lack of suitable equipment. Dried onion tops and roots were trimmed by hand with shears, and bulbs were placed into a commercial 50lb. mesh onion bag. Each plot was placed in an individual bag and labeled. A six-inch buffer between each plot was not harvested to account for any treatment drift. Onions within the six-inch buffer were not collected, or accounted for in yield. Mesh bags remained in the field for an additional 9-12 days for field curing. After field curing, bags were brought into the ARDEC S. storage shed and harvest data was collected.

In the 2020 season, 'Avalon' was harvested on 14 September. Only onions larger than 2 inches with complete bulb development were collected into the bags. 'Vaquero' was harvested on 28 September 2020. All 'Vaquero' plants with a swollen bulb within the designated plot boundaries were collected. There were no decayed bulbs at harvest in 2020 season. Cultivar Snowball was harvested on 15 September 2021 and 'Avalon' and 'Vaquero' were harvested on 28 September 2021. All plants with swollen bulbs, regardless of bulb size, within the designated plot boundaries were collected into mesh bags.

At the time of harvest, marketable and unmarketable (e.g., due to mechanical harvest damage or rot damage) bulbs were collected. Unmarketable bulb count and weight were recorded and bulbs were discarded. The total yield from each plot was recorded. Thirty (30) bulbs (or as many as possible) were randomly collected from each plot and became the representative sample for further study.

The weight and size of representative bulb samples were recorded. Onion sizing followed USDA grades for commercial onions (USDA, 2014). Count and weight of each grade class per plot were recorded. Representative sample bags were placed into storage for further evaluation of bulb rot development.

2.6 Storage Details

Onion bags were placed inside a walk-in cooler at ARDEC South. A racking system was built inside the cooler for air ventilation. Cooler conditions were maintained between 35-40°F and dark. Humidity was not controlled nor recorded. 'Avalon' and 'Vaquero' were placed in the cooler on 23 September 2020 and 7 October 2020, respectively. Cooler temperature was increased to 41-45°F on 9 January 2020 to encourage rot. The bulbs were removed and evaluated monthly (described below) and the last bulbs remained in the cooler for five months total.

In 2021, onion bags were placed inside a walk-in cooler at ARDEC South on 20 September 2021 for 'Snowball' and 7 October 2021 for 'Vaquero' and 'Avalon'. Onions continuously remained in the cooler for four months.

2.7 Disease Evaluation

2.7a Field Evaluation

Fields were evaluated throughout the growing season for foliar symptoms of bacterial disease and evidence of phytotoxicity. Disease incidence was evaluated based on plants per plot showing bacterial disease symptoms (percent infection). The center six feet of each plot was evaluated. If disease symptoms above 5% for an individual plot were present, a severity rating was given to the plot based on the severity of leaf damage per plant. A rating scale for foliar severity based on "Onion Plant-Damage Scale" as presented in *Onion Health Management & Production* (Schwartz, 2013) was used. Similarly, phytotoxicity was reported based on a

percentage of plants per plot showing symptoms. An overall percentage of plants affected per plot was recorded. No severity rating was conducted.

An initial disease incidence rating was conducted one week prior to the first bactericide application; 26 July, 2020 and 7 July, 2021. Thereafter, each plot was rated for incidence and severity weekly for a season total of six evaluations. In 2020 these dates occurred on 6, 13, 20, and 27 August and 3 September, 2020. In 2021, the field evaluations occurred on 13, 20, 27, July and 3, 10 August, 2021. Phytotoxicity rating occurred on 6 and 20 August, 2020. In the 2021 season, ratings occurred on 13 and 27, July and 10 August 2021.

2.7b Storage Evaluation

Stored bulbs were evaluated by two methods. A non-destructive "squeeze test" was conducted by hand-applying pressure around the base of the neck of the onion to determine if there was evidence of rot based on the firmness or softness of the neck. A second, destructive visual inspection was done by using a sharp knife and cutting vertically from the neck to the basal plate through the center of the onion. A "yes" or "no" designation was given to each onion based on if it had symptoms of bacterial rot. The number of rotten onions per sample bag was recorded and a percentage of the total bulbs within the bag was calculated.

In 2020, storage evaluations occurred monthly after sample bags were placed into the cooler. A squeeze test around the onion bulb neck occurred on 29 October, 16 November, 30 November, and 31 December. Count of onions per sample bag with a presumed soft neck was recorded. No squeeze test evaluations occurred in 2021. In the 2020 season, destructive measurements occurred three times with 10 onions per sample bag being evaluated each time. Destructive measurements for 'Avalon' occurred on 17, 31 December, and 22 February 2020. 'Vaquero' destructive measurements occurred on 10, 22 February, and 9 March 2020.

In 2021, a single destructive storage evaluation occurred on 8 and 9 February, four months after the initial storage date. All sample bags were removed from the cooler and onions were cut vertically in half as described above. A count of bulbs with bacterial rot per plot, count of sprouting bulbs, and notes of other postharvest disease symptoms (e.g., basal rot, botrytis neck rot, etc.) were recorded. Individual bulbs with symptoms of bacterial rot were assigned a percent rating for the area of the cut bulb surface that was affected. Ratings were on a 20% increment scale (0%, 1-20%, 21-40%, 41-60%, 61-80%, 81-100%). From this initial rating, a severity index for all the bulbs rated in a plot was calculated to give a single bulb rot severity value per plot using the following formula.

Rot severity index (%) = ((# bulbs with 0 rating * 0%) + (# bulbs with 1-20% rating * 10) + (# bulbs with 21-40% * 30) + (# bulbs with 41-60% * 50) + (# bulbs with 61-80% * 70) + (# bulbs with 81-100% * 90)) **/** (# bulbs with 0 rating + # bulbs with 1-20 rating + # bulbs with 21-40 rating + # bulbs with 41-60 rating + # bulbs with 61-80 rating + # bulbs with 81-100 rating)

2.8 Statistical Analysis

Statistical analysis was conducted using R version 4.0.2 and the following packages; lme4, lemerTest, emmeans, and FSA. Cultivars' means per treatment were analyzed independently. Mixed models were fit using marketable yield (lbs.), the incidence of rot at harvest (percent by total harvest weight), incidence of rot after storage (percent of bulbs per plot), the severity of bulb rot after harvest (percent per bulb) as responses in their own respective models. The fixed effects included treatments, inoculation status, and treatment : inoculation status interaction. Random effects were block, block: treatment interaction, and block: inoculation status interaction. Random effects were included to account for the split plot design with inoculation status as the whole plot factor. A pre-determined *P*-value of 0.05 was used for all analyses. When assumptions for independence, normality, and equal variance were satisfied, the data were subjected to Analysis of Variance (ANOVAs) and means comparisons using

Tukey's adjusted pairwise comparison. Comparisons were based on inoculation status, treatment, and interactions between the two.

If one of the assumptions for independence, normality, or equal variance was not satisfied (such as in the post storage evaluation for 2021), the data was treated as non-parametric. The Kruskal-Wallis rank-sum test was used to indicate any evidence of treatment differences among inoculated and uninoculated per cultivar. If there was evidence of treatment differences, a Bonferroni-Dunn's test (commonly called Dunn's test) was used to confirm which treatments were different from one another. This test allows for multiple rank comparisons while maintaining a 95% confidence (i.e. alpha <0.05).

CHAPTER 3 Results

3.1 Bacterial Pathogenicity Testing

3.1a *Pantoea* spp.

In 2020, the scale assay for *Pantoea* spp. (Appendix 1, Table A) visually indicated three strains (149, 148, O158) had the potential for disease-inducing virulence factors when compared to the water control. Bacterial strains O120, O158, 148, and 149 were selected for inoculation in the field based on scale assay and metadata notes in the summer of 2020. Metadata for the selected strains are located in Appendix 2. However, Dr. Brian Kvitko, University of Georgia, Athens, later confirmed all bacterial isolates of *P. ananatis* strains used as inoculum did not have the Red Scale Necrosis (RSN) phenotype attributed to onion pathogenicity (personal correspondence, Feb 2021).

3.1b *Burkholderia* spp.

In 2021, three types of pathogenicity testing (whole bulb, leaf, and scale) were conducted for archived strains of *Burkholderia* spp. Results indicated various strains had the potential to be a virulent pathogen. The following strains visually showed significant rot compared to the water control for all three types of pathogenicity testing: O125, O170, O186, O 187, O341, and O350. Whole bulb assay photos are shown in Appendix 1, Table B. and the results of the scale and leaf testing are not shown. The four strains selected to make up the inoculum were O125, O170, O186, and O341. Metadata for these strains are in Appendix 2.

3.2 Field Results: Pantoea spp*., 2020*

3.2a Inoculation and Treatment

In 2020, there were no evident symptoms of foliar bacterial leaf blight in the field since the strains used to inoculate were not virulent. Phytotoxicity caused by bactericides was present, however at harvest, yield differences were observed. But, no rot or pathogen decay was present at the harvesting stage. Furthermore, during storage evaluation, bulbs had no bacterial rot development regardless of treatment, inoculation, or cultivar. Of the 30 bulbs sized and collected to represent the sample bags placed in storage, there was no evidence of significant difference regardless of treatment, inoculation, or cultivar.

3.2b Yield

Cultivars means per treatment were analyzed independently. Evidence of significant differences in yield was found between inoculated and uninoculated 'Avalon' bulbs, with inoculated bulbs having a higher yield (Table 3.1). Treatment means were analyzed separately using Tukey's adjusted pairwise comparison of means, and no treatment effects significantly affected the yield (tons/A) at harvest. Alternately, cultivar Vaquero did not show evidence of yield difference based on inoculation effort. As a result, inoculated and uninoculated plots were pooled together to increase the sample size and decrease the standard error. ANOVA indicated no evidence of interaction (P=0.610), nor significant differences between treatments (P=0.099). When looking closer, post hoc analyses using Tukey's pairwise comparisons indicated Actigard 50WG reduced yields when compared to the control. Overall, Actigard 50WG performed at the same relative rate as other bactericide products except for the control, which was the highest yielding treatment. However, it is important to note, ANOVA did not indicate any significant treatment differences. This result could be a practical significance to an onion grower.

3.2c Phytotoxicity

In the 2020 season, phytotoxicity was observed within specific treatments. Although overall incidence was low, three products showed evidence of phytotoxicity when products were applied at two times the label rate. Replicates for inoculated and uninoculated were pooled because no evidence of a difference was seen for inoculation efforts nor was there an interaction between inoculation and treatments. Assumptions for normality and equal variance were not satisfied. Therefore, Kruskal Wallis Rank Sums tests were used to detect significant treatments mean differences with respect to incidence of phytotoxicity. As seen in Table 3.2, the Kruskal Wallis test confirmed there were treatment differences for both cultivars Avalon and Vaquero ($P<0.0001$). For both cultivars, the copper product Kocide 3000 had the highest incidence of phytotoxicity, 9.0% and 5.0%, for Avalon and Vaquero, respectively. In 'Avalon', Kocide 3000 and ManKocide had a significantly higher incidence of phytotoxicity than all the other treatments and the control. Only Kocide 3000 showed significantly higher phytotoxicity than the water-treated control for cultivar Vaquero.

Table 3.1 Onion bulb yield at harvest (tons/acre) for cultivars Avalon and Vaquero in Colorado for 2020 season.

Main plot and split plot treatments	**Yield at harvest (t/A)**		**Yield at harvest (t/A)**
	Avalon		Vaquero
Main plots	Sep 23, 20 (harvest)		Oct 7, 20 (harvest)
Inoculated	20.0 a		17.3 a
Non-inoculated	13.8 b		18.6 a
P value (α=0.05)	**0.018**		0.164
Split plots and rate/A	Inoculated	Non-inoculated	
Actigard 50WG 1.0 oz	18.9 a	14.2 a	14.9 a
BlightBan A506 200g	20.3 a	16.3 a	18.5 a
Control	21.7 a	11.7 a	19.7 a
Kocide 3000 1.5 lb	18.9 a	13.4 a	17.9 a
LifeGard 4.5 oz/100 gal	20.2 a	14.7 a	17.7 a
ManKocide 2.25 lb	20.4 a	13.7 a	18.8 a
Oxidate 5.0 0.39% v/v	19.7 a	13.0 a	17.9 a
P value (α=0.05)	0.83	0.68	0.09

[a] Yield at harvest means compared using Tukey's adjusted pairwise comparison between treatments and by inoculation when evidence of an effect was determined by the ANOVA ($P \leq 0.05$).
[b] Inoculated and uninoculated replications combine due to no evidence of an effect for inoculation, treatment or an interaction between the two.

Table 3.2 Onion leaf incidence of phytotoxicity as a percentage taken at the final rating (August 20, 2020).

Main plot and split plot treatments	**Incidence of phytotoxicity %**	
Main plots		
Inoculated	2.68	1.50
Non-inoculated	2.25	0.89
P value (α=0.05)	0.09	0.49
Split plots and rate/A [a]	Avalon	Vaquero
Actigard 50WG 1.0 oz	0.0 a	0.0 a
BlightBan A506 200g	1.00 a	1.00 a
Control	0.00 a	0.00 a
Kocide 3000 1.5 lb	9.00 b	5.00 b
LifeGard 4.5 oz/100 gal	0.00 a	0.00 a
ManKocide 2.25 lb	8.00 b	3.00 ab
Oxidate 5.0 0.39% v/v	0.00 a	0.00 a
Kruskal-Wallis P value[b]	**0.00**	**0.00**

[a] Inoculated and uninoculated replications were combined due to no evidence of an effect for inoculation, or treatment inoculation interaction.

[b] Kruskal-Wallis rank sum test used to compare mean ranking differences of phytotoxicity. Significant evidence of difference was confirmed. Then a Dunn test was used to compare mean ranks between treatments.

3.3 Field Results: Burkholderia gladioli, 2021

3.3a Success of Inoculation

Artificial inoculation of cultivars Vaquero and Avalon bulbs with *Burkholderia gladioli* caused significant differences in decay at harvest. At harvest time, onion bulbs showing symptoms of bacterial rot were not allowed into storage due to the potential for further degradation. This is a common practice in commercial operations. Inoculation increased bulb rot at harvest by 7.7% for ‘Vaquero’ and 2.8% for ‘Avalon’(Table 3.3). Post storage evaluation showed that artificial inoculation was only successful in cv. Vaquero, as indicated by a significant increase of incidence of bulb rot from 0.19% to 0.89% (Table 3.4). Bacterial inoculation did not affect incidence nor severity in cv. Avalon. Since cv. Snowball did not have an uninoculated control, there was no way to determine success of inoculation effort; however, rot at harvest and incidence of rot post storage was observed (Table 3.3 and 3.5). Additionally, no foliar symptom development was seen in the field during the 2021 growing season.

3.3b Yield

In 2021, significant differences in marketable yield (tons/ac) were found between inoculated treatments for 'Snowball.' For this cultivar, LifeGard WG had significantly higher yields than Actigard 50WG. On average, all other treatments had the same relative effect (Table 3.3). No treatment or inoculation efforts affected yield differences for cultivars Avalon or Vaquero. Not all plots yielded 30 bulbs to represent the sample bags placed in storage. Regardless, there was no evidence of difference regardless of treatment, inoculation, or cultivar.

3.3c Bulb Rot Incidence and Severity

Bacterial bulb decay was observed at harvest. In the inoculated plots, bactericide treatments did not significantly reduce bulb rot at the time of harvest, regardless of onion cultivar (Table 3.3). Post-storage evaluation of bacterial rot showed little evidence of decay (Tables 3.4, 3.5). The exceptions for 'Vaquero' were three replications of Kocide 3000 with bacterial inoculation (6.67, 4.17, 4.76% rot) and a single replication of the control, uninoculated (3.45%). Post-storage evaluation data did not meet the assumptions of a normal distribution or equal variance and was analyzed using non-parametric methods. For 'Vaquero,' there was an interaction between inoculation and treatments (P=0.0001). Kruskal Wallis Rank Sums tests were used to detect significant treatments and rank mean differences among inoculated plots. There was evidence of significant differences between treatments in inoculated plots (P=0.005). Severity was analyzed the same way and produced similar results. The Dunn test compared rank means for incidence and severity of rot across treatments for inoculated plots. Evidence showed that Kocide 3000 ranked significantly higher for incidence rates and rot severity after storage when compared to all other treatments (Table 3.4).

For ‘Avalon’, two bacterial inoculated replicates of Oxidate 5.0 (3.33, 3.33%) and two replications of the control treatment (3.33, 3.33%) had an incidence of rot. Assumptions of normal distribution and equal variance were not met, and non-parametric methods of analysis were used. The mixed model ANOVA results showed evidence of an interaction between inoculation and treatments (P=0.028). Treatment comparisons using Kruskal Wallis Rank Sums test for significant differences did not show any evidence of ranked differences in inoculated plots.

The cultivar ‘Snowball’ had symptoms of rot development after harvest (Table 3.5). A one-way ANOVA indicated significant differences in rot incidence among treatments (P=0.046). Tukey’s adjusted pairwise comparisons of means indicated that ManKocide was the most effective at preventing rot incidence after storage compared to BlightBan A506. BlightBan A506 and ManKocide both performed similarly to the Actigard, Kocide, LifeGard, Oxidate treatments and the control (Table 3.5). No foliar evidence of phytotoxicity was seen during the 2021 growing season.

Table 3.3. Mean ratings for onion bulb rot at harvest and marketable yield in the 2021 season for cultivars Vaquero, Avalon and Snowball in Colorado.

Main plot and split plot treatments	Bulb rot by weight at harvest (%)[z]			Marketable yield at harvest (t/A)[y]		
	Vaquero	Avalon	Snowball[x]	Vaquero	Avalon	Snowball[x]
Main plots						
Inoculated	8.0 a	2.8 a	--	6.7 a	10.1 a	--
Non-inoculated	0.3 b	0.0 b	--	8.0 a	4.5 a	--
P value (α=0.05)	**0.029**	**0.037**	--	0.497	0.060	--
Split plots and rate/A						
Actigard 50WG 1.0 oz	13.0 a	2.0 a	6.8 a	6.1 a	4.8 a	4.6 a
BlightBan A506 200g	NA	NA	14.1 a	NA	NA	5.3 ab
Control	12.4 a	3.5 a	6.1 a	7.0 a	6.9 a	5.0 ab
Kocide 3000 1.5 lb	1.5 a	1.0 a	8.1 a	8.0 a	7.4 a	5.0 ab
LifeGard 4.5 oz/100 gal	11.1 a	6.5 a	10.0 a	7.3 a	4.2 a	6.2 b
ManKocide 2.25 lb	7.2 a	1.6 a	6.8 a	8.6 a	8.6 a	5.4 ab
Oxidate 5.0 0.39% v/v	2.8 a	2.2 a	8.3 a	7.1 a	6.5 a	5.7 ab
P value (α=0.05)	0.120	0.141	0.420	0.334	0.687	**0.048**

[z] Mean ratings for bactericide treatments for bulb rot by weight only for inoculated plots because of a significant inoculation effect for this variable (P =0.029, P=0.037). Very little infection observed in non-inoculated plots, and no significant interaction of inoculation treatments with bactericide treatments in the ANOVAs ($P \leq 0.05$).
[y] Mean marketable yield ratings resulting from bactericide treatments are averaged across inoculated and non-inoculated plots because there was no significant effect of inoculation (P = 0.497, P = 0.060) and no significant interaction of inoculation treatments with bactericide treatments (P >0.05).
[x] Only inoculated plots for cultivar 'Snowball' were included in the study.

Table 3.4. Mean ratings for incidence and severity of onion bulb rot after storage for inoculated plots of cultivars Avalon and Vaquero in the 2021 Colorado growing season.

Main plot and split plot treatments	Internal bacterial bulb rot after storage		Internal bacterial bulb rot after storage	
	Incidence of bulbs (%)	Severity per bulb (%)	Incidence of bulbs (%)	Severity per bulb (%)
Main plots	Vaquero		Avalon	
Inoculated	0.87 a	0.19 a	0.74 a	29.63 a
Non-inoculated	0.19 b	0.02 a	0.00 a	0.00 a
Anova *P* value [z] (α=0.05)	**0.051**	0.185	0.118	0.291
Split plots and rate/A [y]				
Actigard 50WG 1.0 oz	0 a	0 a	0 a	0 a
Control	0 a	0 a	2.22 a	0.89 a
Kocide 3000 1.5 lb.	5.20 b	1.16 b	0 a	0 a
LifeGard 4.5 oz/100gal	0 a	0 a	0 a	0 a
ManKocide 2.25 lb.	0 a	0 a	0 a	0 a
Oxidate 5.0 0.39% v/v	0 a	0 a	2.22 a	0.89 a
Kruskal-Wallis P value	**0.005**	**0.005**	0.084	0.089

[z] ANOVA p-values from mixed model where fixed effects included treatments, bacterial inoculation and treatment:bacteria interaction. Random effects were block: treatment interaction, and block:bacteria interaction. Assumption of normality and/or equal variance not met.
[y] Kruskal-Wallis rank sum test used to compare mean ranking differences. When significant evidence of difference confirmed the Dunn's test was used to compare mean ranks between treatments from inoculated plots.

Table 3.5. Mean ratings for incidence and severity of onion bulb rot after storage for cv. Snowball in Colorado for the 2021 season.

Main plot and split plot treatments	**Internal bacterial bulb rot after storage**	
	Incidence of bulbs (%)[z]	Severity per bulb (%)
Main plots[y]	Snowball	
Actigard 50WG 1.0 oz	1.7 ab	0.4 a
BlightBan A506 200g	5.9 a	1.1 a
Water-treated Control	1.7 ab	0.6 a
Kocide 3000 1.5 lb.	1.7 ab	0.7 a
LifeGard 4.5 oz/100gal	1.7 ab	0.2 a
ManKocide 2.25 lb.	0.0 b	0.0 a
Oxidate 5.0 0.39% v/v	1.7 ab	0.9 a
P value (α=0.05)	**0.046**	0.298

[z] Mean ratings for bactericide treatments for 'Snowball' are only for inoculated plots because no uninoculated plots were present in the study.
[y] Mean ratings done by Tukey's adjusted pairwise comparison due to evidence of significant differences in Treatment effects (*P*=0.046)

Table 3.6. Mean and standard error for incidence and severity of onion bulb rot after storage for cultivars Vaquero and Avalon in the 2021 Colorado growing season.

Main plot and split plot treatments [z]	**Internal bacterial bulb rot after storage**		**Internal bacterial bulb rot after storage**	
	Incidence of bulbs(%)	Severity per bulb (%)	Incidence of bulbs(%)	Severity per bulb (%)
Main plots	Vaquero [y]		Avalon	
Inoculated	0.87 ± 0.19	0.19 ± 0.14	0.74 ± 0.34	0.30 ± 0.18
Non-inoculated	0.19 ± 0.48	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
Split plots and rate/A				
Actigard 50WG 1.0 oz	0.00	0.00	0.00	0.00
Control	0.58 ± 0.58	0.06 ± 0.06	1.11 ± 0.70	0.44 ± 0.38
Kocide 3000 1.5 lb.	2.60 ± 1.21	0.58 ± 0.38	0.00	0.00
LifeGard 4.5 oz/100gal	0.00	0.00	0.00	0.00
ManKocide 2.25 lb.	0.00	0.00	0.00	0.00
Oxidate 5.0 0.39% v/v	0.00	0.00	1.11 ± 0.70	0.44 ± 0.38

[z] Means and standard error shown to indicate the uncertainty around the estimate of the mean measurement. It is useful for calculating a confidence interval.
[y] Values are from the combined inoculated and uninoculated plots.

CHAPTER 4 Discussion

In this series of experiments, marginal differences can be identified between pesticide product types (Table 2.1); coppers, sanitizers, plant defense inducers, and biological control microorganisms. It is also important to recognize cultivar, climate, and regional impacts observed throughout the experiment.

4.1 Pathogen

Some of the varying results from the 2020 to 2021 season were caused by bacterial species selection, age of culture, and virulence status. In 2020, strains for *Pantoea* species were selected based on a national "*Stop the Rot*" request for genome sequencing. The initial intention was to send samples to the University of Georgia, Athens (UGA) for the Red Scale Necrosis (RSN) assay and genome sequencing, then infect research plots with successfully identified pathogenic strains. With the onset of the COVID 19 lockdowns in the Spring of 2020, strains were not able to be sequenced or confirmed for pathogenicity. Since these strains were already segregated from the archive collection, a modified RSN assay was conducted to select the strains. The inoculum was made of four selected *Pantoea* strains and applied to the field. No foliar disease symptoms were seen during the growing season, nor bulb symptoms at harvest or post-storage. After post-storage evaluation, in February 2021, UGA was able to confirm no evidence of virulence for the selected strains. However, valuable treatment effects in the absence of a virulent strain were still investigated.

Yield differences between inoculated and uninoculated plots for cultivar Avalon were significantly different. The significantly higher yield for the inoculated portion, though unexpected, was likely explained by differing harvest crew protocols and techniques. Harvesting

crews were sent down the rows east to west, instead of designated blocking throughout the field. Therefore, we were unable to separate the effect of harvesting crew and inoculation effect to determine the cause of the yield difference. Re-training and randomization of harvest crews did occur for onion cultivar Vaquero and no difference in yield was seen between inoculated and uninoculated.

Before the start of the 2021 growing season, *Burkholderia* spp. strains showed evidence of strong virulence factors during the whole bulb pathogenicity assay. The selected strains all caused bulb rot and were *Burkholderia gladioli* as confirmed by Utah State University. A statistically significant difference between inoculated and non-inoculated in respect to percent decay at harvest for both 'Avalon' and 'Vaquero' indicate a successful effort of inoculation that year. The impact of treatment effect in respect to preventing rot during storage is limited by the lack of statistically significant differences between inoculated and uninoculated and very little rot overall. Further studies should evaluate proper inoculation methodology for their region prior to inoculum application.

4.2 Treatment Types

It was hypothesized that copper-based bactericides and "alternative" product types would both effectively manage bacterial bulb rot disease symptoms. However, results indicated all products responded similarly to the water-treated control. Although our original hypothesis was rejected, the results contribute to a clearer understanding of the implication of using these different bactericide types. Due to the lack of bulb rot development during storage, the results of rot post storage in 2021 cannot confirm any treatment performed better than the water-treated control at preventing storage bulb rot.

4.2a Copper Products

Copper products, Kocide 3000 and ManKocide, had no statistically significant impact on yield differences, grade differences, or preventing bulb rot at harvest regardless of cultivar or inoculation status. When copper-based products were applied at two times the labeled rate in 2020, symptoms of phytotoxicity were seen. These results supported the claims of Schwartz and Gent (2007) who stated, that copper products can be phytotoxic to onion leaves. Similar to results from Gent (2005), even when copper products were applied at two times the label rate in the absence of a virulent pathogen, yield was not significantly impacted (Gent & Schwartz, 2005). Post-storage evaluation of 'Snowball' identified copper product, ManKocide, as having the lowest incidence of rot even though it worked similarly to the water-treated control. It was only significantly more effective at rot management than the worst-performing product, Blightban, a biological.

With respect to managing symptoms of bacterial pathogen *Burkholderia gladioli* at harvest, the copper products were as effective as the water control. Copper-based products have shown efficacy in managing bacterial symptoms caused by *Xanthomonas* spp. (Lang et al., 2007). However, due to low overall rot incidence in this study, efficacy of copper-based products could not be definitively determined to mitigate onion bulb rot symptoms.

Similar bactericide evaluation trials for the efficacy of managing onion bulb rot against *Burkholderia gladioli* have occurred in Georgia, New York, and Washington State during the 2020 season. These single-year bactericide evaluations were published as Plant Disease Management Reports (PDMRs). Results from this thesis build on existing reports from New York and Washington of copper-based bactericides lacking in efficacy at preventing onion rot in the presence of *Burkholderia* spp. (du Toit et al., 2021; Hoepting et al., 2021a). No reports have

been made to indicate *Burkholderia* species have any resistance to copper products, and it is beyond the scope of this study to make any inference.

4.2b Sanitizer Product

The commercially available sanitizer product Oxidate 5.0 had no significant difference in yield, grade, rot at harvest, or incidence of rot post-storage. At every measurement, it performed similar to the water-treated control. These results are further confirmed in reports from Washington State and New York that sanitizer products had the same efficacy as the untreated control when trying to manage symptom development of onion pathogen *Burkholderia gladioli* (du Toit et al., 2021; Hoepting et al., 2021a). Even when applied at double the rate, as done in 2020, no symptoms of phytotoxicity were present. This finding differs from reports from Georgia which indicated Oxidate 5.0 products produce phytotoxicity when applied at the labeled rate (Dutta et al., 2021). Additionally, while in growth chambers, Galal (2017) was able to show peroxyacetic acid treatment effectively suppressed bacterial growth of *Erwinia carotovora* subsp. *carotovora* up to 41%; inhibition was achieved at 0.2 gallons/liter. It is important to consider that efficiency of peroxyacetic acid is more successful when it is applied post-inoculation as compared to a pre-inoculation treatment (Galal, 2017). This is counter to the management practices of most onion growers who generally apply other types of bactericides as a preventative measure. However, behaviors are changing, possibly as an effect of marketing strategy, as some growers have claimed they rely on sanitizer-type products as a “rescue product” and not a preventative like traditional bactericides. Growers seem to apply this type of product after they presume there was a possible inoculation event, such as heavy rain or hail. PAA is generally able to be easily applied with aerial applications in an event like this when entering with a tractor may be difficult. Further research should evaluate application timing to

investigate how sanitizer type bactericide products work as a “rescue product” instead of a preventative for the management of bacterial bulb rot pathogens.

4.2c Biological Products

The only true biological/bacteriophage type product applied in this study was a commercial product BlighBan A506, which contains, *Pseudomonas fluorescens* A506. In other studies, Gent and Schwartz (2005) reported success when using biological control agents *Pantoeas agglomerans* and *Pseudomonas fluorescens* A506 together; yet individual influence of *Pseudomonas fluorescens* A506 was not evaluated. The application of BlighBanA506 was in direct response to these follow up questions. In these experiments, BlightBan A506 statistically performed at a comparable level to copper products at preventing rot at harvest. However, it also responded statistically similar to the water control. Similar results, concerning yield and grade, were seen when Gent and Schwartz (2005) used biological control agents for the control of *Xanthomonas* leaf blight. We also did not observe yield or grade changes in this research.

BlightBan A506 does not hold any claims to effectively manage onion bacterial bulb rots, nor is it labeled for use in onion production. However, by evaluating its efficacy this study was able to answer lingering questions from previous relevant research.

4.2d Systemic Acquired Resistance (SAR) Inducer Products

Two SAR products, Actigard 50WG and LifeGard WG, were applied in this series of experiments. Unlike Actigard, LifeGardWG is not labeled for use in onion production, nor does it claim to prevent disease development of onion bacterial diseases. It was selected based on previous work from de Toit and Walter (du Toit et al., 2020) showing the possibility of efficacy and increasing marketable yield. With regards to bulb size, total marketable yield, and rot at harvest, both products performed similarly to the control regardless of cultivar and inoculation

efforts. As previously explained, very low rot at harvest was seen and treatment effects should not be inferred. During both field seasons, although not statistically significant, Actigard50WG, continuously ranked numerically the lowest in yield in the absence of a pathogenic bacteria in both 'Vaquero' and 'Avalon'. In various studies, decreased yield and successful disease management have been reported when using the Actigard product (Gent & Schwartz, 2005; Lang et al., 2007; Obradovic et al., 2004; Romero et al., 2001; Vallad & Goodman, 2004; Walters et al., 2005). Specifically, work by Gent and Schwartz (2005) demonstrated a notable onion yield reduction when Actigard 50WG was applied at 2.5 the labeled rate in the absence of the target pathogen *Xanthomonas.* Similarly, in the 2020 season, the results of this project indicate that Actigard reduced yield compared to the control in the absence of a pathogenic pathogen. In 2021, inoculated cultivar Snowball under the treatment LifeGardWG had a statistically significant higher yield than Actigard, the lowest yielding treatment. Although the yield differences between SAR products and the control are not statistically significant, they can be considered practically significant. Imagining these results from a grower's perspective, a yield reduction of 5 tons per acre could be, in fact, important to the grower. In scientific research, it is important to hold to statistical significance with respect repeatability of the scientific process, however, as applied researchers, driven by industry needs and questions, practical significance maybe noted as an area for additional investigation.

Actigard did not significantly reduce disease symptoms of bacterial bulb rots as compared to the water control. These results are contrary to the previous work of Stumpf (Stumpf et al., 2021) on the protection of onion bulbs against *Pantoea ananatis* and Gent (Gent & Schwartz, 2005) on management of *Xanthomonas* leaf blight in onions. Similar evaluation trials for efficacy of managing onion bulb rot against *Burkholderia gladioli* occurred in New

York and Washington State. Both trials included SAR products Actigard 50WG and LifeGardWG at the same rate of application as this experiment. Results from New York and Washington State match the results of this study, indicating that these two SAR products may not effectively manage incidence of disease from *Burkholderia gladioli.* Studies in Georgia by Dutta (Dutta et al., 2021), relied on natural infection rather than artificial inoculation and reported that the product LifeGard WG was able to significantly reduce the incidence of center rot. Similarly, they did not see adequate control with using Actigard. Results from these previous studies align with the results presented in this thesis and showed that the Actigard product performed similarly to the control (du Toit et al., 2021; Dutta et al., 2021; Hoepting et al., 2021a).

Extensive research and reviews have reported on defense inducing product types' impact on disease management and yield in various crops. Studies have demonstrated that various factors influence the expression of induced resistance. These reported factors include environmental impacts of greenhouse and field conditions, available salts, nitrogen, and primarily plant species (Walters et al., 2005). It is acknowledged that specific genotype differences among cultivars can affect the activation of resistance in plants treated with these product types, ultimately influencing disease susceptibility of the host (Fu & Dong, 2013; Romero et al., 2001; Vallad & Goodman, 2004; Walters et al., 2005; Zehnder et al., 2001). Likewise, it has been suggested that inducing defense mechanisms cause the plant to reallocate resources elsewhere instead of contributing to overall plant vigor and yield (Vallad & Goodman, 2004). Although this theory seems to be contested it is widely accepted and understood that artificially inducing a SAR response impacts plant signaling and could contribute to possible yield impacts (Bargabus-Larson & Jacobensen, 2007; Kunkel & Brooks, 2002). These responses often depend on more complex interactions between the species, cultivar, the plant's

physiological state, and the pathogen (Kunkel & Brooks, 2002; Rojo et al., 2003; Vallad & Goodman, 2004; Walters et al., 2005).

Instead of focusing on SAR inducer products to be the sole replacement for copper-based products, they can be considered as an addition to an already robust disease management plan. The LifeGardWG specimen label itself states "[it] is most effective when used in combination or alternation with fungicides having other modes of action" (Certis USA, 2018). For example, Gent and Schwartz (2005) were able to demonstrate successful disease management of *Xanthomonas* leaf blight by integrating two applications of acibenzolar-*S*-methyl with six applications of a copper hydroxide-mancozeb product. The addition of this product was more effective than a regiment of copper-based products alone. With this approach, growers can reduce the number of applications or dose rates of copper-based products.

4.3 Cultivar, Climate, and Regional Effects

The two primary cultivars used in the study were Avalon and Vaquero, each with 115 days and 118-120 days to maturity, respectively. This series of experiments was limited by field design regarding statistical comparison of cultivar differences in response to treatment effects. Furthermore, cultivar influence was not a primary hypothesis and therefore, the cultivars were analyzed independently. The results of the 2021 season consistently showed 'Vaquero' having more symptoms of bulb rot at harvest and post storage than cultivar Avalon. This was surprising based on previous experience of growing onions at ARDEC S. (Uchanski and Yoder, 2019 *unpublished)* Although the limitation of this study prevented cultivar statistical analysis, it has been theorized there are cultivar differences based on previous work comparing cultivar susceptibility to bacterial rots. Reports show that some cultivars are more susceptible to bacterial bulb rotting pathogens than others as seen in cultivar trials conducted by Cornell Cooperative

Extension, New York in conjunction with the StR project (Hoepting et al., 2021b) and Schroeder (2010) two-year cultivar study. Although Hoepting did not have a 115 day-length onion cultivar, statistical differences were seen between the 110-112 day length onions and the 118-120 day length onions in respect to natural bacterial bulb rot percent infection and artificially inoculated bacterial bulb rot percent of infection; regardless of protection strategies applied (Hoepting et al., 2021b). These results provide further insight into the relationship between cultivar traits and susceptibility to bacterial diseases.

This study was constrained to the northern Colorado climate and regional effects. Year-to-year differences limited the repeatability of the study. In 2020 during the peak of the growing season, the Cameron Peak Fire blanketed the high plains of Fort Collins with smoke for over 60 days. The Cameron Peak fire is currently recorded as the largest wildfire in Colorado history (U.S. Forest Service, 2020). Additionally, in 2021, the area had relatively heavy spring rains of 11.61cm from April through May that contributed to damping-off of onion seedlings and affected the stand development of this study (Colorado State University, CoAgMET, 2020-2021). Long periods of hot, semi-arid summer days decreased the possibility of natural bacterial infection. Early, heavy snows and threats of frost contributed to the need to undercut onions and roll tops instead of waiting for tops to “fall over” at maturity. This practice aligned with responses from local growers to get the crop out of the field before inclement weather hit. Overall, the results of this study should be considered under northern Colorado conditions. Similarly, when comparing results of PDMRs from other states, the region’s climate and natural infection levels should be considered.

In addition to regional effects, recommendations for managing bacterial bulb rot with bactericide product types should consider how these products will incorporate into existing

disease control programs. Demonstrating how products work under experimental conditions is the first step, but the scope for a proper recommendation should be much broader. Product cost, timing of application, and cohesion with cultural practices should all be considered.

It is yet to be determined how “alternative” type products will be integrated into onion production regions and if they will be used in conjunction with coppers or if they aim to replace them altogether. Therefore, it is important to conduct further research on how different product types can contribute to the onion industry.

CHAPTER 5 Conclusion

Through two field seasons in Northern Colorado, this study evaluated the response of copper-based, sanitizer, biological microorganism and plant defense inducing bactericide types on three onion cultivars' bulb rot development caused by non-pathogenic *Pantoea* spp. in year one, and pathogenic *Burkholderia gladioli*, in year two.

After examination, the results are inconclusive about the efficacy of bactericide types at preventing bacterial rots because there was very low rot development overall. Although alternative products did perform statistically similarly to copper-based products, the copper-based products worked as effectively at preventing rot as the control regardless of cultivar. There was an exception for 'Vaquero' at the poststorage evaluation in 2021 when Kocide 3000 had significantly higher rot incidence than all other treatments at 6%.

Similar to other reports, treatment types do not impact individual bulb size regardless of cultivar. As described in other bacterial studies, copper products are traditionally considered to be the most effective at managing bacterial disease. However, based on results from this research and recent PDMRs, copper products do not seem to be effective at preventing rot against *Burkholderia* spp.

The lack of treatment differences in preventing bacterial bulb rot in this study draws into question whether one product type throughout the whole growing season may or may not be the best at disease prevention of *Burkholderia gladioli.* Further research should consider how the incorporation of multiple product types throughout the growing season can contribute to overall disease management. Cultivar selection, regional effects, product cost, cultural practices, and integration of product type into existing management programs should all be considered before

product recommendations could be made. These results provide growers with additional information should they choose to integrate coppers, sanitizers, microorganisms, and/or plant defense inducers into their strategy for managing bacterial bulb rots of onions.

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APPENDIX

Table A1. Photos of plant pathogen assays for onion scales. Photo results from scale assay "poke test" for *Pantoea* spp. in 2020 season

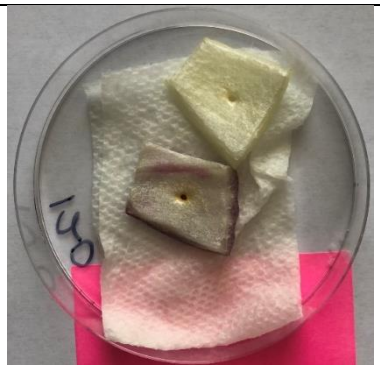
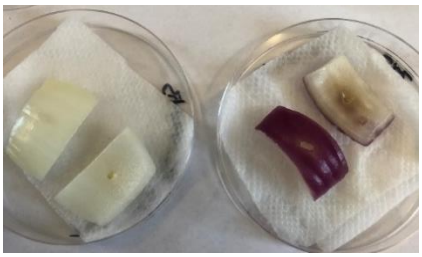
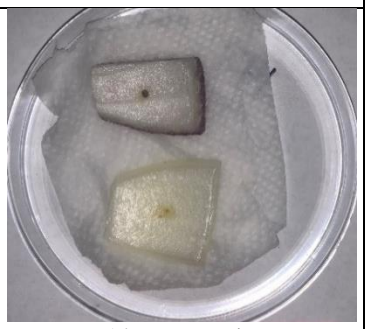
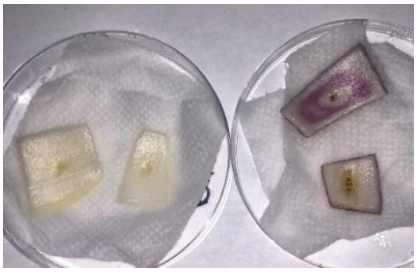
 Control- water inoculated	 O120 *Pantoea* spp	 O145 *Pantoea* spp
 O147 *Pantoea* spp	 148 P. ananatis	 147 P. ananatis
 149 P. ananatis	 O158 *Pantoea* spp	 O161 *Pantoea* spp

Table A2. Photos of plant pathogen assays for onion scales. Photo results from onion bulb inoculation assay for 2021 season.

 O125 *Burkholderia* spp.	 O170 *Burkholderia* spp.
 O186 *Burkholderia* spp	 O187 *Burkholderia gladioli*
 O341 "presumptive" *Burkholderia gladioli*	 O350 "presumptive" *Burkholderia cepacia*
 O159 Pantoea	 Control- water inoculated

Table A3. Metadata for selected bacterial strains from CSU collection

ID #	Description – Location Collected	Found on Host	Date Collected / Stored	Identification method
O120	*Pantoea* spp – Rocky Ford, CO	Onion	?? / 8-5-1994	Field identified
O125	*Burkholderia* spp - ??	Onion	?? / 10-04-1994	Field identified
148 & O158	*Pantoea ananatis* – Rocky Ford, CO	Onion	8-17-1996 / 08-26-1996	PCR confirmed Leach Lab 148 Schwatz Lab O158
149	*Pantoea ananatis* – Rocky Ford, CO	Onion	8-17-1996 / 08-26-1996	PCR confirmed From Schwartz Lab O159
O170	*Burkholderia* spp. – Greeley, CO	Onion	10-21-1996 /11-1-1996	Field identified
O186	*Burkholderia* spp – Pierce, CO	Onion	9-17-1997 / ??	Field identified
O341	*Burkholderia gladioli* – Wiggins, CO	Onion	??/??	Field identified