SEED TRANSMISSION OF *PSEUDOMONAS SYRINGAE* PV. *APTATA*, AND EFFICACY OF BACTERICIDES FOR CONTROL OF THE PATHOGEN IN BEET AND SWISS CHARD SEED PRODUCTION

By

STEPHANIE ANN CRANE

A thesis submitted in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

WASHINGTON STATE UNIVERSITY
College of Agricultural, Human, and Natural Resource Sciences

DECEMBER 2023

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of STEPHANIE ANN CRANE find it satisfactory and recommend that it be accepted.

Lindsey du Toit, Ph.D., Chair

David Weller, Ph.D.

Lyndon Porter, Ph.D.

ACKNOWLEDGMENT

I would like to acknowledge my advisor, Dr. Lindsey du Toit, for the guidance she has provided since I began my journey through WSU's Agriculture program in January of 2020. I greatly appreciate Dr. du Toit's support, effort, patience, and her commitment to her students. I would also like to thank my committee members, Dr. David Weller, and Dr. Lyndon Porter for their guidance and support.

I extend my thanks to Sakata Seed America, for funding my research through their education assistance program, and to all my colleagues who have provided me with both friendship and support throughout this journey. In particular, I would like to extend my gratitude to Dr. Sierra Hartney, for encouraging my academic growth, providing support and resources when needed, and advocating for me throughout this process; Philip Brown, for initiating and including me in the bacterial leaf spot research at Sakata that eventually led to my thesis projects; Jenna Price and Dan Martin, for all their help and support with the many years of field trails and answering my endless questions; Delita Pardue, for championing this research; and Dr. Sanjaya Gyawali, Merrilyn DeLaFuente, and Ben Loper for providing emotional support, guidance, and understanding, as I juggled being a part-time graduate student while working full-time. I am very thankful for my community at Sakata and for the support I received during this process. I would also like to recognize Mike Derie, from the WSU Mount Vernon Research and Extension Center, for his expertise and advice.

I would like to thank my parents William and Belinda, for always encouraging me throughout my academic career and pushing me to succeed. Finally, I extend endless gratitude to Andrew Vander Stoep for encouraging me to begin this journey, being my unofficial field person when I needed a hand, providing a voice of optimism when needed, and for cheering me on along the way.

SEED TRANSMISSION OF *PSEUDOMONAS SYRINGAE* PV. *APTATA*, AND EFFICACY OF BACTERICIDES FOR CONTROL OF THE PATHOGEN IN BEET AND SWISS CHARD SEED PRODUCTION

Abstract

by Stephanie Ann Crane, M.S.
Washington State University
December 2023

Chair: Lindsey du Toit

Bacterial leaf spot (BLS), caused by *Pseudomonas syringae* pv. *aptata* (*Psa*), is an economically important disease in beet and Swiss chard production. *Psa* is a splash-dispersed, seedborne, and seed-transmitted pathogen, and BLS is favored by cool and moist conditions. The economic impact of BLS has increased with expansion of baby leaf beet and chard production because of the dense plantings (>7 million seed/ha) and overhead irrigation. Baby leaf production also has increased the demand for seed. Management practices for BLS are limited, e.g., disinfection of *Psa*-infected seed lots, use of drip irrigation (where feasible, but impractical for baby leaf crops), selection of planting sites, and foliar applications of copper bactericides. Disinfection of seed is costly. Copper bactericides are not systemic or curative, and have limited efficacy, including a risk of pathogen populations developing tolerance to copper. This study evaluated: i) thresholds for seedborne *Psa* that result in development of BLS in baby leaf beet and chard crops, ii) the duration of survival of *Psa* in beet and chard seed, and iii) the efficacy of foliar applications of various bactericides for control of BLS in seed crops. Four baby leaf field trials planted with seed naturally infected at a range of concentrations of *Psa* revealed the threshold for seedborne *Psa* that resulted in ≥5% severity of BLS ranged from 0 to ~6 x 10^4 CFU/g seed, depending on

environmental conditions. Recovery of seedborne *Psa* in naturally infected beet (n = 3) and chard (n = 3) seed lots tested at 3-month intervals after harvest of seed crops revealed the amount of seedborne *Psa* (log_{10}CFU)/g seed) declined by 0.07 to 0.19 X months of storage. Therefore, storing seed for 12 to 24 months may be a viable option to reduce *Psa* to negligible levels prior to selling the seed. Of ten bactericides evaluated in five chard seed crops, none reduced severity of BLS symptoms or *Psa* infection levels of the harvested seed, except ManKocide in only one trial with moderate BLS severity. Further research on seed treatments and bactericides is needed to facilitate effective management of *Psa* in beet and chard production.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER ONE

LITERATURE REVIEW

1.1 *Beta vulgaris*

Significance. *Beta vulgaris* is a flowering plant species in the Chenopodiaceae, which is now included in Amaranthaceae, commonly known as the goosefoot family (Nottingham 2004). This species is split into several cultivar groups of important agricultural crops, all of which are included in *B. vulgaris* subsp. *vulgaris* (Nottingham 2004). The cultivar groups include beet root or table beet in the Conditiva group, spinach beet or leaf beet in the Cicla group, sugar beet in the Altissima group, fodder beet or mangel in the Crassa group, and Swiss chard in the Flavescens group. These crops are used in a wide variety of fresh market products, folk medicine preparations, and dietary supplements (Duke 1983; Traverse 2018). There are wild *B. vulgaris* species growing throughout Europe, northern Africa, and western Asia, and the cultivated species are grown worldwide (Biancardi et al. 2019). Sugar beet is of major importance globally and is counted among other staple crops such as wheat (*Triticum aestivum*) and rice (*Oryza sativa*), but the other *B. vulgaris* crops are considered minor crops in most countries (Agarwal and Sinclair 1997; Neergaard 1977). According to the United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS), >0.46 million ha of sugar beet were harvested in the US in 2022, yielding 6.4 x 10^4 kg/ha (USDA NASS 2022). For table beets destined for fresh market or processing, ~5,800 ha were harvested in 2017 (USDA NASS 2022). In addition, table beet and sugar beet seed production are an essential part of agriculture in the US, specifically in Washington State, which produces 95% of the table beet seed used in the US and 50% of the table beet seed grown worldwide (du Toit 2007). In 2017, ~1,600 ha of sugar beet seed was harvested in the US, of which ~700 ha was produced in Washington State (USDA NASS 2022). For sugar beet growers in the US, the average price received in 2020 was $55/metric ton (USDA NASS 2022).

History. All the *B. vulgaris* cultivars known today came about through domestication of a wild ancestor, *B. vulgaris* subsp. *maritima*, commonly known as seabeet, which grows widely in Europe (Biancardi et al. 2019; Nottingham 2004; Schrader and Mayberry 2003). Beet root, also known as table beet or fresh-market beet, is bred for the large, edible taproot which can be eaten raw, cooked, pickled, or juiced (Schrader and Mayberry 2003). In addition to traditional red beet roots, there are cultivars bred as novelty beets with unusual color or shape, such as golden, white, and cylindrical roots (Biancardi et al. 2019; Schrader and Mayberry 2003). Swiss chard and spinach beet are both bred to have attractive, edible foliage and are grown for fresh markets as mature leaves or baby leaves. They also can be used as decorative vegetation (Biancardi et al. 2019). Fodder beet or mangel is an important source of food for cattle, especially in Europe and New Zealand, and is also the background from which sugar beet was developed (Duke 1983). Sugar beet has been bred to have more than four times the sugar content of its ancestor and produces more than a third of the sugar used throughout the world (Duke 1983; Romieras et al. 2016).

Production. Beet and Swiss chard are biennial species, i.e., two years are required to complete the life cycle and produce seed, unless shortcuts (such as vernalization in cold storage or a greenhouse) are taken (du Toit 2007; Navazio et al. 2010). For seed production, these crops must be exposed to cold enough conditions for the plants to switch from vegetative to reproductive growth (between 4 and 10°C for ~60 to 90 days) but not so cold that the plants are killed, although they can tolerate mild freezes (Schrader and Mayberry 2003). Because of these environmental requirements for flowering and seed set, beet and chard seed crops can only be produced in a very limited number of areas globally. The maritime Pacific Northwest (western Oregon and western Washington) is the only part of the US that has the correct climate for seed production because of the mild, dry summers and cold but temperate winters (Navazio et al. 2010). Other beet and chard seed production areas are Northern Europe, South Africa, New Zealand, and Chile (Jacobsen 2009). For fresh market crops, beet and Swiss chard are grown

as annuals and are a cool season crop (Schrader and Mayberry 2003). In contrast to seed production, the root crops and baby leaf crops can be grown all over the world, including across the European Union, Mexico, India, Brazil, South Africa, Australia, and parts of the United States. Since there are relatively few places in the world where seed can be grown, but fresh market crops can be grown in many areas, collaborators from around the world are needed to produce seed crops and the seed is moved all over the world.

Diseases of *Beta vulgaris*. *B. vulgaris*, like most agricultural crops, is host to a range of plant pathogens that can impact both seed and fresh market production (Harveson et al. 2009; Navazio et al. 2010). Many bacterial and fungal pathogens cause leaf spots, which can negatively affect marketability of fresh market Swiss Chard and spinach beet, in particular (Harveson et al. 2009). Some of the leaf spot diseases include bacterial leaf spot caused by *Pseudomonas syringae* pv. *aptata*, Alternaria leaf spot caused by *Alternaria alternata* and *Alternaria brassicae*, Cercospora leaf spot caused by *Cercospora beticola*, Phoma leaf spot caused by *Phoma betae*, and Stemphylium leaf spot caused by *Stemphylium botryosum* (Harveson et al. 2009). There are also fungal and bacterial pathogens that cause root rots, and nematodes and viruses that are pathogens of beet. Beets planted in soils that flood or that are not properly aerated may be prone to damping-off caused by *Pythium* spp., *Rhizoctonia* spp., and *Aphanomyces* spp. (Harveson et al. 2009).

1.2. *Pseudomonas*

Background. *Pseudomonas* is a gram negative bacterial genus that includes >191 species and belongs to the Pseudomonadaceae (Cornelis 2008). Some defining characteristics of this genus include rod-shaped bacteria with flagella (one or more), that are aerobic and do not form spores (Stanier et al. 1966). This is a very hardy and diverse bacterial genus that can be found in a wide variety of ecological niches (Morris et al. 2008). Flagella allow the bacteria to move through water, which is why many

Pseudomonas species can be found in and spread via water. There are two phylogenetic lineages within *Pseudomonas*, *aeruginosa* and *fluorescens* (Mulet et al. 2010). The *fluorescens* lineage contains six phylogenetic groups, which include most plant pathogens. *P. syringae* is a species within the *fluorescens* lineage that can be found worldwide (Morris et al. 2008; Mulet et al. 2010).

Pseudomonas syringae. *P. syringae* is a diverse species that includes >60 pathovars [a taxonomic group below subspecies that groups organisms according to pathogenicity, either by symptomology or host range (Bradbury 1983; Gutierrez-Barranquero et al. 2019)]. The bacteria can survive in many habitats, including clouds, rain, snow, streams, lakes, wild plants, and agricultural crops (Gutierrez-Barranquero et al. 2019; Monteil et al. 2012; Morris et al. 2008). This species includes many plant pathogens with a wide range of host species that impact agriculture worldwide (Amato et al. 2007; Monteil et al. 2012; Nikolic et al. 2018). Pathogenic strains of *P. syringae* have been found in non-cultivated areas, and the link between this species and the water cycle is thought to have helped spread the bacteria into agricultural areas (Morris et al. 2008). For example, *P. syringae* is commonly found in dead plant material in alpine meadows and can survive the winter in infected plant tissue buried under snow (Monteil et al. 2012; Morris et al. 2008). The bacteria emigrate from leaf litter into the snowpack, traveling into waterways as the snow melts (Monteil et al. 2012). The ability of *P. syringae* to survive in reservoirs outside of agricultural areas, on many wild hosts, and then travel via waterways, makes it difficult to control the diseases caused by some isolates of this species (Montiel et al. 2012).

P. syringae pathovars originally were grouped into seven primary phylogroups, which classify bacterial strains based on the *rpoD* housekeeping gene phylogeny (Baltrus et al. 2017; Lamichhane et al. 2015). More recently Berge et al. (2014) established 13 phylogroups in this species. Phylogroup 2b includes three of the common plant pathogenic pathovars, *aptata*, *atrofaciens*, and *syringae* (Nikolic et al. 2018). Initially, bacterial strains were classified as *P. syringae* if they were fluorescent and had a specific LOPAT profile (Lelliott et al. 1966). LOPAT refers to the five tests developed by Lelliott et al.

(1966) to differentiate *Pseudomonas* strains based on physiological or biochemical processes to help determine if the strains are pathogenic: levan production (L), oxidase activity (O), ability to cause soft rot in potato (P), ability to produce arginine dihydrolase (A), and a hypersensitive reaction on tobacco leaves (T). The LOPAT profile that distinguishes plant pathogenic strains of *P. syringae* is: positive for levan production, negative for oxidase activity, unable to rot potato, unable to produce arginine dihydrolase, and able to cause a hypersensitive response on tobacco (+ - - - +) (Baltrus et al. 2017; Lelliott et al. 1966). Non-fluorescent isolates also can be characterized with the *P. syringae* species classification scheme (Safni et al. 2016). *P. syringae* colonies are cream-colored and round on King's B agar medium (Koike et al. 2003). Lamichhane et al. (2015) stated there have been 72 reported disease outbreaks associated with different strains in the *P. syringae* species complex, on >40 host species in 20 countries since the beginning of the 21st century. In annual plants, *P. syringae* mainly causes foliar diseases known as bacterial leaf spots, and rarely kills plants, which can make it difficult to determine the economic impact if the crop is grown for fruit or root production, although beetroot crops are an exception, since foliar symptoms may affect the ability of the grower to harvest beets via pulling the tops (Lamichhane et al. 2015; Pethybridge et al. 2018). In crops grown for leafy green harvest, the economic impact is more apparent as the blemished leaves often are not marketable.

***P. syringae* on seed.** Some *P. syringae* strains can be seedborne, but it is unknown how long the bacteria can persist on seed of various plant hosts (Jacobsen 2009; Newberry et al. 2017; Rico et al. 2003). Seedborne pathogens such as *P. syringae* cause significant losses worldwide due to infested seeds being moved across international borders, introducing diseases into new areas or new strains into areas where the disease is established (Gitaitis and Walcott 2007). The transport of *B. vulgaris* seed around the world to accommodate the specific environmental conditions needed to grow seed versus the diversity of conditions in which fresh market crops can be grown has exacerbated efforts to control this pathogen. Today, *P. syringae* can be found in fields across the globe from the US to New Zealand

(Derie at al. 2016; Dutta et al. 2014; Ignjatov et al. 2015; Koike et al. 2003; Lamichhane et al. 2015; Rotondo et al. 2020; Safni et al 2016). There are several ways in which seedborne pathogens such as *Psa* cause economic losses. According to Agarwal and Sinclair (1997), these can include losses due to restricted sale of infested seed, the costs of testing seed for the presence of the pathogen and disinfecting infested seed lots, and the increased cost of purchasing seed from areas free of the pathogen. Another significant impact is post-emergence damping-off, which decreases stands (Agarwal and Sinclair 1997). The potentially significant impacts caused by seedborne pathogens have resulted in efforts to improve the selectivity and sensitivity of seed health assays for seedborne *P. syringae* pathogens on various crops (Gitaitis and Walcott 2007).

1.3. Bacterial Leaf Spot

Significance. Bacterial leaf spots caused by *P. syringae* pathovars can affect a wide variety of host species, from ornamentals such as geranium (*Pelargonium* spp.), to a large number of vegetables, including tomato (*Solanum lycopersicum*), kale (*Brassica oleracea*), squash and pumpkin (*Cucurbita spp.*), spinach (*Spinacia oleracea*), melon (*Cucumis melo*), beet, and Swiss chard (Arabiat et al. 2016; Balaz et al. 2014; Dutta et al. 2014; Derie at al. 2016; Ignjatov et al. 2015; Koike et al. 2017; Koike et al. 2002; Koike et al. 2003; Lamichhane et al. 2015; Newberry et al. 2016; Newberry et al. 2017; Nikolic et al 2018; Safni et al 2016; Tymon and Inglis 2017). Strains of *P. syringae* can also colonize many weed species, making the pathogens more difficult to control (Monteil et al. 2012). Bacterial leaf spots have been reported across the US, and across the globe as noted above (Nampijja et al. 2021; Rotondo et al. 2020). The symptoms vary among hosts but typically include round, necrotic lesions that may be water-soaked on some hosts or may have a brown or tan center.

Bacterial leaf spot of *Beta vulgaris*. The first reported case of bacterial leaf spot (BLS) on beet crops in the US was in 1908 on sugar beet in Utah (Brown and Jamieson 1913). More recently, BLS

symptoms were reported on Swiss chard in 1999 in the Salinas Valley, CA (Koike et al. 2003), and in 2021 on baby leaf Swiss chard in Arizona (Nampijja et al. 2021). For many years, *P. syringae* pv. *aptata* (*Psa*) was thought to be the main pathovar infecting beet and Swiss chard crops, but recent mulitilocus sequence analysis (MLSA) using four housekeeping genes suggested there may be multiple *P. syringae* pathovars responsible for BLS on beet and chard in states like Washington, Oregon, and California (Safni et al. 2016). This affects the ability to detect and quantify the diversity of strains of the pathogen on seed and in plants and soil. In addition, some strains of *P. syringae* cause BLS on both beet and Swiss chard, while some only cause BLS on beet (Safni et al. 2016). *Psa* infects plants via the hydathodes on leaf margins, stomata, or areas of physical damage on the foliage, petioles, or stems. On beet, symptoms caused by *Psa* typically include irregular, water-soaked, dark brown or black spots or streaks on leaves, that coalesce over time and may senesce to tan, dry lesions (Harveson et al. 2009). On Swiss chard, lesions begin as water-soaked spots which enlarge into round, necrotic lesions with brown or black borders (Harveson et al. 2009). Symptoms may also include notches on the margins of leaves or cotyledons as tissues expand around lesions, deformed leaves, reduced leaf area, and dieback of seedlings (Derie et al. 2016; Harveson et al. 2009; Jacobsen 2009; Koike et al. 2003). In addition, seedborne *Psa* can cause seedling blight, reducing stands in crops (Harveson et al. 2009). The pathogen can spread quickly and result in major crop losses (Derie et al. 2016; Koike et al. 2003; Pethybridge et al. 2018).

Economic impact. The economic effects of pathogens such as *Psa* can be significant. Significant losses to BLS have been reported on *B. vulgaris* crops in the US over the last two decades. New York table beet growers experienced a prevalence of 75% bacterial leaf spot caused by *Psa* in 2017, with an average incidence of 80% across fields surveyed (Pethybridge et al. 2018). For beet and chard grown for baby leaf markets, BLS can be particularly damaging as small amounts of disease can cause entire crops to be rejected since it is too difficult to sort diseased leaves during processing (Derie et al. 2016; Koike et

al. 2003). Baby leaf crops are also planted at high densities and are overhead-irrigated, resulting in rapid spread of the pathogen in crops (Derie et al. 2016; Koike et al. 2003). A current trend toward growing baby leaf crops indoors may increase BLS prevalence if conditions are moist or humid. In some cases, entire beet or chard fields have had to be replanted as a result of BLS, and many growers use copper foliar sprays to try and limit disease development (Nampijja et al. 2023; Pethybridge et al. 2018; Scheck and Pscheidt 1998).

Environmental influence on BLS. For a pathogen to thrive in a crop, a susceptible host, a sufficient amount of pathogen inoculum, and environmental conditions favorable for disease development are necessary (Agarwal and Sinclair 1997). Cool, wet weather is favorable for development of BLS, including periods of rain or extensive dew (Derie et al. 2016; Hirano and Upper 2000). Under optimal field conditions, a seed lot infected with relatively low levels of *Psa* can result in loss of the entire crop due to the ability of the pathogen to spread quickly via splash dispersal (Derie et al. 2016). In trials by Derie et al. (2016), planting seed lots with <10 CFU/g seed developed BLS under favorable conditions. Conversely, seed lots that are highly infected may not result in development of BLS when the infected seed is planted in conditions not conducive for BLS, such as in warm, dry weather.

1.4 Management of BLS

Several management strategies are used to control BLS in table beet and Swiss chard crops. Planting cultivars with resistance to BLS is one management recommendation, although there currently are no commercial cultivars with complete resistance to BLS (Gaulke and Goldman 2022). When Gaulke and Goldman (2022) screened dozens of beet and Swiss chard cultivars for BLS susceptibility, they identified five cultivars that looked promising for breeding resistance to BLS: Touchstone Gold, Bull's Blood, Ruby Queen, Kestrel, and Rainbow. In the absence of access to resistant cultivars, the next best step to controlling seedborne pathogens like *Psa* is to plant pathogen-free seed (Fatmi and Bolkan 2017;

Kuan 1988). Although *Psa* may be present in the environment in which seed is planted, transmission of seedborne inoculum is thought to be responsible for many incidences of crop losses (Jacobsen 2009). Therefore, beet and chard seed sold commercially should be tested for the presence of *Psa* (Nampijja et al. 2023). Seed lots found to be infected are disinfected and re-tested. Many growers are now requiring a negative test for *Psa* to accept beet or Swiss chard seed lots, and in some countries, such as India, imported seed must be certified *Psa* negative (Phytosanitary Export Database 2023).

Detection of *P. syringae* on seed. According to Gitaitis and Walcott (2007) several methods are used to detect bacterial infections on seed lots, including direct testing of seed via grow-outs, inoculating host plants with bacterial extracts from samples of the seed lot, serology, plating a diluted seed wash onto selective media, polymerase chain reaction (PCR) assays, and flow cytometry. To increase the sensitivity of seed health assays, various techniques can be used individually or in conjunction, such as performing both seed wash and plating with a bioassay for suspect isolates, and a PCR assay of suspect isolates (Gitaitis and Walcott 2007). Seed testing protocols should take into consideration the tolerance or threshold of the pathogen that can result in disease development under field conditions, and the assays should be selective, sensitive, reproducible, economical, and rapid (Schaad 1988).

An example of successful seed testing methods for a *P. syringae* pathogen was reported by Suzuki et al. (2014), who were able to isolate *P. syringae* pv. *japonica* (*Psj*) from wheat seeds using two methods. The first method involved surface-sterilizing the seed sample in 1.5% NaOCl for 3 minutes, inserting the seeds into a semi-selective agar medium, and incubating the seed for 7 days at 25°C. The second method entailed immersing 100 g of wheat seed in sterile distilled water for 4 days at 10°C, and then filtering the bacterial suspension through a membrane filter and plating the filtered suspension onto semi-selective media (Suzuki et al. 2014). Using the membrane filter method, they detected *Psj* in a wheat seed sample when only 0.08% of seeds were contaminated.

Testing for the presence of pathogens on seed lots via grow-out assays is another method used for detection of *Psa.* Grow-out assays are conducted by planting a seed sample, typically 10,000 or more seeds, under conditions conducive for disease development, and evaluating the developing seedlings for symptoms of BLS (Gitaitis and Walcott 2007; Walcott 2003). This method requires a large amount of space in a greenhouse or other suitable area to grow the plants, and can take several weeks or more to complete. In addition, due to variation in symptom expression, it is often necessary to confirm suspected symptomatic seedlings are positive for the disease by isolating the pathogen from lesions, which prolongs the time required to complete the assay, making this a less-than ideal method when timely results are required. Cross-contamination of seed lots is also a concern, since *Psa* is splash dispersed, so additional space is needed to separate adequately assays being performed concurrently (Walcott 2003). There may also be inconsistency in results due to specific growing conditions necessary for disease development, which may be difficult to maintain in a large space like a greenhouse. Due to these constraints, grow-out assays are not the preferred method for detection of *Psa* on beet and chard seed lots.

Using a PCR assay developed for the target seedborne pathogen is another method that has been used for detection of *P. syringae* pathogens. PCR assays typically are used to determine which suspect isolates identified via a seed wash assay are the correct pathogen. After identification of isolates via the PCR assay, the suspect isolates are then subjected to a bioassay to confirm pathogenicity. However, there are several potential limitations to using PCR assays. First, the PCR assay may lack adequate specificity and incorrectly identify some bacterial strains as the pathogen (Gitaitis and Walcott 2007). For example, Rico et al. (2003) found a PCR assay used to detect *P. savastanoi* pv. *phaseolicola* on bean seeds omitted pathogenic strains that lacked the phaseolotoxin gene cluster, the site the PCR assay targeted. As a result, some seed lots were mistakenly certified as pathogen-free. Multiple pathovars that do not group closely together by MLSA have been shown to cause BLS on beet and chard

(Safni et al. 2016). For example, some *P. syringae* isolates pathogenic on beet/chard did not fall into phylogroup 2b to which many pathogenic strains belong, presenting a potential risk if the PCR assay used to detect *Psa* is targeted to regions of DNA unique to strains in phylogroup 2b, i.e., the DNA regions may not be present in all strains of the pathogen. Research is needed to develop PCR primers that are specific and selective for detection of the genetic diversity of *Psa* strains that can infect beet or chard seed lots. In addition, PCR assays detect DNA extracted from both live and dead bacteria, which may result in a false-positive test result when the bacteria are not viable, e.g., following a seed disinfection treatment (Walcott 2003). However, there are ways to address the presence of non-viable bacteria, such as the use of the DNA-binding dye propidium monoazide, which can enable a PCR assay to discriminate DNA extracted from live vs. dead cells (Temple at al. 2013).

Cultural control. Several cultural practices have been used to control *Psa* in beet and chard crops. As discussed above, using certified pathogen-free seed should be the first step in disease control. Transplants should also be certified pathogen-free and inspected for BLS symptoms prior to planting. If symptoms are found, suspect seedlings can be removed and isolated while leaf samples are plated onto a semi-selective agar medium, such as mKBC or KB agar media, to determine the causal agents of the symptoms (King et al. 1954; Mohan and Schaad 1987). Seedlings found to be infected with *Psa* can be discarded. Other cultural control methods for BLS include eradicating possible sources of inoculum. For example, *Psa* can persist on infected host plant residues, volunteer plants, and on some weed hosts, but the bacterium does not appear to persist for long in soil in the absence of host plant tissue (Monteil et al. 2012). Incorporating infested crop residues and potential weed hosts into the soil speeds up microbial decomposition of the residues and, therefore, reduces the duration of survival of *Psa* in soil (Lamichhane at al. 2015). Schultz and Gabrielson (1986) found *Xanthomonas campestris* pv. *campestris*, a pathogenic bacterium on crucifers, persisted on infected cabbage seed crop residues in western Washington for >500 days, resulting in infected residues serving as the primary inoculum source to

infect seed crops the following year. These sources of inoculum can be avoided by following adequate crop rotations, such as not planting a field with beet and chard two years in a row, and not planting into a field that had infected weed hosts the previous year. Since *Psa* is not known to persist in soil in the absence of plant residues, a two-year crop rotation may be sufficient to control this disease, at least for vegetative crops. However, other *B. vulgaris* pathogens may require longer crop rotations, particularly soilborne pathogens such as *Peronospora farinosa*, the causal agent of downy mildew, and Rhizomania caused by *Beet necrotic yellow vein virus* (BNYVV), which is vectored by a soilborne pathogen *Polymyxa betae* (Navazio et al. 2010). Crops rotated with beet and chard should not be alternative hosts of *Psa* or of other beet pathogens.

Other possible environmental sources of inoculum include irrigation water and rain (Riffaud and Morris 2002). Irrigation water sources can be tested for the presence of *Psa*, and treated to eliminate the bacteria. Irrigation water can be disinfected via heat pasteurization, ultraviolet radiation, membrane filtration, electrolysis, or via oxidation reduction using chemicals such as chlorine, ozone, or hydrogen peroxide (Banach and van der Fels-Klerx 2020; Newman 2004). In addition, irrigation methods that do not promote splash dispersal of *Psa* could be used, whenever feasible, such as furrow, drip, or subsurface irrigation (Banach and van der Fels-Klerx 2020). Unfortunately, the use of drip, furrow, or subsurface irrigation is not feasible for baby leaf crops, due to the high planting density.

Chemical control. Another strategy for in-season control of BLS of beet and chard is the use of copper or other bactericide sprays (Lamichhane et al. 2015). Bordeaux mixture, a mixture of copper sulfate pentahydrate and lime, was the first copper-based antimicrobial compound used on plants, in 1885 (Lamichhane et al. 2018). Since its discovery, Bordeaux mixture and other copper compounds have been used widely to control foliar pathogens (Lamichhane et al. 2018). Copper treatments are used strictly as a preventative control tactic, since they are not systemic or curative and have limited efficacy once disease symptoms have developed (Lamichhane et al. 2015). Also, timing of applications is critical,

since latent bacterial infections occur in which plants are infected but not yet showing symptoms, and copper bactericides are less effective against latent infections than at protecting against establishment of bacteria on plants. In addition, some bacterial pathogens have developed resistance to copper (Jones et al. 2007; Jones et al. 2012; Scheck and Pscheidt 1998). Antibiotics, such as streptomycin, also have been used as bactericide treatments, but there has been a significant increase in antibiotic resistance development among plant, animal, and human bacterial pathogens, causing a policy shift away from using antibiotics for chemical control of plant diseases (Jones et al. 2012; Sundin et al. 2016). Because of the limitations to using copper products and antibiotics, biological control treatments are being researched for control of bacterial diseases of plants (Lamichhane at al. 2015).

Biological control. The term biological control can be defined as the use of beneficial microbes or their byproducts, or the byproducts and extracts from plants or animals, to suppress plant pathogen activity (Sundin et al. 2016). Bacteria from a variety of genera are used for biological control, including *Agrobacterium*, *Bacillus*, *Erwinia*, *Pseudomonas*, and *Streptomyces* (Bonaterra et al. 2022). Bacteria used as biological control agents act upon a target bacterial or fungal pathogen via a variety of mechanisms, such as competitive exclusion, antagonistic activity due to the release of antimicrobials or enzymes, or the induction of a host plant immune response (Bonaterra et al. 2022). One example of a byproduct produced by bacteria that is used for biological control is bacteriocins. Bacteriocins are peptides secreted by bacteria for the purpose of either preventing the growth of, or killing, other bacteria or unrelated microorganisms (Abriouel et al. 2011; Benitez-Chao et al. 2021; Subramanian and Smith 2015). The host bacterial strain that produces a bacteriocin is protected from the bacteriocin activity via an immunity protein that is co-produced with the bacteriocin (Montesinos et al. 2022). *Bacillus subtilis* is an example of a bacterium used for biocontrol that produces bacteriocins, along with other antimicrobial compounds (Fira et al. 2018). *B. subtilis* can be found in several biocontrol products, such as Serenade ASO and Serenade OPTI (Bayer CropScience LP, St. Louis, MO), and Cease (BioWorks, Inc.,

Victor, ID). Similarly, other *Bacillus* species have been studied as biological control agents, such as *B. pumilus* and *B. amyloliquefaciens*, which were evaluated by Nikolić et al. (2019) for biological control of *Psa* on sugar beet, who found that pure cultures of *B. amyloliquefaciens* strain SS-12.6 caused significant suppression of *Psa*.

Plant extracts, such as the extract produced by the giant knotweed plant, *Reynoutria sachalinensis*, are in products such as Regalia (Marrone Bio Innovations, Davis, CA) and induce a host plant immune response. Esquivel-Cervantes et al. (2022) found the incidence of gray mold caused by *Botrytis cinerea,* and powdery mildew caused by *Leveillula taurica,* were reduced significantly in tomato plants following foliar applications of *R. sachalinensis* extracts. Schneider and Ullrich (1994) found applications of these extracts resulted in less severe powdery mildew (*Sphacelotheca fuliginea*) on cucumber and less severe bacterial speck *(P. syringae* pv*. tobaci* and *P. syringae* pv. *pisi*) on tobacco.

Another type of biocontrol agent is bacteriophages, viruses that infect bacteria, causing the bacterial host cells to lyse (Sundin et al. 2016). The use of bacteriophages as biocontrol agents is called phage therapy and has been the subject of much research. Effective phage therapies have been established for bacterial speck of tomato caused by *P. syringae* pv. *tomato*, and bacterial spot of pepper (*Capsicum* spp.) and tomato caused by *Xanthomonas campestris* pv. *vesicatoria* (Balogh et al. 2003; Flaherty et al. 2000). More recently, Rombouts et al. (2016) isolated phages to combat leek (*Allium ampeloprasum*) bacterial blight caused by *P. syringae* pv. *porri*, and Frampton et al. (2014) identified potential phages to combat kiwifruit (*Actinidia deliciosa*) bacterial canker caused by *P. syringae* pv. *actinidiae*. Biocontrol using phage therapy potentially is useful in areas where antibiotics and copper treatments are not permitted or have failed due to pathogen populations developing resistance, but there are many obstacles to establishing effective phage biocontrol of plant diseases like BLS. The biggest obstacle, according to Jones et al. (2012), is bacteriophages do not persist very long on the plant surface because they are inactivated by ultraviolet light. Also, phages need to be close to the pathogen

at certain stages in the pathogen life cycle, so it is critical to provide environmental conditions that enable phages to persist on plant surfaces. Strategies developed to combat the persistence issues include altering the timing of applications and using protective formulations, such as adding skim milk to phage suspensions, that prolong phage viability on the leaf surface (Jones et al. 2012).

An additional control measure for BLS is the use of seed treatments prior to planting to control seedborne inoculum. Currently, only proprietary seed disinfection treatments are available to reduce or eradicate seedborne *Psa* inoculum, such as the ProBio Gopure treatment used by Germains Seed Technology (Gilroy, CA), and the Clean Start organic disinfection process used by Universal Seeds LLC (Independence, OR). Seed disinfection methods have been evaluated and found to be effective at eradicating or reducing other *P. syringae* pathogens from seed. For example, Yamashiro et al. (2011) found a combination of dry heat and vinegar treatment was highly effective at disinfecting barley seed infected with *P. syringae* pv. *japonica*, the causal agent of black node disease.

Thresholds for *Psa* on beet and chard seed. Another method to limit the economic impact caused by *Psa* on beet and chard seed is to determine thresholds for seedborne inoculum that result in economically significant losses. The inoculum threshold of a seedborne pathogen can be defined as the amount of seed infestation or infection that will result in disease development in the field and cause economic losses under environmental conditions favorable for disease (Kuan 1988). To identify relevant inoculum thresholds, the correlation between seedborne infection levels, determined via seed health testing, and the severity of disease observed in the field when seed is planted, must be determined (Kuan 1988). Determining thresholds for a pathogen on seed lots enables companies and consumers to quantify the risk associated with planting infected seed lots, and facilitates efforts focused on reducing pathogen levels to below the threshold instead of trying to eliminate the pathogen from seed lots altogether, which can be costly, time-consuming, and may not be feasible logistically or economically (if the threshold is determined to be >0) (Kuan 1988). Developing accurate thresholds for seedborne

pathogens is particularly important as infection of seedlings typically occurs in seed germination and seedling development, and can spread quickly through a greenhouse or field, resulting in severe crop loss, even from very low seedborne infection levels (Derie et al. 2016; Schaad 1988). For example, epiphytotics of halo blight of bean resulted from seedborne infection levels as low as 0.02% (Walker and Patel 1964). In trials by Derie et al. (2016) in western Washington, a Swiss chard seed lot infested with *Psa* at <10 CFU/g seed was able to transmit the pathogen and establish BLS when planted as a baby leaf crop under favorable environmental conditions for BLS, including sprinkler irrigation. Under such conditions, it is possible that any detectable amount of pathogen on the seed may be enough to cause disease, i.e., the threshold for the pathogen on seed lots may be zero. One example is the black rot pathogen on crucifers, *Xanthomonas campestris* pv. *campestris*, for which the threshold for seed infection is 0 out of 30,000 seeds (Schaad 1980). However, for some crops and diseases, the threshold for seedborne inoculum may be very high. Umesh et al. (1998) determined, for *X. hortorum* pv. *carotae*, the cause of bacterial leaf blight of carrot, the threshold for seed contamination that resulted in an economic loss at harvest in a field trial in central California was 10^4 to 10^5 CFU/g seed, which they described as "unexpectedly high." They found a positive correlation between seed contamination levels and *X. hortorum* pv. *carotae* populations detected on leaves as well as the incidence and severity of bacterial leaf blight (Umesh et al. 1998).

It can be difficult to establish inoculum thresholds because many factors influence thresholds, including cropping systems, weather conditions, and cultivar susceptibility (Kuan 1988). For example, baby leaf crops grown in a relatively dry greenhouse environment may tolerate a higher threshold of seedborne inoculum versus baby leaf crops direct-seeded in rainy field conditions. For *Lettuce mosaic virus* (*LMV*), the threshold for infected seed was determined to be significantly higher (9 out of 2,000 seeds) in the Netherlands than in California where seed lots must have 0 infected seeds out of a sample of 30,000 to be certified (Agarwal and Sinclair 1997). The extreme difference in these two thresholds is

due to the distinct weather patterns in these two areas and how temperature affects the aphid populations that vector the pathogen (Agarwal and Sinclair 1997). The different cropping system in the two regions also play a role in these thresholds. In California, lettuce is grown almost year-round, while in the Netherlands there is a longer lettuce-free period between crops, which breaks the disease cycle (Agarwal and Sinclair 1997). Other factors that can impact disease development, making it challenging to determine relevant thresholds, include the presence of alternative inoculum sources, such as infected weed hosts or crop residues (Kuan 1988).

***Psa* persistence on beet and chard seed.** In addition to seedborne thresholds, another important aspect in determining the level of risk associated with a seedborne pathogen is the duration over which a pathogen can survive on seed (Agarwal and Sinclair 1997). In some cases, seedborne pathogens may survive longer than the host seeds remain viable, such as certain embryo-borne viruses (Agarwal and Sinclair 1997). There are several factors that affect the persistence and viability of seedborne pathogens, including host genotype, amount of inoculum present, location of inoculum in or on the seed, storage environment (including the type of container in which seed is stored), length of time seed is stored, and the presence of other antagonistic microflora in/on the seed, such as bacteriophages (Agarwal and Sinclair, 1997). Brodal and Asdal (2021) studied the longevity of 15 seedborne pathogens over a 30-year period. All 15 pathogens were still detectable at the end of the study, although some pathogen levels had decreased (e.g., *Septoria nodorum* in wheat), some increased (e.g., *Phoma betae* in beet), and some had no significant change over the 30 years (e.g., *Drechslera* spp. in barley, *Hordeum vulgare*).

1.5. Conclusions and Future Research.

BLS on beet and Swiss chard has gained much attention over the past few decades and research avenues are being explored related to this disease. In 2019, Specialty Crops Research Initiative (SCRI)

grant No. 2019-51181-30019 was funded by the USDA National Institute of Food and Agriculture (NIFA) to support research on diseases caused by *P. syringae* pathogens of cucurbits and chenopods (Bull 2019). The grant is titled "Integrated Management of Emerging Seedborne Bacterial Diseases of Cucurbits and Chenopods (IMDCC)". The overall goal is to develop economically feasible integrated management systems for diseases caused by *P. syringae* pathogens of cucurbits and chenopods, with six main objectives:

1. Develop diagnostic methods for detection and quantification of the pathogens in seed and from environmental inoculum sources;
2. Develop novel IPM practices for crop production and seed production to reduce seed contamination/infection and disease;
3. Develop seed testing protocols and treatments for quality assurance;
4. Identify novel sources of disease resistance to these pathogens;
5. Analyze the cost-effectiveness for all practices developed;
6. International seed health extension, training, and mentorship.

The significant number of seed companies across the globe involved in this project in an advisory capacity is indicative of the impact this pathogen has on the agriculture industry worldwide (Bull 2019).

The research explored in the following two chapters falls under several of these objectives. The first objective of this MS in Agriculture research project was to determine seedborne thresholds for *Psa* in baby leaf beet and chard crops. The second objective was to determine the duration of survival of *Psa* on beet/chard seed placed in typical commercial storage conditions. These two aspects of the project are described in Chapter 2. The third objective was to evaluate the efficacy of biological and chemical treatments used preventatively in seed crops for management of *Psa*, as detailed in Chapter 3. These combined projects will help fill in some of the gaps in our understanding of how *Psa* can be managed in vegetative and reproductive chenopod crops.

1.6. REFERENCES

Abriouel, H., Franz, C. M., Omar, N. B., and Gálvez, A. 2011. Diversity and applications of *Bacillus* bacteriocins. FEMS Microbiol. Rev. 35(1):201–232.

Agarwal, V. K., and Sinclair, J. B. 1997. Principles of Seed Pathology, 2nd Ed. Lewis Publishers, Boca Raton.

Amato, P., Parazols, M., Sancelme, M., Laj, P., Mailhot, G., and Delort, A. M. 2007. Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dôme: major groups and growth abilities at low temperatures. FEMS Microbiol. Ecol. 59(2):242-254.

Arabiat, S., Chanda, A. K., Chittem, K., and Khan, M. F. R. 2016. First report of *Pseudomonas syringae* pv. *aptata* causing bacterial blight of sugar beet (*Beta vulgaris*) in Oregon. Plant Dis. 100(11):2334.

Assad, S., Sands, D. C., and Mohan, S. K. 2017. Detection of *Pseudomonas syringae* pv. *syringae* in wheat seeds. Pp. 21-26, In: Detection of Plant-Pathogenic Bacteria in Seed and Other Planting Material, 2nd Ed. M. Fatmi, R. R. Walcott, and N. W. Schaad, eds. American Phytopathological Society, St Paul.

Balaž, J., Iličić, R., Maširević, S., Jošić, D., and Kojić, S. 2014. First report of *Pseudomonas syringae* pv. *syringae* causing bacterial leaf spots of oil pumpkin (*Cucurbita pepo*) in Serbia. Plant Dis. 98(5):684.

Balogh, B., Jones, J. B, Momol, M. T., Olson, S. M., Obradovic, A., King, P., and Jackson, L. E. 2003. Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. Plant Dis. 87(8):949-954.

Baltrus, D. A., McCann, H. C., and Guttman, D. S. 2017. Evolution, genomics and epidemiology of *Pseudomonas syringae*: Challenges in bacterial molecular plant pathology. Molec. Plant Pathol. 18(1):152-168.

Banach, J. L., and van der Fels-Klerx, H. J. 2020. Microbiological reduction strategies of irrigation water for fresh produce. J. Food Prot. 83(6):1072-1087.

Benitez-Chao, D. F., Leon-Buitimea, A., Lerma-Escalera J. A., and Morones-Ramirez, J. R. 2021. Bacteriocins: an overview of antimicrobial, toxicity, and biosafety assessment by in vivo models. Front. Microbiol. 12:630695.

Berge, O., Monteil, C. L., Bartoli, C., Chandeysson, C., Guilbaud, C., Sands, D. C., and Morris, C. E. 2014. A user's guide to a data base of the diversity of *Pseudomonas syringae* and its application to classifying strains in this phylogenetic complex. PLoS One 9(9):e105547.

Biancardi, E., Panella, L. W., and McGrath, J. M. 2019. *Beta maritima*: The Origin of Beets, 2nd Ed. Springer International Publishing AG, New York.

Bonaterra, A., Badosa, E., Daranas, N., Francés, J., Roselló, G., and Montesinos, E. 2022. Bacteria as biological control agents of plant diseases. Microorganisms 10(9):1759.

Bradbury, J. F. 1983. The new bacterial nomenclature - What to do. Phytopathology 73(10):1349-1350.

Braun-Kiewnick, A., and Sands, D. C. 2001. II. Gram negative bacteria. C. *Pseudomonas*. Pp. 84-120, In: Laboratory Guide for Identification of Plant Pathogenic Bacteria, 3rd Ed. N. W. Schaad, J. B. Jones, and W. Chun, eds. American Phytopathological Society, St. Paul.

Brodal, G., and Asdal, A. 2021. Longevity of plant pathogens in dry agricultural seeds during 30 years of storage. Microorganisms 9(10):2175.

Brown, N. A., and Jamieson, C. O. 1913. A bacterium causing a disease of sugar beet and nasturtium leaves. J. Agric. Res. 1:189-210.

Bull, C. T. 2019. Integrated management of emerging seedborne bacterial diseases of cucurbits and chenopods (IMDCC). United States Department of Agriculture National Institute of Food and Agriculture Specialty Crops Research Initiative Project No. 2019-51181-30019. https://www.pseudosonseed.org/ (accessed 10 Oct. 2023).

Cornelis, P. 2008. *Pseudomonas*: Genomics and Molecular Biology. Caister Academic Press, Norfolk.

Derie, M. L., Holmes, B. J., Safni, I., Bull, C. T, and Derie, L. J. 2016. Seedborne inoculum thresholds of *Pseudomonas syringae* pv. *aptata*, causal agent of bacterial leaf spot, in 'baby leaf' Swiss chard crops. Phytopathology 106:S4.142 (Abstract).

Duke, J. A. 1983. *Beta vulgaris* L. Handbook of Energy Crops. Center for New Crops and Plants Products, Purdue University, Lafayette. www.hort.purdue.edu/newcrop/duke_energy/Beta_vulgaris.html (accessed 10 Oct. 2023).

Dutta, B., Gitaitis, R., Smith, S., and Langston, D. Jr. 2014. Interactions of seedborne bacterial pathogens with host and non-host plants in relation to seed infestation and seedling transmission. PLoS One 9(6):e99215.

Dutta, B., Ingram, T., Gitaitis, R. D., Langston, D. B., Brenneman, T., Webster, T. M., and Davis, R. F. 2014. First Report of bacterial blight of sugar beet caused by *Pseudomonas syringae* pv. *aptata* in Georgia, USA. Plant Dis. 98(10):1423.

du Toit, L. 2007. Crop profile for table beet seed in Washington. U.S. Department of Agriculture National Pest Management Regional IPM Center, Davis, CA, U.S.A. https://ipmdata.ipmcenters.org/documents/cropprofiles/WAbeetseed2007.pdf (accessed 10 Oct. 2023).

Esquivel-Cervantes, L. F., Tlapal-Bolaños, B., Tovar-Pedraza, J. M., Pérez-Hernández, O., Leyva-Mir, S. G., and Camacho-Tapia, M. 2022. Efficacy of biorational products for managing diseases of tomato in greenhouse production. Plants 11(13):1638.

Fatmi, B. M., and Bolkan, H. 2017. Bacterial disease of plants: Epidemiology and management strategies. Pp. 3-10, In: Detection of Plant-Pathogenic Bacteria in Seed and Other Planting Material, 2nd Ed. M. Fatmi, R. R. Walcott, and N. W. Schaad, eds. American Phytopathological Society, St Paul.

Fira, D., Dimkić, I., Berić, T., Lozo, J., and Stanković, S. 2018. Biological control of plant pathogens by *Bacillus* species. J. Biotechnol. 285:44-55.

Flaherty, J. E., Somodi, G. C., Jones, J. B., Harbaugh, B. K., and Jackson, L. E. 2000. Control of bacterial spot on tomato in the greenhouse and field with H-mutant bacteriophages. HortSci. 35(5):882-884.

Frampton, R. A., Taylor, C., Moreno, A. V. H., Visnovsky, S. B., Petty, N. K., Pitman, A. R., and Fineran, P. C. 2014. Identification of bacteriophages for biocontrol of the kiwifruit canker phytopathogen *Pseudomonas syringae* pv. *actinidiae*. Appl. Environ. Microbiol. 80(7):2216-2228.

Gaulke, E., and Goldman, I. L. 2022. Screening table beet and Swiss chard for resistance to *Pseudomonas syringae* pathovar *aptata*. HortSci. 57(11):1436-1446.

Gitaitis, R., and Walcott, R. 2007. The epidemiology and management of seedborne bacterial diseases. Annu. Rev. Phytopathology 45:371-397.

Gitaitis, R., and Walcott, R. 2017. Review of seed sample size determination and methods for extracting phytopathogenic bacteria from seeds. Pp. 13-17, In: Detection of Plant-Pathogenic Bacteria in Seed and Other Planting Material, 2nd Ed. M. Fatmi, R. R. Walcott, and N. W. Schaad, eds. American Phytopathological Society, St Paul.

Goldman, I. L., and Navazio, J. P. 2008. Table beet. In: Vegetables I. Handbook of Plant Breeding, Vol. 1. J. Prohens, F. Nuez, eds. Springer, New York.

Gutierrez-Barranquero, J. A., Cazorla, F. M., and de Vicente, A. 2019. *Pseudomonas syringae* pv. *syringae* associated with mango trees, a particular pathogen within the “hodgepodge” of the *Pseudomonas syringae* complex. Front. Plant Sci. 10:570.

Harveson, R. M., Hanson, L. E., and Hein, G. L. 2009. Compendium of Beet Diseases and Pests, 2nd Ed. American Phytopathological Society, St. Paul.

Hesse, C., Schulz, F., Bull, C. T., Shaffer, B. T., Yan, Q., Shapiro, N., Hassan, K. A., Varghese, N., Elbourne, L. D. H., Paulsen, I. T., Kyrpides, N., Woyke, T., and Loper, J. E. 2018. Genome-based evolutionary history of *Pseudomonas* spp. Environ. Microbiol. 20(6):2142-2159.

Hirano, S. S., and Upper, C. D. 2000. Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*-a pathogen, ice nucleus, and epiphyte. Microbiol. Mol. Biol. Rev. 64(3):624-653.

Ignjatov, M., Gvozdanović-Varga, J., Milošević, D., Nikolić, Z., Ivanović, Ž., and Popović, T. 2015. First report of bacterial leaf spot of chard (*Beta vulgaris* subsp. *cicla*) caused by *Pseudomonas syringae* pv. *syringae* in Serbia. Plant Dis. 99(5):723.

Jacobsen, J. 2009. Bacterial leaf spot. Pp. 59-60, In: Compendium of Beet Diseases and Pests, 2nd Ed. R. M. Harveson, L. E. Hanson, and G. L. Hein, eds. American Phytopathological Society, St. Paul.

Jones, J. B., Jackson, L. E., Balogh, B., Obradovic, A., Iriarte, F. B., and Momol, M. T. 2007. Bacteriophages for plant disease control. Annu. Rev. Phytopathol. 45(1):245-262.

Jones, J. B., Vallad, G. E., Iriarte, F. B., Obradović, A., Wernsing, M. H., Jackson, L. E., Balogh, B., Hong, J. C., and Momol, M. T. 2012. Considerations for using bacteriophages for plant disease control. Bacteriophage 2(4):e23857.

King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44(2):301-307.

Koike, S. T., Alger, E. I., Sepulveda, L. R., and Bull, C. T. 2017. First report of bacterial leaf spot caused by *Pseudomonas syringae* pv. *tomato* on kale in California. Plant Dis. 101(3):504.

Koike, S. T., Azad H. R., and Cooksey, D. C. 2002. First report of bacterial leaf spot of spinach caused by a *Pseudomonas syringae* pathovar in California. Plant Dis. 86(8):921.

Koike, S. T., Henderson, D. M., Bull, C. T., Goldman, P. H., and Lewellen, R. T. 2003. First report of bacterial leaf spot of Swiss chard caused by *Pseudomonas syringae* pv. *aptata* in California. Plant Dis. 87(11):1397.

Kuan, T. L. 1988. Inoculum thresholds of seedborne pathogens. Overview. Phytopathology 78(6):867-868.

Lamichhane, J. R., Messéan, A., and Morris, C. E. 2015. Insights into epidemiology and control of diseases of annual plants caused by the *Pseudomonas syringae* species complex. J. Gen. Plant Pathol. 81(5):331-350.

Lamichhane, J. R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J. B., and Aubertot, J. N. 2018. Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. Agron. Sustain. Develop. 38(3):1-18.

Lelliott, R. A., Billing, E., and Hayward, A. C. 1966. A determinative scheme for the fluorescent plant pathogenic Pseudomonads. J. Appl. Bacteriol. 29(3):470-489.

Mohan, S. K., and Schaad, N. W. 1987. An improved agar plating assay for detecting *Pseudomonas syringae* pv. *syringae* and *P. s.* pv. *phaseolicola* in contaminated bean seed. Phytopathology 77(10):1390-1395.

Monteil, C. L., Guilbaud, C., Glaux C., Lafolie F., Soubeyrand S., and Morris, C. E. 2012. Emigration of the plant pathogen *Pseudomonas syringae* from leaf litter contributes to its population dynamics in alpine snowpack. Environ. Microbiol. 14(8):2099-2112.

Montesinos, E., Badosa, E., Pla, M., Montesinos, L., and Bonaterra, A. 2022. Antimicrobial and defense elicitor peptides as biopesticides for plant disease control. Pp. 121-158, In: Biocontrol of Plant Disease: Recent Advances and Prospects in Plant Protection. C. Prigent-Combaret, and B. Dumas, eds. John Wiley and Sons, Inc., Hoboken.

Morris, C. E., Sands, D. C., Vinatzer, B. A., Glaux, C., Guilbaud, C., Buffiere, A., Yan, S., Dominguez, H., and Thompson, B. M. 2008. The life history of the plant pathogen *Pseudomonas syringae* is linked to the water cycle. ISME J. 2(3):321-334.

Mulet, M., Lalucat, J., and García-Valdés, E. 2010. DNA sequence-based analysis of the *Pseudomonas* species. Environ. Microbiol. 12(6):1513-1530.

Nampijja, M., Crane, S., du Toit, L., and Ocamb, C. 2023 March. Beet, red (*Beta vulgaris*)-Bacterial Leaf Spot. Pacific Northwest Plant Management Handbook. Oregon State University. https://pnwhandbooks.org/plantdisease/host-disease/beet-red-beta-vulgaris-bacterial-leaf-spot (accessed 10 Oct. 2023).

Nampijja, M., Derie, M. L., and du Toit, L. J. 2021. First report of bacterial leaf spot caused by *Pseudomonas syringae* pv. *aptata* on Swiss chard, *Beta vulgaris* subsp. *vulgaris*, in Arizona. Plant Dis. 105:3738.

Navazio, J., Colley, M., and Zyskowski, J. 2010. Principles and practices of organic beet seed production in the Pacific Northwest. Organic Seed Alliance, Port Townsend.

Neergaard, P. 1977. Seed Pathology. Wiley, New York.

Newberry, E. A., Jardini, T. M., Rubio, I., Roberts, P. D., Babu, B., Koike, S. T., Bouzar, H., Goss, E. M., Jones, J. B., Bull, C. T., and Paret, M. L. 2016. Angular leaf spot of cucurbits is associated with genetically diverse *Pseudomonas syringae* strains. Plant Dis. 100(7):1397-1404.

Newberry, E. A, Ritchie, L., Babu, B., Sanchez, T., Beckham, K. A., Jones, J. B., Freeman, J. H., Dufault, N. S., and Paret, M. L. 2017. Epidemiology and management of bacterial leaf spot on watermelon caused by *Pseudomonas syringae*. Plant Dis. 101(7):1222-1229.

Newman, S. E. 2004. Disinfecting irrigation water for disease management. 20th Annu. Conf. Pest Management Ornamentals, 20-22 Feb, 2004, Society of American Florists, San Jose.

Nikolić, I., Stanković, S., Dimkić, I., Berić, T., Stojšin, V., Janse, J. and Popović, T. 2018. Genetic diversity and pathogenicity of *Pseudomonas syringae* pv. *aptata* isolated from sugar beet. Plant Pathol. 67:1194-1207.

Nikolić, I., Berić, T., Dimkić, I., Popović, T., Lozo, J., Fira, D., and Stanković, S. 2019. Biological control of *Pseudomonas syringae* pv. *aptata* on sugar beet with *Bacillus pumilus* SS-10.7 and *Bacillus amyloliquefaciens* (SS-12.6 and SS-38.4) strains. J. Appl. Microbiol. 126(1):165-176.

Nottingham, S. 2004. Beetroot. Archived from the original (e-book) on 2012-02-09. Retrieved 15 Aug. 2010.

Parkinson, N., Bryant, R., Bew, J., and Elphinstone, J. 2011. Rapid phylogenetic identification of members of the *Pseudomonas syringae* species complex using the rpoD locus. (Report). Plant Pathol. 60(2):338-344.

Pethybridge, S. J., Kikkert, J. R., Hanson, L. E., and Nelson, S. C. 2018. Challenges and prospects for building resilient disease management strategies and tactics for the New York table beet industry. Agron. J. 8(7):112.

Rico, A., Lopez, R., Asensio, C., Aizpun, M. T., Asensio-Manzanera M. C., and Murillo, J. 2003. Nontoxigenic strains of *Psuedomonas syringae* pv. *phaseolicola* are a main cause of halo blight of beans in Spain and escape current detection methods. Phytopathology 93:1553-1559.

Riffaud, C. M. H., and Morris, C. E. 2002. Detection of *Pseudomonas syringae* pv. *aptata* in irrigation water retention basins by immunofluorescence colony-staining. Eur. J. Plant Pathol. 108(6):539-545.

Rombouts, S., Volckaert, A., Venneman, S., Declercq, B., Vandenheuvel, D., Allonsius, C. N., Van Malderghem, C., Jang, H. B., Briers, Y., Noben, J. P., Klumpp, J., Van Vaerenbergh, J., Maes, M., and Lavigne, R. 2016. Characterization of novel bacteriophages for biocontrol of bacterial blight in leek caused by *Pseudomonas syringae* pv. *porri*. Front. Microbiol. 7:279.

Romeiras, M. M., Vieira, A., Silva, D. N., Moura, M., Santos-Guerra, A., Batista, D., Duarte, M. C., and Paulo, O. S. 2016. Evolutionary and biogeographic insights on the Macaronesian *Beta-Patellifolia* species (*Amaranthaceae*) from a time-scaled molecular phylogeny. PLoS One 2016(3):e0152456.

Rotondo, F., Vrisman, C. M., Rani, R., Testen, A. L., Deblais, L., and Miller, S. A. 2020. First report of *Pseudomonas syringae* pv. *aptata* causing bacterial leaf spot on common beet (*Beta vulgaris*) in Ohio. Plant Dis. 104(2):561.

Safni, I., Ramos-Sepulveda, L., Goldman, P., Derie, M., du Toit, L., Koike, S., Stockwell, V., and Bull, C. 2016. Genetic diversity of *Pseudomonas syringae* causing bacterial leaf spot on table beet (*Beta vulgaris*) and Swiss chard (*Beta vulgaris* subsp. *cicla*). Phytopathology 106:S4.143 (Abstract).

Sanders, E. R. 2012. Aseptic laboratory techniques: plating methods. J. Vis Exp. (63):e3064.

Sarkar, S. F., and Guttman, D. S. 2008. Evolution of the core genome of *Pseudomonas syringae*, a highly clonal, endemic plant pathogen. Appl. Environ. Microbiol. 74(6):1961.

Schaad, N. W. 1980. Relationship of incidence of seedborne *Xanthomonas campestris* to black rot of crucifers [cabbage, broccoli]. Plant Dis. 64(1):91.

Schaad, N. W. 1988. Inoculum thresholds of seedborne pathogens. Bacteria. Phytopathology 78(6):872-875.

Schaad, N. W., Schuenzel, E., Jones, J. B. 2017. Taxonomy of plant-pathogenic bacteria. Pp 11-12 In: Detection of Plant-Pathogenic Bacteria in Seed and Other Planting Material, 2nd Ed. M. Fatmi, R. R. Walcott, and N. W. Schaad, eds. American Phytopathological Society, St Paul.

Scheck, H. J. and Pscheidt, J. W. 1998. Effect of copper bactericides on copper-resistant and -sensitive strains of *Pseudomonas syringae* pv. *syringae*. Plant Dis. 82(4):397-406.

Schneider, S., and Ullrich, W. R. 1994. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. Physiol. Mol. Plant Pathol. 45(4):291-304.

Schrader, W. L., and Mayberry, S. K. 2003. Beet and Swiss chard production in California. University of California Division of Agriculture and Natural Resources Publication 8096.

Schultz, T., Gabrielson, R. L. 1986. *Xanthomonas campestris* pv. *campestris* in western Washington crucifer seed fields: occurrence and survival. Phytopathology 76:1306–1309.

Seedborne cucurbits and chenopods diseases caused by *Pseudomonas syringae*. 2021. Seedborne *Pseudomonas* on Cucurbits and Chenopods. USDA NIFA 2019-51181-30019. https://www.pseudosonseed.org/ (accessed 10 Oct. 2023).

Stanier, R. Y., Palleroni, N. J., and Doudoroff, M. 1966. The aerobic Pseudomonads. A taxonomic study. J. Gen. Microbiol. 43(2):159-271.

Subramanian, S., and Smith, D. L. 2015. Bacteriocins from the rhizosphere microbiome - from an agriculture perspective. Front. Plant Sci. 6:909.

Sundin, G. W., Castiblanco, L. F., Yuan X., Zeng, Q., and Yang, C-H. 2016. Bacterial disease management: challenges, experience, innovation and future prospects. Challenges in bacterial molecular plant pathology. Mol. Plant Pathol. 17(9):1506-1518.

Suzuki, H., Kuroda, K., Yamakawa, T., Matsumoto, K., Hashizume, F., and Tsuji, T. 2014. Highly sensitive detection of *Pseudomonas syringae* pv. *japonica* from wheat seeds by selective medium and membrane filter method. Kansai Byōchūgai Kenkyūkaihō 56:43–47.

Temple, T. N., du Toit, L. J., Derie, M. L., and Johnson, K. B. 2013. Quantitative molecular detection of *Xanthomonas hortorum* pv. *carotae* in carrot seed before and after hot-water treatment. Plant Dis. 97(12):1585-1592.

Traverse, J. H. 2018. Beet it. Circ. Res. 123(6):635-637.

Tymon, L., and Inglis, D. 2017. Identification and pathogenicity of *Pseudomonas syringae* genomospecies 1 phylogroup 2b causing leaf spots and fruit warts on cucurbits in western Washington, US. J. Plant Pathol. 99 (3):713-722.

Umesh, K., Davis, R., and Gilbertson, R. 1998. Seed contamination thresholds for development of carrot bacterial blight caused by *Xanthomonas campestris* pv. *carotae*. Plant Dis. 82(11):1271-1275.

United States Department of Agriculture National Agricultural Statistics Service. 2022. United States Library of Congress. https://quickstats.nass.usda.gov/ (accessed 10 Oct. 2023).

United States Department of Agriculture Phytosanitary Export Database. 2023. Phytosanitary Certificate Issuance and Tracking System. https://pcit.aphis.usda.gov/PExD/faces/ViewPExD.jsf (accessed 10 Oct. 2023).

Walcott, R. R. 2003. Detection of seedborne pathogens. HortTechnol. 13(1):40-47.

Walcott, R. R., and Gitaitis, R. D. 2017. Review of seed sample size determination and methods for extracting phytopathogenic bacteria from seeds. Pp. 13-18, In: Detection of Plant-Pathogenic Bacteria in Seed and Other Planting Material, 2nd Ed. M. Fatmi, R. R. Walcott, and N. W. Schaad, eds. American Phytopathological Society, St Paul.

Waker, J. C., and Patel, P. N. 1964. Splash dispersal and wind as factors in epidemiology of halo blight of bean. Phytopathology 54:140-141.

Yamashiro, M., Waki, T., Morishima, M., and Fukuda, T. 2011. Occurrence of barley bacterial black node (*Pseudomonas syringae* pv. j*aponica*) in Tochigi prefecture and its control by barley seed disinfection treatments. Annu. Rep. Kanto-Tosan Plant Prot. Soc. 2011 (58):9-12.

CHAPTER TWO

THRESHOLDS FOR SEEDBORNE INOCULUM OF *PSEUDOMONAS SYRINGAE* IN *BETA VULGARIS* BABY LEAF CROPS, AND PERSISTENCE OF THE PATHOGEN ON SEED

2.1 Introduction

Beta vulgaris is a flowering plant species in the Chenopodiaceae, which is now included in Amaranthaceae, commonly known as the goosefoot family (Nottingham 2004). The cultivated *B. vulgaris* crops have been split into five groups, all of which are included in *B. vulgaris* subsp. *vulgaris.* These include beet root or table beet in the Conditiva group, spinach beet or leaf beet in the Cicla group, sugar beet in the Altissima group, fodder beet or mangel in the Crassa group, and Swiss chard in the Flavescens group (Nottingham 2004). *B. vulgaris* crops have various uses, including fresh market and processed foods, fodder for livestock, folk medicine preparations, and dietary supplements (Duke 1983; Traverse 2018). The different *B. vulgaris* subsp. *vulgaris* groups are considered minor crops in the US, but millions of acres of farmland are devoted to these crops throughout the country. According to the United States Department of Agriculture (USDA) National Agricultural Statistics Service (USDA NASS 2020), >0.46 million ha of sugar beet were harvested in the US in 2022, weighing in at >31 million metric tons. In addition, >5,600 ha of table beet were harvested for fresh market and processing (USDA NASS 2022). Seed production for *B. vulgaris* crops is also important in the US, specifically in the Pacific Northwest. Approximately 260 to 280 ha of table beet seed crops are grown each year in Washington State, with a commercial value of $5.5 million (du Toit 2007), and ~700 ha of sugar beet seed were produced in Washington State in 2017, representing over 40% of the total 1,600 ha produced in the US (USDA NASS 2022).

B. vulgaris subsp. *vulgaris* is biennial, i.e., two years are required to complete the life cycle and produce seed, unless vernalization in cold storage or a greenhouse is used to shorten the duration needed to achieve flowering and seed set (du Toit 2007). For seed production, the plants must be

exposed to a cold enough period for long enough to induce bolting, i.e., a switch from vegetative to reproductive growth (between 4 and 10°C for ~60 to 90 days), but not so cold the plants are killed (Schrader and Mayberry 2003). *B. vulgaris* plants can tolerate mild freezing conditions (Schrader and Mayberry 2003). As a result of these environmental requirements for flowering and seed set, beet and chard seed crops can only be produced in a very limited number of areas globally. The maritime Pacific Northwest (western Oregon and western Washington) is the only part of the US that has the correct climate for beet and chard seed production, with mild, dry summers and cold but temperate winters (Navazio et al. 2010). In contrast to seed production, fresh market beet and Swiss chard crops are grown as cool season annuals in many areas of the US and the world (Schrader and Mayberry 2003).

B. vulgaris crops can suffer from multiple foliar diseases, one of which is bacterial leaf spot (BLS) caused by *Pseudomonas syringae* pv. *aptata* (*Psa*) (Jacobsen 2009). BLS has been reported across the US, including in Washington, California, Georgia, Ohio, New York, and Florida, and in Europe, Australia, New Zealand, and Asia (Dutta et al. 2014; Ignjatov et al. 2015; Jacobsen 2009; Koike et al. 2002; Koike et al. 2003; Rotondo et al. 2020). The first report of BLS on *B. vulgaris* crops in the United States was in 1908 on sugar beet leaves in Utah (Brown and Jamieson 1913). More recently, BLS symptoms were reported in 1999 on Swiss chard in the Salinas Valley, CA (Koike et al. 2003), and in 2021 on baby leaf crops of this host in Arizona (Nampijja et al. 2021). For many years, *Psa* was thought to be the pathogen causing BLS on Chenopodiaceae crops, but recent multilocus sequence analysis using four housekeeping genes suggested there may be multiple *P. syringae* pathovars, clades, or other subspecies groups responsible for BLS on beet and chard, based on isolates from Washington, Oregon, and California (Safni et al. 2016). For ease of communication, the pathogens causing BLS are referred to collectively as *Psa* in this study.

Psa infects plant tissue via stomata, hydathodes on leaf margins, or wounds caused by physical damage to leaves, cotyledons, or stems (Jacobsen 2009; Nampijja et al. 2023). Symptoms caused by *Psa*

on beet and chard can include water-soaked lesions, often with a black or brown margin, irregular, black lesions that can lead to notches on the margins of leaves or cotyledons expanding around the dead tissue, deformed leaves, and reduced leaf area (Derie et al. 2016; Koike et al. 2003). In addition, *P. syringae* can be seedborne and seed-transmitted, causing seedling blight and reduced stands in crops, and can spread quickly, resulting in major crop losses (Derie et al. 2016; Gitaitis and Walcott 2007; Harveson et al. 2009; Koike et al. 2003).

One approach to limiting the economic impact caused by *Psa* is to establish if there are thresholds for seedborne inoculum on beet and chard so that seed lots infected below the threshold can be selected for planting (Derie et al. 2016). The inoculum threshold of a seedborne pathogen can be defined as the amount of seed infestation or infection that can result in sufficient disease development under production conditions to cause economic losses (Kuan 1988). To identify such inoculum threshold(s), the correlation must be determined between seedborne infection levels, determined via seed health testing, and severity of BLS observed in field conditions (Kuan 1988). Developing accurate thresholds for seedborne pathogens is important as infection of seedlings usually occurs during seed germination and seedling development, and the pathogen can spread quickly, potentially resulting in severe crop losses under favorable conditions, even from very low seedborne infection levels (Schaad 1988). However, identification of specific thresholds for individual seedborne pathogens is complicated by the fact that risk of seed transmission is influenced by numerous factors, such as susceptibility of cultivars to the pathogen and to seed infection and seed transmission, weather conditions at planting and during seedling emergence, virulence of strain(s) of the pathogen present in/on seed, prevalence of the pathogen in a seed lot, planting density of the crop (e.g., baby leaf vs. bunching vs. root crops of beet), etc. (Agarwal and Sinclair 1997; Kuan 1988). In addition, other inoculum sources, such as infected crop residues and weed hosts, can influence disease development, which makes it challenging to determine thresholds relevant for the diversity of potential production conditions (Kuan 1988). The

factors that can influence seed transmission risk are illustrated well by *Lettuce mosaic virus* (LMV), for which the threshold for infected seed in the Netherlands is 9 in 2,000 lettuce (*Lactuca sativa*) seeds, which is much less stringent than in California where seed needs to be certified to have 0 infected seeds out of a sample of 30,000 (Agarwal and Sinclair 1997; Kuan 1988). Such extreme differences in thresholds are associated with distinct weather conditions in these two areas of lettuce production, including the effects of prevalent temperature on aphid vector populations (Agarwal and Sinclair 1997; Kuan 1988). The different cropping systems also impact the thresholds determined to be acceptable in each region. In California, lettuce is grown almost year-round, except for a one-month lettuce-free period required to manage LMV, whereas lettuce cannot be grown year-round in the Netherlands, providing a longer period to interrupt the LMV disease cycle (Agarwal and Sinclair 1997).

For some crops and diseases, the threshold for seedborne inoculum may be very high. For example, Umesh et al. (1998) determined the threshold for carrot (*Daucus carota*) seed contamination by *X. hortorum* pv. *carotae*, the cause of bacterial leaf blight of carrot, was 10^4 to 10^5 CFU/g seed in field trials in central California. Conversely, under favorable environmental conditions, any detectable amount of pathogen present on the seed may be enough to cause a disease outbreak, i.e., the threshold for a pathogen on seed may be zero. For example, for halo blight of bean (*Phaseolus vulgaris*), caused by *Pseudomonas syringae* pv. *phaseolicola*, disease outbreaks resulted from seed infection levels as low as 0.02% incidence of seed infected (Walker and Patel 1964). For black rot of crucifers caused by *Xanthomonas campestris* pv. *campestris*, the seedborne threshold for crucifer stock seed used in seed production is 0 out of 30,000 seeds (Schaad 1980). In trials conducted by Derie et al. (2016) in western Washington, planting a Swiss chard seed lot infested with *Psa* at <10 CFU/g seed resulted in BLS in field plots planted at a baby leaf density of 7.5 million seed/ha. This suggested the threshold for *Psa* on beet and chard seed may be very low or even zero to avoid disease development under highly conducive conditions often encountered in baby leaf crops, with dense seeding rates, sequential plantings, and

overhead irrigation. For baby leaf beet and Swiss chard crops, the threshold for marketability of the harvested leaves is usually 5% incidence, although this can vary based on the amount of product available in the market (Delita Pardue, *personal communication*).

The duration of survival of a pathogen on seed also plays an important role in determining the level of risk for seedborne pathogens (Agarwal and Sinclair 1997). Several factors can affect the viability and survival of seedborne pathogens, including host genotype, amount of inoculum present in or on seed, location of inoculum in or on the seed, seed storage environment (including the type of containers in which seed are stored), length of time in storage, and presence of other microflora on the seed, such as bacteriophages (Agarwal and Sinclair 1997). Some seedborne pathogens can survive longer than the host seed remain viable, such as certain embryo-borne viruses (Agarwal and Sinclair 1997). Brodal and Asdal (2021) studied the longevity of 15 seedborne pathogens over a 30-year period. All 15 pathogens were still detectable at the end of the study, although some pathogen levels decreased over time (e.g., *Septoria nodorum* in wheat, *Triticum aestivum*), some increased (e.g., *Phoma betae* in beet), and some had no significant change (e.g., *Drechslera* spp. in barley, *Hordeum vulgare*). Beet and chard seed typically maintain good germination rates in storage for at least four years (Ells et al. 2020). Therefore, it may be viable to store beet and chard seed that is infected with *Psa*, with periodic re-testing of the lots, in order to sell the lots once the *Psa* levels have declined adequately, assuming *Psa* levels decline more rapidly than seed shelf-life (germination rate and vigor). This could potentially enable seed producers to avoid the cost and potential risk of seed disinfection for *Psa*, thus reducing the economic impacts of BLS on the seed industry and growers who purchase the seed. Establishing a threshold(s) for *Psa* on beet and Swiss chard seed could allow seed companies to save expense and time by only having to disinfect seed lots infected or infested above the threshold(s).

There were two overall objectives to this study: i) to determine seedborne thresholds for *Psa* on table beet and Swiss chard baby leaf crops, and ii) to determine the duration of survival of *Psa* on table

beet and Swiss chard seed lots held in commercial storage conditions. The results of this study will help fill some of the gaps in our understanding of how *Psa* can be managed in beet and chard crops.

2.2 Materials and Methods

Field trials: Treatments and experimental design. Four field trials were completed in Skagit Co., WA over two years to determine seedborne thresholds for *Psa* on beet and chard that result in BLS in baby leaf crops. For each trial, two seed lots of the same proprietary cultivar were selected, one that tested negative for the presence of *Psa* and one that tested positive at $>10^4$ CFU/g seed. The positive and negative seed lots were mixed in various ratios to obtain six sub-lots with a range from 0 to approximately 10^6 CFU/g seed, at 10-fold increments (Table 1). The *Psa* concentration in each of the positive seed lots was determined using a seed health assay (described below). Four replications of each treatment (seed sub-lot) were planted in each trial in a randomized complete block design (RCBD). The same positive and negative seed lots were used in Trials 1 and 2, while the same negative lot but different positive lots of the same cultivar were used in Trials 3 and 4 based on the amount of seed available.

Determining seed infection levels. Two semi-selective agar media were used for detection and quantification of *Psa* on each beet and chard seed lot. The first was a modified version of Medium B described by King et al. (1954), which is commonly referred to as King's B (KB) agar medium. The modifications included increasing the amount of glycerol from 1.0 to 1.5% and increasing the amount of Bacto agar from 1.5 to 1.7%. The second medium was a derivation of KB agar medium called KBC, described by Mohan and Schaad (1987). Modifications included those described for KB agar medium, plus a decrease in the amount of boric acid from 1.5 to 0.15 g/ml, and addition of 67 mg of nystatin/liter in place of cycloheximide. This is referred to as mKBC agar medium. The *Psa* infection level of each seed lot was determined by soaking samples of each seed lot, followed by plating a serial dilution of the seed

rinsate onto mKBC agar medium, and transferring suspect isolates onto KB agar medium, as described below (Bull and Koike 2017; Sanders 2012; Walcott and Gitaitis 2017). Suspect *Psa* colonies from each seed wash were then tested for pathogenicity on beet seedlings to confirm which isolates were pathogenic to beet, i.e., the BLS pathogen, as described below. Pathogenicity tests of representative colonies from each seed wash were necessary because beet and chard seed lots are colonized readily by non-pathogenic strains of *P. syringae* that cannot be differentiated from *Psa* morphologically, even on semi-selective agar media (Bull and Koike 2017).

Seed wash. A 10,000-seed sample of each Swiss chard seed lot to be tested was weighed based on a predetermined, 1,000-seed weight sample. The sample was placed in a sterilized Erlenmeyer flask. If the total weight for 10,000 seeds was >140 g, the sample was split into two subsamples of 5,000 seeds. Sterile 0.85% saline was added to each flask until all seeds were barely fully immersed. If seed began to float when saline was added, the flask was agitated gently to ensure all seeds were wet. Seeds were incubated in the saline at room temperature (24 ± 1°C) for 4 h, adding extra saline as needed to keep all the seeds submerged. The total volume of saline added was recorded to calculate CFU/g seed. Each flask of seed was then placed on an oscillating shaker for 10 minutes at 150 rpm. The seed rinsate was removed from the flask and placed in a sterile flask. A 1 ml aliquot of the rinsate was then added to 9 ml of 0.85% sterile saline (10^{-1} dilution) in a test tube, and three more 10-fold dilutions were prepared. A 100 µl aliquot of each dilution was plated onto mKBC agar medium in each of three replicate, 100-mm diameter Petri plates. Each aliquot was spread over the surface of the agar medium with a sterile glass rod.

For the positive control sample, a slightly turbid suspension [~0.3 optical density (OD) at 600 nm = ~10^6 CFU/ml] of a control strain of *Psa*, isolate BS324 (obtained from Carolee Bull, Pennsylvania State University), was diluted in sterile, deionized water to 10^3 CFU/ml (three 10-fold dilutions). A 10 µl aliquot of this suspension was added to a 1 ml sample of sterile 0.85% saline, incubated at room temperature

for 30 minutes, and plated onto mKBC agar medium to assess the quality of the medium and saline used to soak the seed. In addition, spiked seed wash dilutions were prepared to detect if there were inhibitors of *Psa* in each seed wash, including saprophytic fungi and bacteria. A 1.0 ml aliquot of rinsate from each seed wash dilution was dispensed into a sterile test tube. For seed samples split into two subsamples, a 1.0 ml aliquot of rinsate from each sub-sample was pooled for each dilution. A 10 µl aliquot of the 10^3 CFU/ml suspension of the positive control isolate was added to the 1 ml subsample of each seed wash dilution to create the spiked samples. The spiked dilutions were incubated for at least 30 minutes at room temperature, and then 100 µl of each spiked dilution was spread onto a plate of mKBC agar medium, as described above. The number of *Psa* colonies that grew from the spiked saline on mKBC agar medium was compared to the spiked seed wash dilutions to determine if competition or antagonism may have affected the detection and quantification of *Psa*. The plates were incubated in the dark at 27°C and examined after 3, 4, and 5 days. Suspect colonies of *Psa* were counted, and a sample of ~20 representative colonies per seed wash transferred to KB agar medium. Isolates on KB agar medium were examined after 24 to 48 h, subcultured for purification, and colony morphology compared to that of the BS324 control strain. At least 10 suspect *Psa* isolates per seed wash were tested for pathogenicity on beet seedlings (if less than 10 suspect isolates were detected, then all suspect isolates were tested).

Pathogenicity tests. Seeds of the beet cv. Red Ace were planted in potting medium (Sunshine Mix #5, SunGro, Agawam, MA) in flats (28 x 55 x 6 cm tall) and placed in a growth chamber set at 23 ± 1°C with a 9 h/15 h day/night cycle for 14 ± 2 days, until the first set of true leaves was approximately 1 cm long. Plants were fertilized three times per week using a 15-5-15 liquid fertilizer (Ultrasol, SQM North America Corp., Atlanta, GA) at a rate of 394 ppm N, 131 ppm P, and 394 ppm K. Seeds were planted in 5 to 7 rows per flat, with 20 seeds/28 cm row, and plants thinned 1 to 2 days prior to inoculation to 12 to 15 seedlings/row to ensure uniform seedling size and spacing. The suspect *Psa* strains and the BS324 control strain were each streaked onto KB agar medium 2 days prior to inoculation, and incubated in the

dark as described above. A suspension of each of the putative *Psa* strains and the positive control strain was prepared the day seedlings were inoculated, using a sterile, mini-tip, long-handled cotton swab (Puritan model # 826-WC) to transfer bacterial colonies from each strain into sterile, deionized water in a test tube. The bacterial concentration for each strain was adjusted to an OD_{600} of 0.5 (~10^8 CFU/ml). For the negative control treatment, seedlings were inoculated by swabbing sterile water gently onto the top and lower surfaces of the first set of true leaves of 5 to 10 beet seedlings per replication (12 to 16 days after emergence), using sterile, large-tip, long-handled, cotton swabs (Puritan model # 806-WCL). The same protocol was used for each of the suspect *Psa* isolates and the positive control strains, with 6 plants inoculated per isolate. Inoculated plants were maintained for 7 days in a growth chamber at the temperature and lighting noted above. Plants were watered from below to avoid splash dispersal of bacteria. After 7 days, the seedlings were rated for severity of BLS symptoms using a 1 to 5 scale, as described in Table 1. Only inoculated leaves were evaluated, with the leaf that had the most severe symptoms rated on each plant. The average rating for all inoculated plants per strain was used as the final rating for determination of pathogenicity and virulence of each isolate.

Isolates with an average score >3.3 were considered pathogenic on beet, isolates with a mean severity score of 3.8 to 5.0 were considered highly virulent, and isolates with a score between 3.3 and 3.8 considered moderately virulent. The control strain BS324 consistently scored an average of 4 to 5 (highly virulent) in pathogenicity tests on the cv. Red Ace. To confirm the cause of symptoms, symptomatic leaf samples were macerated in sterile water and streaked onto mKBC agar medium. The plates were incubated as described above, and suspect colonies transferred to KB agar medium. The morphology of these re-isolated strains was compared to that of the original isolates and the control strain BS324 to confirm the identity of the pathogen.

Field trial design and BLS rating. In each field trial, each plot was 0.6 m wide (9 rows at a 7.5-cm row spacing) x 4.6 m long and planted at a baby leaf density of 8.65 million seeds/ha. A Hedge 1000

cone seeder was used to plant each trial with a custom-made attachment to allow for baby leaf row spacing. Each plot was surrounded on all four sides by a 3.1 m border of bare soil. In all trials, the plants were sprayed approximately 1 to 2 weeks after emergence with the insecticide AzaDirect (1.2% azadirachtin, Gowan Co., Yuma, AZ) for leafminer (*Liriomyza sativae*) control, as severe leafminer symptoms make it difficult to evaluate BLS symptoms. Plants in each plot were evaluated for symptoms of BLS every 2 to 3 days after the plants emerged. Once BLS symptoms were identified, BLS severity was rated every 4 to 6 days until 30 to 40 days after planting, when plants reached the size at which baby leaf crops are harvested. Overhead rotating sprinklers were used to irrigate the trial during the first two weeks of growth, as needed to ensure enough moisture for germination. The amount of rainfall was recorded for the duration of each trial (Fig. 2). Irrigation was not needed over the full duration in which plots were rated for BLS severity. BLS was evaluated in terms of percentage of the canopy in each plot with symptoms. After the final rating, a sample of 2 to 4 symptomatic leaves was collected from each plot in which symptoms were observed. Symptomatic leaves were not sampled earlier to avoid disrupting spread of the pathogen in each plot. Each lesion was macerated in sterilized water, and the macerate plated onto KB and mKBC agar media, as described above. Isolates were compared to known pathogenic isolates of *Psa*, and tested for pathogenicity on beet seedlings to confirm the identity of the bacteria causing symptoms in the field trial to be *Psa*.

2020 trials. Two trials were planted in 2020, one in the spring (planted 1 May) and one in the fall (planted 31 August). Each trial was 25.0 by 33.5 m, including the buffer around each plot. For the spring trial, stand counts were completed on 27 May, concurrent with the second BLS severity rating, to determine if there were differences in stand count among treatments or replications, by counting the plants in each of two, random, 46-cm long sections of different rows in each plot. The two counts were averaged to calculate the average stand count per plot. If either of the two stand counts in a plot showed poor stand (<15 plants), a third stand count was taken, and all three counts averaged. For Trial 1

in spring 2020, the first BLS severity rating was conducted 19 days after planting (dap), the second rating 26 dap, and the third and final rating 33 dap. For Trial 2 in fall 2020, the first, second, and final BLS ratings were done 22, 26, and 29 dap, respectively.

2021 trials. Two spring 2021 trials (Trial 3 and Trial 4) were each planted in the same field on 5 May. Trial 3 was located at the north end of the field, and Trial 4 on the south end. Stand counts were recorded three times for Trial 3, and twice for Trial 4, congruent with the first, second, and third BLS severity ratings in Trial 3, and the second and third ratings in Trial 4. Stand counts were conducted by counting plants in two replications of 30.5 x 61 cm sections of each plot, and averaging the two counts, as an estimate for the entire plot. The protocol for collecting stand count data was expanded in the 2021 trials because there were obvious differences in stand counts among plots. The first BLS severity rating for Trials 3 and 4 took place 20 dap (25 May), and the subsequent ratings occurred 28, 34, and 40 dap. In addition, the size of the plants relative to the size at which baby leaf crops are typically harvested was recorded concurrently with the ratings 34 and 40 dap to determine if there was a significant correlation of plant size with BLS severity and/or *Psa* seed infection level.

Threshold trials data analysis. Disease progression was measured using the BLS severity ratings for each plot to calculate the area under the disease progress curve (AUDPC) (Madden et al. 2007). The data for each threshold trial were analyzed statistically using a 2-way analysis of variance (ANOVA) to determine if the seedborne *Psa* infection level had significant effects on stand count, plant size, BLS severity ratings over the duration of the trial, or AUDPC values (Table 2). Proc GLM in SAS Studio (SAS Insitute, Inc., Cary, NC) was used to conduct the analyses. Replication and seed infection level were treated as random effects. Log_{10} and reciprocal square root transformations were used to meet assumptions for parametric analysis, if needed. If data for a dependent variable did not meet the assumptions for parametric analysis, the ANOVA was conducted using ranked data. Proc CORR in SAS Studio was also used to assess if any of the variables were correlated significantly. The threshold for

seedborne inoculum in each trial was identified as the *Psa* seed infection level planted at which BLS severity ratings were significantly different compared to plots planted with *Psa* seed infection levels that did not result in BLS symptoms or that resulted in <5% severity of BLS, which is used often as a threshold for marketability of baby leaf crops.

Persistence of *Psa* on beet and Swiss chard seed. Six seed lots, each with a different level of natural *Psa* infection, were used to assess the duration of survival of *Psa* in beet seed (three lots) and Swiss chard seed (three lots). Three replications of 10,000 seeds of each lot were tested every 3 months, starting 6 to 12 months after harvest, using the seed wash plating assay and pathogenicity test described above, to quantify viable *Psa* infestation levels over time. Three of the lots (B1, B3, and S2) were produced in western Washington and harvested in September 2020, and the other three lots (B2, S1, and S3) were produced in New Zealand and harvested in March 2020, accounting for the differences in harvest dates relative to when seed testing for this study was initiated. Estimated harvest dates for each lot and the initial seed health assay results for a seed wash of one replication of 10,000 seeds are provided in Table 2.3. The six seed lots were tested over a duration of 21 months, except for lot S2, for which testing ended after 15 months because of insufficient seed for further testing. If a seed lot tested negative for *Psa* in all three replicate samples for four sequential 3-month periods, the lot was considered negative for viable *Psa*, and testing was discontinued for that lot.

***Psa* persistence on seed data analysis.** Results for the *Psa* seedborne persistence study were analyzed using repeated measures analysis in PROC MIXED of SAS to determine if there were significant main effects of duration of storage (time) or host (beet vs. chard) on the amount of *Psa* detected on the seed lots, and a significant interaction of time and host. Seed lot was treated as a random effect, while time and host were fixed effects in the model. Linear regression analysis was completed using PROC MIXED to calculate and compare the linear slope of the change in CFU *Psa*/g of seed/month of storage for each lot.

2.3 Results

Trial 1, spring 2020. The weather during the spring 2020 trial was mild with temperatures averaging 16.8°C during the day and 8.8°C at night (Fig. 2A). Rain occurred at least once a week, with a period of more rainy weather (>25 mm) 30 dap, 3 days before the final BLS severity rating. Conditions were ideal for spread of the pathogen and BLS symptom development (rainy and windy, with cool temperatures). BLS symptoms were first observed 19 dap, with BLS severity averaging 0.5 ± 0.29% (mean ± standard error, SE) of the canopy across all plots. The most severe initial BLS rating 19 dap was 5%, observed in one plot planted with seed that had the highest *Psa* infection level in that trial (4.7×10^5 CFU/g seed). In the following two weeks, BLS symptoms increased rapidly in plots planted with the two most highly infected seed lots (9.75×10^4 and 4.7×10^5 CFU/g seed), resulting in final mean BLS severity ratings 33 dap of 13.3 ± 7.6 and 13.8 ± 2.4%, respectively, and a range from 3 to 35% of the canopy symptomatic for these eight plots (Fig. 2A). In contrast, the severity of symptoms in plots planted with seed infected at the four lower levels of *Psa* remained ≤1% throughout the trial (Fig. 2A).

There were significant differences in mean AUDPC values for plots planted with seed infected with *Psa* at different levels (ANOVA $P = 0.0014$, Table 2.2). Plots planted with seed that had the two highest infection levels (9.7×10^4 and 4.7×10^5 CFU/g seed) had significantly greater AUDPC values (69.0 ± 25.8 and 86.4 ± 14.4, respectively) than plots planted with the four seed lots with the lowest infection levels, and there was no significant difference in mean AUDPC values among plots with these four lower seed infection levels (range of 14.4 to 21.8 mean AUDPC, Table 2.1). AUDPC values were significantly positively correlated with *Psa* infection levels on the seed planted ($r = 0.6468$, $P = 0.0006$) and with all three BLS severity ratings ($r = 0.3756$, $P = 0.0705$; $r = 0.8909$, $P < 0.0001$; and $r = 0.9081$, $P < 0.0001$, for ratings 19, 26, and 33 dap, respectively) (Table 2.2). The threshold for seedborne *Psa* in this trial, at which the leaves at harvest had <5% BLS symptoms, was 9.8×10^3 CFU *Psa*/g seed, i.e., only plants that grew from seed infected above this threshold were non-marketable at harvest.

Suspect *Psa* isolates were obtained from symptomatic leaf samples collected in 8 of the 24 plots at the final rating, 33 dap, all of which were planted with seed that had the two highest *Psa* infection levels. All 10 of the isolated strains proved pathogenic on beet seedlings and were highly virulent (*data not shown*). Leaf samples from plots planted with the 0 CFU/g seed lot and the 9.7 x 10^3 CFU/g seed lot did not result in isolation of suspect *Psa* isolates, suggesting that the few symptoms observed in these plots may not have been caused by *Psa*, but by other factors such as mechanical injury from wind. Stand counts were not correlated significantly with BLS severity ratings or AUDPC values, and there was no significant difference in stand counts among plots planted with seed lots that had different *Psa* infection levels or among the replications (Table 2.2).

Trial 2, fall 2020. During the 2020 fall-planted trial, conditions were warm and dry for the first two weeks after planting, with mean air temperatures significantly warmer than in spring Trial 1. Day and night temperatures averaged 21.6°C and 10.2°C, respectively (Fig. 2B). Symptoms were observed 22 dap in plots planted with seed at all six *Psa* infection levels, including the 0 CFU/g seed lot, and BLS severity averaged 1.6 ± 0.11% across all plots. A storm occurred 24 dap, with significant rainfall (15 mm) and winds for several days, which spread *Psa* throughout the trial (Fig. 2B). Between the second and third ratings, there was a significant increase in severity of BLS symptoms across all plots, from an average of 4.0 ± 0.23% at the second rating (26 dap) to 15.4 ± 2.1% at the third rating (29 dap) (Fig. 2B). Even the negative control plots planted with seed that had 0 CFU *Psa*/g seed had BLS symptoms (8.0 ± 1.8%) by the final rating, reflecting spread of the pathogen from adjacent plots via splash dispersal during the storm, despite the 3.1-m-wide buffer between adjacent plots. As a result, none of the plots was marketable for commercial harvest, as all had ≥5% BLS by 29 dap. The AUDPC values ($P < 0.0001$) and BLS ratings 22 and 29 dap ($P = 0.0151$ and <0.0001, respectively) differed significantly among plots planted with seed infected at different levels (Table 2.2). There was no significant difference in AUDPC values for plots planted with seeds lots at the lowest four infection rates (range of 40.4 to 52.0)

compared to plots with the two most infected seed lots, and no difference in AUDPC values between the latter two treatments (71.3 ± 7.9 and 84.9 ± 7.9 for lots with 9.8 x 10^4 and 4.7 x 10^5 CFU/g seed, respectively) (Table 2.1). AUDPC values were positively correlated with *Psa* seed infection levels (r = 0.6108, *P* = 0.0015) and with the final two BLS ratings (r = 0.7022 at *P* = 0.0001, and r = 0.9257 at *P* < 0.0001, 26 and 29 dap, respectively). The two final BLS ratings were also positively correlated with the *Psa* seed infection levels (r = 0.4976 at *P* = 0.0134 and r = 0.7023 at *P* = 0.0001). For this trial, in which highly conducive weather after the first two weeks resulted in high disease pressure, none of the seed lots resulted in an average BLS severity below the 5% threshold for marketability, implying a threshold of 0 CFU *Psa*/g seed under such favorable conditions for BLS.

Symptomatic leaves were collected from 23 plots, from which suspect *Psa* isolates were obtained from 16 plots. Of those 16, 15 samples produced pathogenic isolates based on inoculation of beet seedlings. All leaves sampled from the eight plots planted with seed that had the two highest seedborne Psa infection levels (9.7 x 10^4 and 4.7 x 10^5 CFU/g seed) produced highly virulent isolates, and the other seven pathogenic isolates were from plots planted with seed that had the four lower infection levels, including the negative lot.

Trials 3 and 4, spring 2021. During the two spring 2021 trials, air temperatures were similar to those in Trial 1 in spring 2020, with average day and night temperatures of 18.6°C and 7.7°C, respectively. Seedling emergence and stand count were inconsistent throughout the field, with slower emergence in plots along the eastern side of the trial (*data not shown*). In addition, plants did not emerge uniformly in some rows within plots, possibly from seed getting planted too deep on that side of the affected plots. Stand counts were taken throughout the trial to determine if *Psa* infection levels on the seed were correlated with emergence. Very few BLS symptoms were observed in plots of either trial during the first two ratings, 20 and 28 dap (<5% symptoms; Fig. 2C and 2D). However, by 34 dap, foci of

BLS were observed, that spread rapidly before the final rating 40 dap, particularly in Trial 4 (Fig. 2C and 2D).

In Trial 3, BLS symptoms were mild, with a final BLS rating of 2.8 ± 0.5% across all plots 40 dap. Even in plots planted with seed that had the highest *Psa* infection level (2.9 x 10^4 CFU/g seed), the mean BLS severity rating 40 dap was only 6.3 ± 1.9%. Nonetheless, the ANOVA revealed a marginally significant effect of *Psa* seed infection levels on AUDPC values (*P* = 0.0835) and on BLS severity 40 dap (*P* = 0.0798) (Table 2.2). The mean AUDPC values for each seed infection level (from 0 to 10^4 CFU/g seed) were: 15.4 ± 2.3, 15.4 ± 2.2, 15.3 ± 1.8, 16.6 ± 2.7, 17.1 ± 3.5, and 28.5 ± 6.1, respectively (Table 2.1). The correlation between stand counts and *Psa* seed infection level was not significant, but the first stand count (20 dap) was positively correlated with the final BLS severity rating (r = 0.4241, *P* = 0.0389) and with AUDPC values (r = 0.4323, *P* = 0.0349). This suggests that final BLS severity was influenced by initial stands, i.e., greater initial stands resulted in a more dense canopy with more severe disease. Similarly, *Psa* seed infection level was positively correlated with AUDPC values (r = 0.4133, *P* = 0.0447) and with the final BLS rating (r = 0.4629, *P* = 0.0227). The threshold for seedborne *Psa* in this trial that resulted in <5% BLS severity was 1.2 x 10^4 CFU *Psa*/g seed, i.e., all plots planted with seed at or below this infection level had marketable leaves 40 dap.

In Trial 4, significant BLS development occurred in plots planted with the two most highly infected seed lots (6.4 x 10^5 and 1.5 x 10^6 CFU/g seed) by the end of the trial (40 dap), with one plot planted with the 1.5 x 10^6 CFU/g seed lot having 50% BLS severity (Fig. 2D). The mean BLS severity at the final rating ranged from 2 ± 1% for plots planted with non-infected seed (0 CFU/g seed), to 31 ± 8% for plots planted with seed at the highest infection level (1.5 x 10^6 CFU/g seed). The ANOVA showed a significant effect of the *Psa* seed infection levels on BLS ratings 28, 34, and 40 dap (*P* = 0.0591, 0.1067, and 0.0001, respectively, Table 2.2), and a significant effect of *Psa* seed infection level on AUDPC values (*P* = 0.0001; Table 2.2). Plots planted with seed infected at the two highest levels (6.4 x 10^5 and 1.5 x 10^6

CFU/g seed) had significantly greater AUDPC values (79.5 ± 31.5, and 122.8 ± 35.6, respectively) than plots planted with seed infected at the four lower levels (range of 12.5 to 15.4, Table 1). There was a positive correlation between stand counts 28 and 34 dap and the first BLS rating 20 dap (r = 0.3647 at *P* = 0.0797 and r = 0.4516 at *P* = 0.0267, respectively), suggesting that initial stand in the plots affected BLS severity over the duration of the trial, i.e., more dense stands resulted in more severe BLS. There was also a significant correlation of the *Psa* seed infection levels with AUPDC values (r = 0.5482 at *P* = 0.0055) and BLS severity ratings 28, 34, and 40 dap (r = 0.4652, *P* = 0.0220; r = 0.3871, *P* = 0.0617; r = 0.5640, *P* = 0.0041, respectively). In addition, AUDPC values were strongly positively correlated with BLS severity ratings 28, 34, and 40 dap (r = 0.8763, 0.8595, and 0.9905 at *P* <0.001 for all three correlations, respectively). The stand counts and plant size ratings did not differ significantly among plots planted with seed that had different *Psa* seed infection levels (Table 2.2). The threshold for seedborne *Psa* in Trial 4 that resulted in <5% severity of BLS was 6.4 x 10^4 CFU Psa/g seed, i.e., only plots planted with seed above this threshold had non-marketable crops.

***Psa* persistence on seed.** The initial *Psa* seed infection levels of the six beet and chard seed lots, before initiating testing every three months, ranged from $\log_{10}$4.10 to $\log_{10}$6.11 CFU *Psa*/g seed (Fig. 2.4). The 0-month test results for September 2021 ranged from 0 to $\log_{10}$5.39 CFU/g seed for individual subsamples of the six lots. Time (duration of storage) and *Psa* seed infection levels were negatively correlated (r = -0.5105, *P* = <0.0001), i.e., *Psa* levels detected on the six seed lots decreased over the 21-month duration of testing compared to the initial test results used to identify infected lots for this study. Based on repeated measures analysis, the amount of *Psa* detected on the six seed lots was affected significantly by time (duration of storage, *P* <0.0001), marginally significantly by host (beet vs. chard, *P* = 0.0961), and significantly by the interaction of time and host (*P* = 0.0017). The effects of host and host-by-time largely reflected the fact that two beet seed lots, B1 and B3, had far less *Psa* at the 0-month test (<10^2 CFU/g seed) compared to the other four lots (10^4 to 10^5 CFU/g seed). Regression analyses revealed

the amount of *Psa* detected (log_{10}CFU/g seed) declined by 0.07 to 0.19 per month in storage across the six seed lots (Fig. 2.4). The slowest rate of decline was for the two beet seed lots that had the least amount of infection at the start of the study.

Lot S2, for which there was inadequate seed to continue testing beyond 15 months, had a rate of decline in seedborne *Psa* detected that was similar over the 15-months of testing to the rate of decline for lots B2, S1, and S3 (Fig. 2.4). The average *Psa* infection level after 21 months was log_{10}0.82 CFU/g seed for the remaining five seed lots. Lot B1, for which 12 months had elapsed from harvest to the initial, 0-month testing, had *Psa* infection levels ranging from 0 to log_{10}1.56 CFU/g seed at the 0-month test. This lot tested negative for *Psa* in all three replicate subsamples for four consecutive 3-month periods (from 12 to 21 months), so this lot was considered 'negative' for *Psa* by the end of this study. Lot B3, which also had a low average infection level at the 0-month period (log_{10}0.92 CFU/g seed), tested negative after 6, 9, 12, 18, and 21 months, but Psa was detected in one replicate subsample of 10,000 seeds at the 15-month test (log_{10}2.67 CFU/g seed) (Fig. 2.4). Although seed lot S3 had log_{10}4.91 CFU/g seed at the 0-month test, infection decreased to log_{10}0.94 CFU/g seed after 21 months, i.e., <10 CFU/g seed.

2.4 Discussion

This study aimed to establish seedborne thresholds for *Psa* infection in baby leaf crops of *B. vulgaris* at which severity of BLS is below the marketable threshold of 5%. *Psa* infection levels on the beet and Swiss chard seed lots planted ranged from 0 to 4.7 x 10^5 CFU/g seed in the first two trials, from 0 to 2.9 x 10^4 CFU/g seed in the third trial, and from 0 to 1.5 x 10^6 CFU/g seed in the fourth trial. The seed contamination levels that resulted in BLS symptoms on <5% of the canopy were 9.8 x 10^3 CFU/g seed in Trial 1, 1.2 x 10^4 CFU/g in Trial 3, and 6.4 x 10^4 CFU/g in Trial 4. However, in Trial 2, all the plots developed >5% BLS by harvest, i.e., all were non-marketable, regardless of the level of *Psa* infection of

the planted seed. This reflected the fact that wet and windy conditions during that trial spread *Psa* among all the plots, including to plots planted with non-infected seed. These results demonstrate the significant effect of environmental conditions on the risk of seed transmission of *Psa*, and the difficulty of developing a single threshold for seedborne *Psa* that is relevant across all cultivars, environmental conditions, and production practices.

When multiple seed lots are planted in the same field, or farmers use sequential plantings of seed, as is typical for baby leaf beet and Swiss chard production, BLS could spread readily under highly conducive conditions from crops planted with *Psa* infected lots to nearby crops planted with seed lots that tested negative. The seedborne thresholds observed in Trials 1, 3, and 4 that resulted in <5% BLS severity were comparable to the threshold observed for bacterial leaf blight of carrot caused by *X. hortorum* pv. *carotae* (*Xhc*) (Umesh et al. 1998), i.e., 10^4 to 10^5 CFU/g seed were needed for bacterial leaf blight to become severe enough to impact harvested yield of the roots in field trials in the Central Valley of California, as more severely symptomatic carrot tops break off when the roots are pulled out of the ground by the tops at harvest. However, Umesh et al. (1998) noted this threshold may be lower in environments with more conducive conditions for the disease (e.g., the Midwestern or Eastern USA, with greater relative humidity and rainfall), or where agronomic practices may be more favorable for seed transmission and spread (e.g., when seeding at greater densities).

The results from Trial 2 of this *Psa* study suggest in highly conducive conditions the threshold for seedborne *Psa* may be zero. However, these results also reflected secondary spread of *Psa* from plots planted with highly infected seed to adjacent plots located ~3 m apart, i.e., a threshold of 0 CFU *Psa*/g seed may only be relevant in highly conducive conditions, since the severity of BLS was <5% in plots planted with seed infected at <1 x 10^4 *Psa* CFU/g seed in the other three field trials. Overall, the results of this study suggest that *B. vulgaris* seed lots should be tested to quantify *Psa* infection levels, and disinfected if infection levels exceed 10^4 CFU/g seed to avoid >5% BLS severity that generally renders

baby leaf crops unmarketable. It may be reasonable to use a conservative seedborne *Psa* threshold of 1 x 10^3 CFU/g seed, especially for crops grown in conditions less favorable for disease. This threshold may be adequate for beet root crops, which are planted at much lower densities than baby leaf crops, and for which a limited amount of foliar disease may be acceptable as long as the tops do not break off when the roots are pulled out of the ground during harvest (Pethybridge et al. 2018). Although seed lots infected with *Psa* above the thresholds demonstrated in this study could be diverted to plant root crops, breeding programs typically develop cultivars with traits required for specific markets, i.e., cultivars developed for baby leaf crops may not be suitable for bunching or root crop production.

The trials in this study in western Washington were planted at baby leaf densities, under weather conditions conducive for seed transmission and development of BLS. Seedborne thresholds may be higher in growing regions or conditions that are less favorable (e.g., during dry conditions in semi-arid regions such as Yuma, AZ). In addition, the risk of planting seed infected with *Psa* could be reduced by avoiding overhead irrigation, and growing crops in drier environments, such as indoors where wind and rain will not cause pathogen spread and environmental conditions can be controlled to a greater degree. Watering plants from below to avoid splash dispersal has the benefit of limiting how much the canopy is wet during production, which is also less conducive for other *B. vulgaris* diseases, such as Cercospora leaf spot (*C. beticola*), which is favored by high humidity and is splash- and wind-dispersed (Jacobsen and Franc 2009). Ultimately, growers have to decide if the added security of purchasing disinfected seed and/or seed tested negative for *Psa* is worth the additional cost of the disinfection and testing. Growers could request beet or Swiss chard seed lots be tested and shown to be infected at or below a threshold of ~10^3 CFU/g seed, since planting just one lot highly infected with *Psa* could result in spread of the pathogen to nearby crops, resulting in other crops becoming unmarketable. For seed producers, stock seed lots should be only planted if they test negative for *Psa,* or the lots

should be disinfected if they test positive, since it is far easier to prevent *Psa* infection in seed crops if the initial seed and transplants are pathogen-free.

Another consideration concerning seedborne thresholds is the uncertainty of what a positive test result means in terms of seed infection and disease expression. A seed lot that tests positive could have low levels of *Psa* infection across a majority of the seeds, or only a few seeds infected at very high levels (Agarwal and Sinclair 1997). The distribution of a pathogen in a seed lot can affect development of the disease in the field, e.g., one focal point of BLS vs. many foci. This could have contributed to some of the discrepancy in thresholds across the field trials in this study. *Psa* infection may not have been uniform throughout the seeds in each subsample, even with the steps taken to ensure the samples were collected representatively from the entire lot. This is also a risk when testing seed for the presence of pathogens, since no destructive seed health test can certify absence of the pathogen from the lot (Kuan 1988). Additionally, there can be variation in virulence among strains of *Psa* that cause BLS, which could result in some lots infected at low levels with highly virulent strains developing more severe BLS, while other seed lots highly infected with moderately or weakly virulent isolates may not result in development of BLS when planted, or may result in mild or moderate symptoms. Development of beet and chard varieties with resistance to BLS may complicate this issue further, since resistant varieties may not respond in the same manner to strains of *Psa* that differ in virulence.

Another potential method to reduce the amount of seedborne *Psa* on beet and chard seed crops is to store seed until the level of *Psa* has declined to non-detectable levels or to below established thresholds, as reflected in the seedborne *Psa* persistence study. This may not be appealing to seed production companies, however, because of the potential loss in sales from delayed selling of seed. However, it may be viable economically to store seed lots that are highly infected until the *Psa* levels have declined to below thresholds relevant to the environmental conditions and planting conditions into which they will be sold. Alternatively, seed lots that are highly infected could be prioritized for

disinfection, while lots that are below or close to the threshold could be sold or stored for limited durations, since the *Psa* level may only need to decline slightly to reach below the threshold. In this study, *Psa* infection levels on seed declined in all of six lots tested, at a range of rates from 0.07 to 0.19 (log_{10}CFU)/g seed/month. The time between when the seed was harvested and the final 21-month testing period ranged from 34 to 40 months. If a threshold is set at 1 x 10^3 CFU/g seed, then all six of the lots were below the threshold by the end of the study. The time between seed harvest and when the *Psa* infection level declined to <1.0 x 10^3 CFU *Psa*/g seed ranged from 12 to 25 months. This suggests that *Psa* infection levels of beet and chard seed lots decline in storage faster than the rate of decline in seed quality (germination and vigor). With a seedborne threshold of 1.0 x 10^3 CFU/g seed, seed lots infected between 10^3 to 10^4 CFU/g seed could be stored for a limited duration to reach below the threshold. However, if it is necessary for *Psa* levels on seed to decline to 0 CFU/g seed, storing seed may only be viable if initial *Psa* infection levels are <1 x 10^3 CFU/g seed. The two lots in this study that had initial infection levels of ~1 x 10^4 CFU/g seed, lots B1 and B2, declined to 0 CFU/g seed in 34 to 40 months after harvest.

Additional steps can be taken to minimize the economic impact of BLS on beet and chard crops, including development of effective protocols for disinfection of *Psa*-infected seed. Most seed companies and seed treatment facilities use proprietary disinfection protocols to treat *Psa*-infected seed lots. There are no publicly available seed treatment protocols for *Psa*, so smaller seed companies and growers may not have the means to test and/or disinfect seed lots, and using third-party seed disinfection services can be prohibitively expensive, potentially resulting in smaller-scale seed producers selling infected seed lots, charging higher prices to offset the cost of seed disinfection, or disposing of seed lots if they cannot justify the cost of seed disinfection.

2.5 REFERENCES

Agarwal, V. K., and Sinclair, J. B. 1997. Principles of Seed Pathology, 2nd Ed. Lewis Publishers, Boca Raton.

Brodal, G., and Asdal, A. 2021. Longevity of plant pathogens in dry agricultural seeds during 30 years of storage. Microorganisms 9(10):2175.

Brown, N. A., and Jamieson, C.O. 1913. A bacterium causing a disease of sugar beet and nasturtium leaves. J. Agric. Res. 1:189-210.

Bull, C. T., and Koike, S. T. 2017. Detection of *Pseudomonas* pathogens from crucifer seeds. Pp. 165-172, In: Detection of Plant-Pathogenic Bacteria in Seed and Other Planting Material, 2nd Ed. M. Fatmi, R. R. Walcott, and N. W. Schaad, eds. American Phytopathological Society, St Paul.

Derie, M. L., Holmes, B. J., Safni, I., Bull, C. T, and du Toit, L. J. 2016. Seedborne inoculum thresholds of *Pseudomonas syringae* pv. *aptata*, causal agent of bacterial leaf spot, in 'baby leaf' Swiss chard crops. Phytopathology 106:S4.142 (Abstract).

Duke, J. A. 1983. *Beta vulgaris* L. Handbook of Energy Crops. Center for New Crops and Plants Products, Purdue University, West Lafayette, IN. www.hort.purdue.edu/newcrop/duke_energy/Beta_vulgaris.html (accessed 10 Oct. 2023).

Dutta, B., Ingram, T., Gitaitis, R. D., Langston, D. B., Brenneman, T., Webster, T. M., and Davis, R. F. 2014. First report of bacterial blight of sugar beet caused by *Pseudomonas syringae* pv. *aptata* in Georgia, USA. Plant Dis. 98(10):1423.

du Toit, L. 2007. Crop profile for table beet seed in Washington. U.S. Department of Agriculture National Pest Management Regional IPM Center, Davis, CA. https://ipmdata.ipmcenters.org/documents/cropprofiles/WAbeetseed2007.pdf (accessed 10 Oct. 2023).

Ells, J. E., Bass, L. N., and Whiting, D. 2020. Storing vegetable and flower seeds. Colorado State University Extension Fact Sheet 7.221, Fort Collins, CO.

Gitaitis, R., and Walcott, R. 2007. The epidemiology and management of seedborne bacterial diseases. Annu. Rev. Phytopathol. 45:371-397.

Harveson, R. M., Hanson, Linda, E., and Hein, G. L. 2009. Compendium of Beet Diseases and Pests, 2nd Ed. American Phytopathological Society, St. Paul.

Ignjatov, M., Gvozdanović-Varga, J., Milošević, D., Nikolić, Z., Ivanović, Ž., and Popović, T. 2015. First report of bacterial leaf spot of chard (*Beta vulgaris* subsp*. cicla*) caused by *Pseudomonas syringae* pv. *syringae* in Serbia. Plant Dis. 99(5):723-723.

Jacobsen, J. 2009. Bacterial leaf spot. Pp. 59-60, In: Compendium of Beet Diseases and Pests, 2nd Ed. R. M. Harveson, L. E. Hanson, and G. L. Hein, eds. American Phytopathological Society, St. Paul.

Jacobsen, B. J., and Franc, G. D. 2009. Cercospora leaf spot. Pp. 7-10, In: Compendium of Beet Diseases and Pests, 2nd Ed. R. M. Harveson, L. E. Hanson, and G. L. Hein, eds. American Phytopathological Society, St. Paul.

King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44(2):301-307.

Koike, S. T., Azad, H. R., Cooksey, D. C. 2002. First report of bacterial leaf spot of spinach caused by a *Pseudomonas syringae* pathovar in California. Plant Dis. 86(8):921.

Koike, S. T., Henderson, D. M, Bull, C. T., Goldman, P. H., Lewellen, R. T. 2003. First report of bacterial leaf spot of Swiss chard caused by *Pseudomonas syringae* pv. *aptata* in California. Plant Dis. 87(11):1397.

Kuan, T. L. 1988. Inoculum thresholds of seedborne pathogens. Overview. Phytopathology 78(6):867-868.

Madden, L. V., Hughes, G., and van den Bosch, F. 2007. The Study of Plant Disease Epidemics. The American Phytopathological Society, St. Paul.

Mohan, S. K., and Schaad, N. W. 1987. An improved agar plating assay for detecting *Pseudomonas syringae* pv. *syringae* and *P. s.* pv. *phaseolicola* in contaminated bean seed. Phytopathology 77(10):1390-1395.

Nampijja, M., Crane, S., du Toit, L., and Ocamb, C. 2023. Beet, Red (*Beta vulgaris*)-Bacterial Leaf Spot. Pacific Northwest Plant Disease Management Handbook. Oregon State University. https://pnwhandbooks.org/plantdisease/host-disease/beet-red-beta-vulgaris-bacterial-leaf-spot (accessed 10 Oct. 2023).

Nampijja, M., Derie, M. L., and du Toit, L. J. 2021. First report of bacterial leaf spot caused by *Pseudomonas syringae* pv. *aptata* on Swiss chard, *Beta vulgaris* subsp. *vulgaris*, in Arizona. Plant Dis. 105:3738.

Navazio, J., Colley, M., and Zyskowski, J. 2010. Principles and Practices of Organic Beet Seed Production in the Pacific Northwest. Organic Seed Alliance, Port Townsend, WA.

Neergaard, P. 1977. Seed Pathology. Wiley, New York.

Nottingham, S. 2004. Beetroot. Archived from the original (e-book) on 2 Sep. 2012.

Rotondo, F., Vrisman, C. M., Rani, R., Testen, A. L., Deblais, L., and Miller, S. A. 2020. First report of *Pseudomonas syringae* pv. *aptata* causing bacterial leaf spot on common beet (*Beta vulgaris*) in Ohio. Plant Dis. 104(2):561.

Safni, I., Ramos-Sepulveda, L., Goldman, P., Derie, M., du Toit, L., Koike, S., Stockwell, V., and Bull, C. 2016. Genetic diversity of *Pseudomonas syringae* causing bacterial leaf spot on table beet (*Beta vulgaris*) and Swiss chard (*Beta vulgaris* subsp. *cicla*). Phytopathology 106(4):143 (Abstract).

Sanders, E. R. 2012. Aseptic laboratory techniques: plating methods. J. Vis. Exp. 63:e3064.

Schaad, N. W. 1980. Relationship of incidence of seedborne *Xanthomonas campestris* to black rot of crucifers [cabbage, broccoli]. Plant Dis. 64(1):91.

Schaad, N. W. 1988. Inoculum thresholds of seedborne pathogens. Bacteria. Phytopathology 78(6):872-875.

Schrader, W. L., and Mayberry, S. K. 2003. Beet and Swiss chard production in California. University of California Division of Agriculture and Natural Resources Publication No. 8096.

Traverse, J. H. 2018. Beet It. Circ. Res. 123(6):635-637.

Umesh, K., Davis, R., and Gilbertson, R. 1998. Seed contamination thresholds for development of carrot bacterial blight caused by *Xanthomonas campestris* pv. *carotae*. Plant Dis. 82(11):1271-1275.

United States Department of Agriculture National Agricultural Statistics Service. 2022. Quick Stats. https://quickstats.nass.usda.gov/ (Accessed 10 Jan. 2022).

Waker, J. C., and Patel, P. N. 1964. Splash dispersal and wind as factors in epidemiology of halo blight of bean. Phytopathology 54:140-141.

Walcott, R. R., and Gitaitis, R. D. 2017. Review of seed sample size determination and methods for extracting phytopathogenic bacteria from seeds. Pp. 13-18, In: Detection of Plant-Pathogenic Bacteria in Seed and Other Planting Material, 2nd Ed. M. Fatmi, R. R. Walcott, and N. W. Schaad, Eds. American Phytopathological Society, St Paul.

Table 2.1. *Pseudomonas syringae* pv. *aptata* (*Psa*) infection levels (CFU/g seed) of Swiss chard seed lots in each of four field trials used to determine thresholds of seedborne inoculum that result in development of bacterial leaf spot, with Tukey's grouping of the area under the disease progress curve (AUDPC) values for each *Psa* seed infection level.

	Trial (year)							
	1 (2020)		2 (2020)		3 (2021)		4 (2021)	
Seed lot[a]	CFU/g seed	AUDPC	CFU/g seed	AUDPC	CFU/g seed	AUDPC	CFU/g seed	AUDPC
1	0	14.4 a	0	51.4 a	0	15.4 a	0	14.5 a
2	195	19.1 ab	195	40.4 ab	12	15.4 a	639	12.5 a
3	975	20.6 ab	975	49.5 ab	122	15.3 a	6,390	15.4 a
4	9,751	21.8 ab	9,751	52.0 ab	1,216	16.6 a	63,900	15.3 a
5	97,510	69.0 bc	97,510	71.3 bc	12,157	17.1 a	639,000	79.5 b
6	470,000	86.4 c	470,000	84.9 c	29,300	28.5 a	1,540,000	122.8 b

[a] In each trial, a seed lot of a proprietary Swiss chard cultivar infected with *Psa* was mixed in different ratios with a non-infected seed lot of the same cultivar to generate seed samples with the levels of infection (CFU/g seed) shown.

Table 2.2. Analyses of variance (ANOVA) for stand counts, bacterial leaf spot (BLS) severity ratings, plant size, and area under the disease progress curve (AUDPC) of BLS ratings in seedborne threshold field trials planted with Swiss chard seed lots infected with *Pseudomonas syringae* pv. *aptata* (*Psa*)

					Mean square probability		
Trial and dependent variable[a]	**Normality test[b]**	**Test of variances[c]**	**R^{2}[d]**	**CV[e]**	**Repli-cation**	**Log_{10}*Psa*[f]**	**Trans-formation[g]**
Trial 1							
BLS rating 19 dap[h]	0.9929	0.3860	0.27	62.26	0.8829	0.4659	Log_{10}
BLS rating 26 dap	0.4123	0.0667	0.47	76.06	0.6424	0.0918	
BLS rating 33 dap	0.9674	0.6180	0.49	54.24	0.6609	**0.0750**[k]	Log_{10}
AUDPC[i]	0.5076	0.0688	0.73	18.31	0.1716	**0.0014**	Log_{10}
Stand count	0.6621	0.0703	0.55	17.80	0.0697	0.1561	
Trial 2							
BLS rating 22 dap			0.58	36.98	1.0000	**0.0151**	Rank
BLS rating 26 dap	0.6139	0.2140	0.43	26.12	0.8747	0.1243	
BLS rating 29 dap	0.8226	0.0948	0.91	25.35	**0.0034**	**<.0001**	
AUDPC[i]	0.1919	0.3094	0.82	16.60	**0.0359**	**<.0001**	
Trial 3							
BLS rating 20 dap	0.2272	0.1076	0.24	48.27	0.6222	0.7288	
BLS rating 28 dap			0.25	17.50	1.0000	0.4509	Rank
BLS rating 34 dap	0.3140	0.0484	0.44	32.66	**0.0638**	0.7008	
BLS rating 40 dap	0.7503	0.3835	0.51	77.13	0.4177	**0.0798**	
AUDPC[i]	0.7049	0.3414	0.52	36.83	0.2906	**0.0835**	
Stand count 20 dap			0.08	59.07	1.0000	0.9325	Rank
Stand count 28 dap	0.1879	0.0972	0.11	16.75	0.8021	0.9691	
Stand count 34 dap	0.4540	0.0405	0.28	18.98	0.2622	0.9073	
Plant size 34 dap[j]	0.7375	0.8451	0.24	33.99	0.8796	0.5585	
Plant size 40 dap	0.7252	0.2214	0.23	27.85	0.7301	0.6867	
Trial 4							
BLS rating 20 dap			0.20	25.56	1.0000	0.5988	Rank
BLS rating 28 dap			0.48	33.50	1.0000	**0.0591**	Rank
BLS rating 34 dap	0.1743	0.0722	0.46	25.45	0.6953	0.1067	Reciprocal square root
BLS rating 40 dap	0.1644	0.0638	0.80	41.27	0.3982	**0.0001**	Log_{10}
AUDPC[i]	0.0437	0.1543	0.80	21.51	0.7050	**0.0001**	Reciprocal square root
Stand count 28 dap	0.9458	0.2235	0.13	27.44	0.8006	0.9263	
Stand count 34 dap	0.6151	0.7231	0.15	27.03	0.8747	0.8388	
Plant size 34 dap	0.0876	0.3537	0.06	33.35	0.9175	0.9941	
Plant size 40 dap	0.3734	0.3426	0.18	31.72	0.6962	0.8544	

[a] Each trial consisted of a randomized complete block design with four replications of plots planted with Swiss chard seed samples infected with a range in levels of *Psa* (see Table 1).

[b] Shapiro-Wilk test for normality of residuals, *P*-value.
[c] Levene's test for homogeneity of variances, *P*-value.
[d] R^2 = coefficient of determination.
[e] CV = coefficient of variation.
[f] Log_{10}Psa = Log_{10}CFU/g seed *Psa* infection levels in Swiss chard seed samples planted.
[g] Transformation to fulfill the assumptions for ANOVA.
[h] Rating for severity of BLS symptoms at various days after planting (dap).
[i] AUDPCN calculated for three BLS ratings in each of Trials 1 and 2, and four ratings in each of Trials 3 and 4.
[j] Size of plant measured as average seedling height in each plot (score of 2 = average 5 cm height).
[k] Bolded values represent statistically significant or marginally significant data.

Table 2.3. Seed lots used in a persistence study to determine the rate of decline of seedborne *Pseudomonas syringae* pv. *aptata* (*Psa*) on *Beta vulgaris* seed in storage. Includes the estimated harvest date, initial date of seed test used to establish *Psa* infection levels, and the results of the initial seed test showing the *Psa* infection level of the seed.

Lot	Crop	Estimated harvest date	Initial test date	CFU *Psa*/g seed
B1	Beet	September 2020	19 March 2021	14,800
B2	Beet	March 2020	5 June 2020	8,710,000
B3	Beet	September 2020	30 April 2021	12,700
S1	Swiss chard	March 2020	11 June 2020	123,000
S2	Swiss chard	September 2020	12 February 2021	270,000
S3	Swiss Chard	March 2020	17 April 2020	1,300,000

Rating	Symptoms
1	No symptoms.
2	Small lesion or spot on <5% of leaf surface.
3	Multiple spots or small lesions covering 6 to 20% of leaf surface.
4	Many spots or large lesions covering 21 to 50% of leaf surface. Leaf may have large notches in the margins from distortion as the leaf expanded after infection.
5	>50% of leaf covered by lesions or spots, leaf shriveled/mostly dead.

Fig 2.1. Rating scale for bacterial leaf spot symptoms on beet seedling leaves inoculated to test pathogenicity of isolates of *Pseudomonas syringae* pv. *aptata* obtained from beet or Swiss chard seed or leaves.

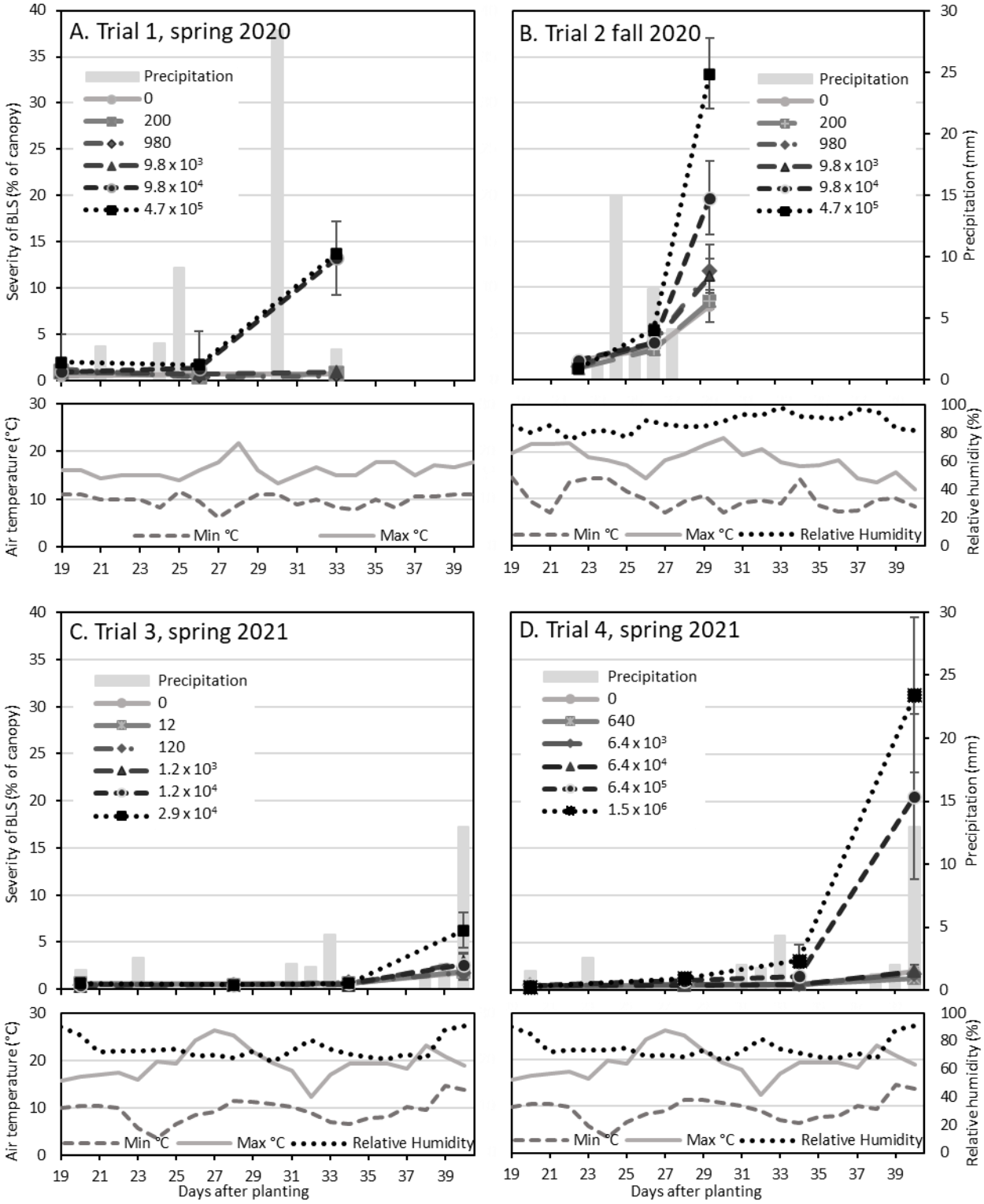

Fig 2.2. Development of bacterial leaf spot (BLS) in each of four baby leaf Swiss chard field trials to determine thresholds for seedborne inoculum of *Pseudomonas syringae* pv. *aptata* (*Psa*). In each trial, two Swiss chard seed lots were used, one that tested positive and one that tested negative for *Psa*. The two lots were mixed in different ratios to obtain six sub-lots with the levels of *Psa* infection shown,

which were planted at a baby leaf density (8.65 million seeds/ha) using a randomized complete block design with four replications. Severity of BLS symptoms was recorded three to four times in each trial as a percentage of the total canopy affected per plot. Precipitation, minimum and maximum daily air temperature, and relative humidity were recorded for the duration of each trial, except for relative humidity in Trial 1.

Fig. 2.3. Trials conducted in 2020 and 2021 to determine the seedborne thresholds of *Pseudomonas syringae* pv. *aptata* (*Psa*) on beet and Swiss chard that will cause bacterial leaf spot (BLS) symptoms when grown as baby leaf crops. **A-C)** Plots from the trial conducted in May 2020, including a close-up photo of BLS symptoms. **D-F)** Plots from the trial conducted in May 2021, including a close-up photo of BLS symptoms.

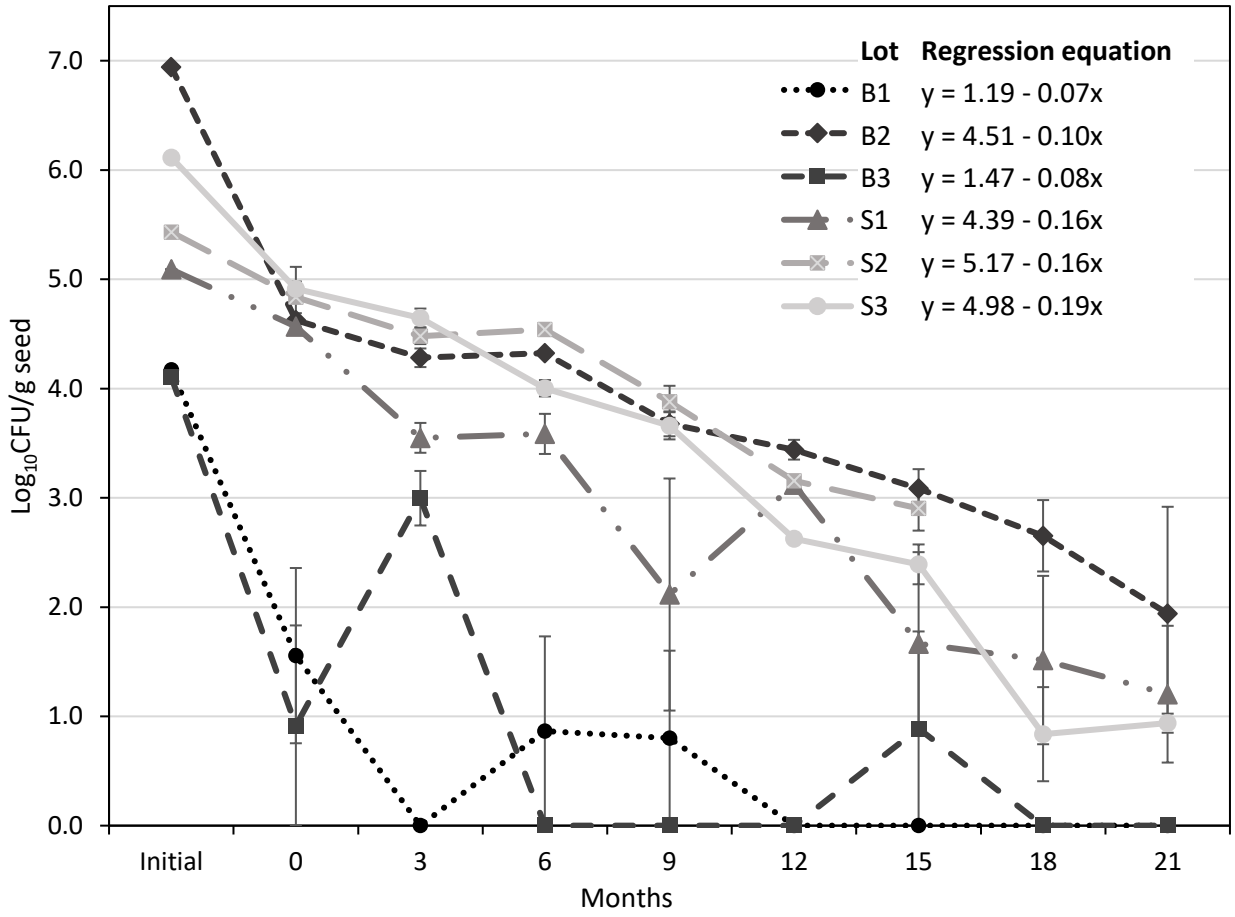

Fig. 2.4. Three seed lots each of beet (B1, B2, B3) and Swiss chard (S1, S2, S3) tested for the presence of *Pseudomonas syringae* pv. *aptata* (*Psa*), causal agent of bacterial leaf spot on beet and Swiss chard, every three months over 21 months to determine the rate at which levels of *Psa* infection on beet and chard seed declines over time. Three replicate susbsamples of 10,000 seeds of each lot were tested each 3-month interval. The duration between seed harvest and the final 21-month test ranged from 34 to 40 months among lots due to different harvest dates of the seed lots. Lot S2 was only tested through 15 months, which was 20 months from the date the seed lot was harvested. Regression equations are provided for predicting the level of *Psa* infection in each lot over time (months in storage).

CHAPTER THREE

EFFICACY OF BACTERICIDES TO MANAGE *PSEUDOMONAS SYRINGAE* PV. *APTATA* INFECTION IN BEET AND SWISS CHARD SEED CROPS

3.1 Introduction

Beta vulgaris, a flowering plant species in the goosefoot family (subfamily Chenopodiaceae within Amaranthaceae), includes several cultivar groups of important agricultural crops, all of which are included in *B. vulgaris* subsp. *vulgaris* (Nottingham 2004). These include Conditiva (beet root/table beet), Cicla (spinach beet or leaf beet), Altissima (sugar beet), Crassa (fodder beet or mangel), and Flavescens (Swiss chard). There are millions of hectares of farmland devoted to these crops across the US. According to the United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS), >0.46 million ha of sugar beet were harvested in the US in 2022, yielding 64,000 kg/ha (USDA NASS 2022). For table beets grown as fresh market or processing crops, ~5,800 ha were harvested in the US in 2017 (USDA NASS 2022). In addition, *B. vulgaris* subsp. *vulgaris* seed production is a crucial industry in the US, specifically in western Washington and Oregon States. In 2017, ~1,600 ha of sugar beet seed crops were harvested in the US, of which 700 ha were produced in Washington State (USDA NASS 2022). In addition, western Washington and western Oregon produce 95% of the table beet seed grown in the US, and approximately 50% of the table beet seed grown worldwide (du Toit 2007).

B. vulgaris is a biennial species, i.e., two years are required to complete the life cycle and produce seed, unless shortcuts (such as vernalization in cold storage or a greenhouse) are taken (du Toit 2007; Navazio et al. 2010). For beet and Swiss chard seed production, the crops must be exposed to a cold enough environment, between 4 and 10°C, for 60 to 90 days for the plants to switch from vegetative to reproductive growth. *B. vulgaris* can tolerate mild freezes, but the plants will die if exposed to prolonged freezing temperatures or excessively cold temperatures (Schrader and Mayberry 2003). Beet and chard seed crops can only be produced in a limited number of areas globally because of

these environmental requirements for flowering and seed set. The maritime Pacific Northwest (western Oregon and western Washington) is the only part of the US that has the correct climate for seed production because of the mild, dry summers and cold but temperate winters (du Toit 2007; Navazio et al. 2010).

Many bacterial and fungal pathogens cause foliar diseases of *B. vulgaris* and can negatively affect marketability of fresh market crops and yield of both fresh market and root crops (Harveson et al. 2009). One of these diseases is bacterial leaf spot (BLS) caused by *Pseudomonas syringae* pv. *aptata* (*Psa*) (Jacobsen 2009). BLS has been reported around the globe in Europe, Australia, New Zealand, Asia, and the US (Ignjatov et al. 2015; Jacobsen 2009; O'Brien and Sparshott 1999). In the latter, BLS has been reported in Arizona, California, Georgia, New York, Ohio, Utah, and Washington (Arabiat et al. 2016; Brown and Jamieson 1913; Dutta et al. 2014; Koike et al. 2003; Nampijja et al. 2021; Pethybridge et al. 2018; Rotondo et al. 2020). BLS was first reported on *B. vulgaris* crops in the US in Utah in 1908 on sugar beet leaves (Brown and Jamieson 1913). More recent reports of BLS include in 1999 on Swiss chard in the Salinas Valley, CA (Koike et al. 2003), and in 2021 in Arizona on baby leaf Swiss chard (Nampijja et al. 2021). *Psa* was described as the pathogen causing BLS on beet and Swiss chard for many years but, based on recent multilocus sequence analysis using four housekeeping genes, Safni et al. (2016) determined that isolates of multiple *P. syringae* pathovars, clades, or subspecies may be responsible for causing BLS on beet and chard. However, the pathogens causing BLS are referred to, collectively, as *Psa* in this study for ease of communication.

Psa infects plant tissue by colonizing and entering the hydathodes on leaf margins, stomata, and wounds on leaves, cotyledons, or stems (Jacobsen 2009; Nampijja et al. 2023). Typical symptoms of BLS on beet and chard can include water-soaked lesions, often with a black or brown margin, irregular black lesions or notches on the margins of leaves or cotyledons, deformed leaves as the leaf continues to expand around the necrotic lesions, and reduced leaf area (Derie et al. 2016; Koike et al. 2003). *Psa* can

spread quickly via splash-dispersal, resulting in major crop losses, and can persist on infected host plant residues, volunteer plants, and on some weed hosts (Derie et al. 2016; Gitaitis and Walcott 2007; Harveson et al. 2009; Koike et al. 2003; Monteil et al. 2012). In addition, *P. syringae* can be seedborne and seed-transmitted, causing seedling blight and reduced stands (Derie et al. 2016; Gitaitis and Walcott 2007; Harveson et al. 2009; Koike et al. 2003).

The restrictive environmental conditions needed for production of biennial *B. vulgaris* seed crops vs. the conditions optimum for fresh market crops results in *B. vulgaris* seed being transported across the globe from the few regions of the world with appropriate conditions for seed production (primarily the Pacific Northwest US, New Zealand, South Africa, Chile, and northern Europe). Given the seedborne nature of *Psa*, this can result in movement of this pathogen around the world on infected seed, with the potential to cause economic losses (Gitaitis and Walcott 2007). BLS has become particularly problematic with the rapid increase in popularity of baby leaf products over the past few decades, which are harvested from crops planted at very dense seeding rates (7 to 9 million seed/ha) with a short cropping cycle of 30 to 60 days, and sequential plantings for daily harvest, which has also increased the demand for seed (Grahn et al. 2015; Nampijja et al. 2023).

For many fungal and bacterial pathogens, chemical or biological control can be an effective way to prevent disease outbreaks or slow disease development. Copper formulations, such as Bordeaux mixture, and cuprous oxide and cupric hydroxide products, are used widely for bacterial disease control (Lamichhane et al. 2018; Scheck and Pscheidt 1998; Sundin et al. 2016). However, copper treatments are not systemic or curative and have limited efficacy once disease symptoms have developed, i.e., they are effective only for preventative disease control (Lamichhane et al. 2015). Additionally, resistance to copper has developed among some bacterial pathogens, reducing the effectiveness of these products for disease control (Jones et al. 2007; Jones et al. 2012). Another chemical approach to the management of bacterial pathogens is the application of antibiotics, such as streptomycin, but widespread

development of resistance to antibiotics in populations of plant, human, and veterinary pathogens has led to widespread disuse of antibiotics in crop production (Jones et al. 2012; Sundin et al. 2016). Due to these limitations of copper and antibiotics, and the lack of highly effective antibacterial products for bacterial plant diseases, biological control agents (BCAs) have been researched widely for control of bacterial diseases such as BLS (Lamichhane et al. 2015). BCAs entail a variety of active ingredients, including beneficial microbes or their byproducts, and extracts from plants, insects, or animals (Sundin et al. 2016). Many BCAs contain live bacteria such as *Agrobacterium, Bacillus, Erwinia, Pseudomonas,* and *Streptomyces* species that use a variety of mechanisms of control of the target bacterial or fungal pathogens, such as competitive exclusion, antagonistic activity from antibiotics or enzymes, or induction of host plant immune response (Bonaterra et al. 2022). One example of a BCA that contains a plant extract is the product Regalia, which contains an extract of the giant knotweed plant, *Reynoutria sachalinensis* (*Rs*), which induces a host plant immune response (Daayf et al. 2000).

Many studies have been conducted to evaluate the efficacy of various BCAs on *P. syringae* pathogens, some of which have proved efficacious (Bonaterra et al. 2022; Fousia et al. 2015; Mora et al. 2015). Mora et al. (2015) evaluated the ability of various *Bacillus* strains to inhibit eight plant pathogens *in vitro*, including *P. syringae* pv. *syringae*. Using nutrient agar assays, all the *Bacillus* strains showed inhibitory effects on pathogen growth, and over half the strains were highly inhibitory. Fousia et al. (2015) evaluated the efficacy of *B. subtilis* strain QST 713, the active ingredient in Serenade Aso and Serenade Opti, for control of bacterial speck of tomato, caused by *P. syringae* pv. *tomato* (*Pst*), in greenhouse conditions. They found *B. subtilis* QST 713 effectively reduced bacterial speck severity. Nikolić et al. (2019) found strain SS-12.6 of *Bacillus amyloliquefaciens* caused significant suppression (60 to 92%) of *Psa* on sugar beet cultivars. Schneider and Ullrich (1994) found applications of the extracts from *Rs* resulted in less severe powdery mildew (*S. fuliginea*) on cucumber and bacterial speck *(P. syringae* pv. *tobaci* and *P. syringae* pv. *pisi*) on tobacco. To our knowledge, no one has evaluated BCAs in

beet and chard seed crops under field conditions for management of BLS, i.e., there is a gap in our knowledge of how to control effectively *P. syringae* pathogens in seed crops.

One of the obstacles to effective use of BCAs is the microorganisms or other active ingredients may not survive or persist for long on plant surfaces, especially under conditions of high UV light exposure (Jones et al. 2012). Some strategies that have been developed to combat persistence issues of BCAs include altering the timing of applications and using protective formulations to prolong viability of the living organism or active ingredient on plant surfaces (Jones et al. 2012). In addition, BCAs can be applied as seed treatments prior to planting to control seedborne or soilborne inoculum of some pathogens. Furthermore, several companies have developed proprietary seed disinfection methods that are effective against seedborne inoculum of some pathogens, such as the proprietary disinfectant treatments used by Germains Seed Technology (Gilroy, CA) for control of seedborne *Psa* (Organic Seed Disinfection ProBio Gopure).

Currently, recommendations for control of *Psa* in the field include the use of bactericides, such as copper products like Badge (copper hydroxide + copper oxychloride), Champ WG (copper hydroxide), or Nordox 75WG (cuprous oxide), or the use of Oxidate 5.0 (hydrogen peroxide + peroxyacetic acid) (Nampijja et al. 2023). However, there have been no published studies on the use of these products for control of BLS on beet and chard. The objective of this study was to evaluate the efficacy of select biological and chemical products used preventatively as foliar applications in beet and chard seed crops for management of *Psa*. The goal was to determine if any of the treatments reduce BLS symptom development, increase harvested seed quantity (yield) and quality (germination), and reduce *Psa* infection of the harvested seed.

3.2 Materials and Methods

Experimental design. Five bactericide trials (one each in 2017-2021) were conducted in Skagit Co., western Washington, to evaluate the efficacy of chemical treatments typically used for foliar disease control in table beet and chard seed crops. Trials 1 to 4 (2017 to 2020, respectively) were planted near Mount Vernon, WA, and Trial 5 (2021) was planted near Day Creek, WA. Vernalized Swiss chard seedlings of the same proprietary cultivar were transplanted into the field in April of each year, using a two-row drop transplanter (Model 5000, Mechanical Transplanter Co., Holland, MI). Planting dates were based on weather conditions (Table 3.1). The experimental design for each trial was a randomized complete block (RCB) with four replications. Each plot was a single row (2.7 to 3.0 m long) with 8 to 10 plants, with a 0.6 to 0.9 m wide buffer of bare soil between adjacent plots in the row, and a 1.2 to 1.5 m long buffer at the ends of each row. The rows were spaced 2.1 m apart to minimize the risk of *Psa* spread between plants in adjacent plots. Transplants were evaluated on a weekly basis, and spacing was adjusted, if necessary, by transplanting additional vernalized seedlings into areas where plants did not survive, to ensure each plot had at least eight plants, prior to the first bactericide application. The bactericide applications were initiated approximately one month after transplanting, i.e., once the transplants had established and resumed growth. Plots were weeded by hand, as needed, throughout the growing season.

Bactericides. The bactericide treatments evaluated over the five trials included four copper products (Cueva, Nordox, Badge, and ManKocide), a general disinfectant (KleenGrow), and five biological treatments (Regalia, Serenade Opti, Serenade Aso, Double Nickel, and Lifegard) (Table 3.1). The specific products used in each trial differed slightly, and details on the products used, the active ingredients, application dates, and foliar application rates are provided in Tables 3.1 and 3.2.

Treatment layout. For Trials 1 to 3 (2017 to 2019), two control treatments were included, an inoculated control treatment and a non-inoculated control treatment. Trials 1 and 2 (2017 and 2018)

also included four bactericide products, and Trial 3 (2019) included five bactericide products. For each of Trials 4 (2020) and 5 (2021), a factorial treatment design was used with bactericide treatments and a control treatment (no bactericide) each applied to plots inoculated with *Psa* and to plots not inoculated. Each treatment was applied one to three times at approximately one-week intervals (weather permitting), using a backpack pump sprayer (model 61800N, Chapin Pro Series, Batavia, NY) for Trials 1 and 2, and a CO_2-pressurized backpack sprayer for Trials 3 to 5. Three of the bactericide products required the addition of a spreader-sticker, for which NuFilm 17 was used (Table 3.2).

Plants in each plot were evaluated monthly after inoculation to rate the percentage of canopy with BLS symptoms, and samples of symptomatic leaves were collected during the final evaluation to confirm the presence of *Psa*, as described for the threshold trials (Chapter 2). At the end of each growing season, once the plants were senescing and the seed had matured, the plants in each plot were harvested manually, laid on tarps in a greenhouse to dry for 1 to 2 weeks, and the seed manually stripped from the plants, winnowed, and decorticated using either a coffee-can grinder (metal coffee can with sandpaper on the inside) or a Hoffman SC100 scarifier (Hoffman Manufacturing, Inc., Corvallis, OR). Seed cleaning equipment was sanitized using 70% isopropyl alcohol between each plot to prevent cross-contamination with *Psa*. A sample of 10,000 seeds per plot was tested via a seed wash dilution plating assay to determine the CFU *Psa*/g harvested seed. In addition, at least 10 suspect *Psa* isolates from each seed wash were tested for pathogenicity on beet seedlings of the cv. Red Ace, with two replications of six plants per isolate. The seed wash and pathogenicity testing protocols can be found in Chapter 2. A blotter germination assay was conducted for a subsample of the harvested seed from each plot, as described below, and total marketable seed yield/plant was calculated to determine if the bactericide treatments had any effects on seed quality (normal seed germination and thousand seed weight, TSW) or quantity (yield), respectively.

2017 trial. For the 2017 trial, bactericide applications began 26 days after transplanting (dat) using a hand-pump backpack sprayer. Plots were inoculated with a Washington isolate of *Psa* three days after the first bactericide application (29 dat). The second bactericide application took place two weeks later (43 dat). Plants were harvested in September 2017 (exact date not recorded), dried as described above, winnowed, cleaned using a coffee-can grinder, and winnowed again to remove dirt and debris. Seed wash dilution plating to quantify infection by *Psa* began approximately two months after harvest and took three months to complete (by replication).

2018 trial. The 2018 trial included the same number of treatments and experimental design as the 2017 trial, except that two of the bactericides were substituted, as detailed in Table 2. The two treatments from the 2017 trial that appeared to have had the least significant effect on *Psa* infection of the seed were omitted. The first bactericide application took place 57 dat due to the plants recovering slowly from transplanting. *Psa* inoculation occurred 60 dat, and a second application of the bactericides occurred two weeks later. Plants were harvested on 7 September (159 dat), and the seed threshed, cleaned, and tested for *Psa* as described for the 2017 trial.

2019 trial. The 2019 trial proceeded as described for the previous two trials, with several minor changes. An additional bactericide was included (5 bactericides and 2 control treatments) and the bactericides were applied with a CO_2-pressurized backpack sprayer. The first and second chemical applications occurred 36 and 42 dat, followed by *Psa* inoculation 8 days later (50 dat). A third bactericide application occurred 66 dat, and seed was harvested on 11 September (156 dat). The trial was weeded by hand four times through the growing season, on 19 June, and 15, 23, and 30 July. Seed was cleaned using a Hoffman SC100 scarifier.

2020 trial. The treatment design for the 2020 trial differed from the previous three trials since each treatment was applied to both inoculated and non-inoculated plots (6 x 2 factorial treatment design). Each chemical treatment was applied three times. The first two applications occurred 41 and 48

dat, respectively. *Psa* inoculation occurred 54 dat, and the third chemical application was done 61 dat. Seed was harvested 146 dat and cleaned with the Hoffman SC100 scarifier. In addition, the area of the field in which the trial was planted had been treated with a herbicide the previous fall that resulted in the transplants experiencing varying levels of herbicide damage, especially the male row. Therefore, each plot was rated for herbicide damage after transplanting and before bactericide treatments began, and the plots were rated again for herbicide damage before each chemical application. This rating was also used to assess if any phytotoxicity occurred from the bactericide applications.

2021 trial. The 2021 trial entailed a 7 x 2 factorial treatment design, with one additional bactericide evaluated in addition to the five evaluated in the 2020 trial. The bactericide applications took place 46, 55, and 64 dat, and the *Psa* inoculation occurred 66 dat. At the end of the growing season, seed was harvested from five plants in the center of each plot. In contrast, all plants were harvested per plot in the previous four trials.

***Psa* inoculation.** Strains of *Psa* isolated from seed grown in Washington and that were highly virulent when inoculated onto beet seedlings were used for each of the field trial inoculations. The timing of inoculation relative to the bactericide applications varied among trials, as described in Table 1. Inoculations were done 3 to 7 days after the second or third chemical application, based on local weather conditions. Different isolates were used each year and are available from Lindsey du Toit's program at the Washington State University Mount Vernon Northwestern Washington Research and Extension Center, except for the Sakata isolate used in the 2017 trial, which is no longer available. The isolate used in 2018 was 18-004A 0-3, in 2019 was 18-187 1-9, in 2020 was 19-010 2-8, and in 2021 was a rifampicin-resistant Washington isolate generated by Carolee Bull's program at Pennsylvania State University. For each inoculation, a suspension of 5 to 8 liters of the *Psa* isolate was prepared at 0.3 to 0.5 optical density (OD at 600 nm) by growing lawns of *Psa* on KB agar medium. Sterile cotton swabs were then used to transfer the bacteria into test tubes of sterile de-ionized water, the suspension vortexed

and transferred to large, sterile 2-liter bottles, and the OD of the suspension measured using a spectrophotometer and adjusted to 0.3 to 0.5. Each plant was sprayed with the *Psa* suspension for approximately 3 to 5 seconds, with 15 to 30 ml of inoculum/plant, based on the size of the plant.

Seed germination assay. A blotter germination assay (Association of Official Seed Analysts 2022) was completed for a sample of the seed harvested from each plot in each trial, by placing two replications of 100 seeds on germination blotter paper (heavy-weight, brown, Kraft towel, 76-pound weight, Anchor Paper, St. Paul, MN) dampened with deionized water. The germination paper was then folded, placed in clear plastic bags, each bag sealed with a rubber band, and the seed incubated in a growth chamber with LED lights at 26°C by day and 20°C by night with a 10 h/14 h day/night cycle. The number of seeds that germinated normally was counted after 7 and 14 days, to determine the percentage total (cumulative) germination for each replication of 100 seeds. After the 7-day reading, the germination paper was moistened with deionized water using a spray bottle to maintain adequate moisture for continued germination. The incidence of normal germination for harvested seed in both replications of 100 seeds was averaged to calculate the percent normal seed germination for each plot in each trial.

Data analyses. In each trial, replication was treated as a random effect and inoculation and bactericide treatments as fixed effects. The data were analyzed using SAS PROC GLM to evaluate whether the bactericide treatments or inoculation treatment had any effects on BLS severity, area under the disease progress curve (AUDPC), seed yield, seed quality, or the amount of *Psa* infection of the harvested seed. In addition, SAS PROC CORR was used to calculate the correlations between dependent and independent variables. Transformations were used to fulfill the assumptions for parametric data analysis, including $\log_{10}$, square root $\log_{10}$, and ranking of the data, as necessary.

3.3 Results

2017 trial. In the 2017 trial, BLS symptoms appeared within one week of *Psa* inoculation. Disease pressure was high, and BLS spread throughout the plots, including to the non-inoculated control plots. Seed harvested from all plots tested positive for *Psa*, and the infection levels of the seed harvested from all plots ranged from $\log_{10}$0.95 to $\log_{10}$4.86 CFU *Psa*/g seed. The mean CFU/g seed differed significantly among bactericide treatments (P = 0.0131, Table 3.3) with the seed harvested from ManKocide treated plots having significantly less infection ($\log_{10}$2.93 CFU/g seed) than seed harvested from plots treated with Cueva ($\log_{10}$4.33 CFU/g seed) and the inoculated control plots ($\log_{10}$4.48 CFU/g seed). Therefore, only ManKocide reduced *Psa* infection levels on the harvested seed compared to inoculated control plots. Non-inoculated control plots had similar levels of infection of the harvested seed to the inoculated control plots and all other plots, demonstrating there had been dispersal of *Psa* among plots. There was no significant effect of the bactericide treatments on thousand seed weight (Tables 3.3 and 3.4).

2018 trial. Similar to the 2017 trial, BLS in the 2018 trial was visible within several days of inoculation with *Psa*. Disease pressure was very high. By harvest, all plots had BLS symptoms and the seed harvested from all plots tested positive for *Psa* (Tables 3.3 and 3.4). The level of infection of harvested seed ranged from $\log_{10}$5.84 to $\log_{10}$6.60 CFU *Psa*/g seed across all plots. Even non-inoculated control plots averaged $\log_{10}$6.10 CFU/g seed. There were no significant differences in mean CFU *Psa*/g seed among bactericide treatments, including between inoculated and non-inoculated control plots, and no difference in thousand seed weight among plots that received different treatments (Tables 3.3 and 3.4). None of the bactericide treatments was effective at reducing *Psa* infection of the seed.

2019 trial. *Psa* symptoms were observed in the 2019 trial a week after inoculation (3 June) and BLS was severe, with symptoms observed in all plots, including the non-inoculated control plots. The level of *Psa* infection on the harvested seed ranged from $\log_{10}$4.19 to $\log_{10}$5.89 CFU/g seed (Table 3.4).

The ANOVA showed a marginal effect of the bactericide treatments on the level of *Psa* infection of the harvested seed (*P* = 0.0716, Table 3.3), but only seed harvested from the inoculated and non-inoculated control plots had significantly different levels of *Psa* infection of the harvested seed, with seed from the inoculated control plots having a higher level of infection (log_{10}5.51 CFU/g seed) than seed from the non-inoculated plots (log_{10}4.75 CFU/g seed) (Table 3.4). None of the bactericide treatments reduced the level of infection of harvested seed.

2020 trial. In the 2020 bactericide trial, the location of plants in the field affected how badly plants were damaged by residual herbicide from the previous season. In some plots, beet plants grew out of the herbicide damage, but in others the herbicide phytotoxicity did not decrease over the season (*data not shown*). There was no significant correlation between herbicide damage ratings and seed yield or the amount of *Psa* detected on the harvested seed (*data not shown*). BLS symptoms were observed within a week after inoculation. In July, the severity of BLS across all plots ranged from 15 to 40% with a mean ± standard error of 28.0 ± 0.9%. When symptomatic leaf samples were plated onto selective agar media, suspect *Psa* isolates were detected as well as a *Stemphylium* sp. from a symptomatic leaf in the male row. There was no difference in BLS severity due to either the bactericide treatments (*P* = 0.8144) or the inoculation treatments (*P* = 0.9612), and there was no interaction between bactericide and inoculation treatments (*P* = 0.1852). This lack of significance was possibly due, in part, to the presence of other pathogens in the field causing similar symptoms, such as the *Stemphylium* isolate obtained from a symptomatic male plant (Harveson et al. 2009).

The seed yield for all plots ranged from 126.9 to 294.1 g seed/plant with an overall mean of 204.76 ± 4.7 g seed/plant. The bactericide treatments affected seed yield (*P* = 0.0309), with the control plots (222.7 g) having significantly greater yield then the Serenade Opti plots (180.4 g). Seed yield was also affected by an interaction between the bactericide and inoculation treatments (*P* = 0.0094). Seed yield for non-inoculated plots with the Serenade treatment (168.2 g seed/plant) was significantly less

than that of the non-inoculated control plots (238.3 g/plant) and the inoculated Badge plots (242.9 g/plant), suggesting the Serenade Opti bactericide treatment adversely affected seed yield. The germination rate of the harvested seed ranged from 78.0 to 98.5% with a mean of 90.5 ± 0.7% across all plots. There was no effect of inoculation treatments (P = 0.3855) or bactericide treatments (P = 0.8174) on seed germination, and the interaction of these factors also was not significant (P = 0.1974, Table 3.3). The TSW of seed harvested from all plots ranged from 9.85 to 15.24 g, with an average of 12.55 ± 0.17 g. TSW was positively correlated with normal seed germination (r = 0.3176, P = 0.0278), but there were no significant effects of bactericide or inoculation treatments on TSW and no significant interaction of these factors (Table 3.5).

Psa infection levels of the harvested seed ranged from $\log_{10}$3.64 to $\log_{10}$6.31 CFU/g seed, with an average across all plots of $\log_{10}$5.56 CFU/g seed. The non-inoculated control plots had an average *Psa* infection of $\log_{10}$4.65 CFU/g seed, while the inoculated control plots averaged $\log_{10}$5.68 CFU/g seed (Table 3.5). The main effect of inoculation had no significant effect on the *Psa* seed infection levels (P = 0.131). The ANOVA showed that the bactericide treatment had a marginal effect on the CFU *Psa*/g harvested seed (P = 0.0764), with the control plots having the lowest infection level ($\log_{10}$5.17 CFU/g seed) and plots treated with Nordox having the highest infection ($\log_{10}$5.86 CFU/g seed, Table 3.5). There was also a significant interaction between the inoculation and bactericide treatments on CFU *Psa*/g seed (P = 0.0507). The non-inoculated control plots had the least average *Psa* infection ($\log_{10}$4.65 CFU/g seed), with significantly less infection than seed harvested from plots treated with several bactericide products, both inoculated and non-inoculated, i.e., Badge, Nordox, and Double Nickel. This suggested these bactericides may have resulted in an increase in *Psa* infection of the seed (Table 3.5). The inoculated plots treated with Double Nickel had the highest average infection ($\log_{10}$6.01 CFU/g seed, Table 3.5). None of the bactericide treatments was effective at reducing *Psa* infection of harvested seed compared to the control plots.

2021 trial. Weather conditions during the 2021 trial were hot and dry, with very few BLS symptoms observed. The initial BLS severity rating on 22 June ranged from 0 to 0.71%, with a mean of 0.32 ± 0.02%. By the final BLS severity rating on 27 July, the percent canopy with symptoms ranged from 1 to 5% across all plots, and AUDPC values ranged from 6.5 to 68.9 with a mean of 37.3 ± 2. There was a significant effect of inoculation treatment on the first and second BLS severity ratings on 22 and 30 June (*P* = 0.0427 and 0.0512, respectively), as inoculated plots had more severe BLS than non-inoculated plots (0.10 vs. 0.16% on 22 June, respectively, and 0.20 vs. 0.32% on 30 June, respectively). The bactericide treatments had no effect on BLS severity (*P* = 0.5812, 0.2653, 0.5056, and 0.5020, from the first to fourth ratings, respectively) or the AUDPC values (*P* = 0.8981), and there was no significant interaction between bactericide and inoculation treatments (Tables 3.3 and 3.6).

Seed yield across all plots in the 2021 trial ranged from 63.6 to 262.6 g seed/plant, with a mean of 115.2 ± 4.7 g. The non-inoculated and inoculated control plots had average seed yields of 109.0 and 98.7 g, respectively. There was a marginal main effect of inoculation treatments on seed yield (*P* = 0.1056), with inoculated plots having slightly higher average seed yield (122.5 g/plant) compared to the non-inoculated plots (107.9 g/plant). There was a significant negative correlation between seed yield and the final BLS rating (r = -0.39809, *P* = 0.0024), but the ANOVA showed no significant differences in seed yield among plots treated with different bactericides (*P* = 0.3795), and no significant interaction between bactericide and inoculation treatments (*P* = 0.3758). Seed germination rates across all plots ranged from 83 to 96%, with a mean of 89.7 ± 0.4%. Neither the bactericide treatments (*P* = 0.7949) or inoculation treatments (*P* = 0.1408) affected seed germination significantly, and there was no significant interaction between the two factors (*P* = 0.2241).

The *Psa* infection level of the seed harvested ranged from 0 to $\log_{10}$3.82 CFU/g seed, with a mean of $\log_{10}$1.63 ± $\log_{10}$0.20 CFU/g seed. The non-inoculated control plots had an average *Psa* infection level of $\log_{10}$ 1.66 CFU/g seed, while inoculated control plots averaged $\log_{10}$2.11 CFU/g seed (Table 3.6).

The ANOVA showed a significant effect of inoculation treatments on CFU *Psa*/g seed (P = 0.0043), with inoculated plots ($\log_{10}$2.19 CFU/g seed) having higher levels of *Psa* infection than non-inoculated plots ($\log_{10}$1.07 CFU/g seed, Table 3.6). Bactericide treatments had no effect on *Psa* infection of harvested seed (P = 0.6899), but there was a marginal interaction between inoculation treatments and bactericide treatments (P = 0.1048), with the lowest average *Psa* infection detected on seed from the non-inoculated plots treated with Badge or Serenade Aso (both 0 CFU/g seed), and the highest average infection on seed harvested from the inoculated plots treated with Serenade Aso ($\log_{10}$2.69 CFU/g seed, Table 3.6).

3.4 Discussion

BLS can result in significant economic losses in *B. vulgaris* crops, especially when infected seed is planted at high densities for baby leaf beet and Swiss chard production. A crucial step used to control BLS is the identification and treatment of *Psa*-infected seed lots. However, seed disinfection is costly and time-consuming and, as a result, other approaches are being explored to reduce the levels of *Psa* infection in seed crops. One possible means of managing BLS in seed crops is through foliar applications of chemical or biological control products. In this study, five field trials in Swiss chard seed crops were used to evaluate multiple copper formulations and BCAs for control of BLS and for reducing infection of the harvested seed. However, none of the treatments evaluated was effective consistently at preventing or reducing *Psa* infection of the harvested seed.

Recommendations for control of BLS typically are focused on the applications of copper products, even though these treatments have limited efficacy for control of *P. syringae* pathogens (Fouisa et al. 2016; Nampijja et al. 2023). In this study, results of the first trial (2017) suggested the copper bactericide ManKocide had some efficacy, albeit limited, at reducing the amount of *Psa* infection of the harvested seed, but the subsequent trials failed to demonstrate similar efficacy of ManKocide or

other copper products at controlling BLS and reducing infection of the harvested seed. Applications of ManKocide (copper hydroxide plus mancozeb) reduced the amount of *Psa* on harvested seed in the 2017 trial by >10-fold compared to inoculated control plots and non-inoculated control plots, but applications of ManKocide failed to reduce *Psa* seed infection in the 2018 trial. BLS severity differed significantly between the two trials, with much higher disease pressure in the 2018 trial, based on the amount of *Psa* infection detected on the harvested seed. Overall, *Psa* infection levels on harvested seed in the 2017 trial were much less ($\log_{10}$0.95 to $\log_{10}$4.86 CFU/g seed) than in the 2018 trial ($\log_{10}$5.84 to $\log_{10}$6.60 CFU/g seed). Under the moderate disease pressure in the 2017 trial, ManKocide had some efficacy at reducing *Psa* infection of seed, but not under the high disease pressure in the 2018 trial.

Disease pressure also was greater in the 2019 and 2020 trials than the 2017 trial ($\log_{10}$4.19 to $\log_{10}$5.89 CFU/g seed in the 2019 trial, and $\log_{10}$3.64 to $\log_{10}$6.31 CFU/g seed in the 2020 trial), with the copper treatments in those trials also not effective at reducing *Psa* infection of seed. Conversely, disease pressure was very low in the 2021 trial, with BLS severity ratings not exceeding 5% in any plot, and an average overall *Psa* infection of only $\log_{10}$1.63 CFU/g seed. Even though *Psa* was not detected on seed harvested from 24 of the 56 plots in the 2021 trial, there was no efficacy of the copper treatments on *Psa* seed infection, likely because of inadequate disease pressure to discriminate among treatments. In future studies, multiple *Psa* inoculations should be used if conditions are not conducive to BLS to ensure adequate disease pressure to evaluate bactericide treatments.

Another factor that may have affected the efficacy of copper bactericides in these trials is the use of different *Psa* strains in each trial. Copper resistance has been documented in many bacterial plant pathogens, including in several pathovars of *P. syringae* (Griffin et al. 2017; Lamichhane et al. 2018; Scheck and Pscheidt 1998). It is possible that the isolate used in the 2017 trial, in which ManKocide had some efficacy against *Psa*, was sensitive to copper, while the isolate used in the 2018 trial may have been tolerant of copper. To avoid confounding this issue when screening treatments for efficacy against

BLS, the isolates used to inoculate plants should be screened for copper tolerance (Husseini and Akköprü 2020; Zhang et al. 2017). In addition, multiple isolates of *Psa* could be used to assess whether treatments are efficacious against a diversity of strains that can cause BLS of beet and chard.

The 2017 and 2018 field trials were planted in the same field, with the 2018 trial close to the location of the 2017 trial (~12 m). Infected plant residues from the 2017 trial or infected weed hosts may have harbored *Psa* over the winter, creating a secondary source of *Psa* inoculum for the 2018 trial, since splash-and wind-dispersed bacterial pathogens such as *Psa* can spread over relatively long distances, as documented by Upper et al. (2003). In that study evaluating factors that affect the spread of *P. syringae* pv. *syringae* (*Pss*), *Pss* spread 20 m from infected 'source' plants to initially pathogen-free 'sink' plants. In one experiment, there was no significant difference in the population size of *Pss* on plants placed in sinks surrounded by a 6- or 20-m barrier (Upper et al. 2003). In future field trials, planting locations should, ideally, be isolated from locations where beet and chard crops have been grown to avoid potential alternative sources of inoculum. Field options were limited for this study, resulting in the necessity of using fields in relatively close proximity for several trials.

In addition to lack of efficacy demonstrated for most of the copper treatment evaluated, none of the BCAs evaluated in these trials proved effective at reducing BLS severity or *Psa* infection of seed. As previously discussed, there have been many studies evaluating the efficacy of BCAs for control of *P. syringae* pathogens (Bonaterra et al. 2022; Fousia et al. 2015; Mora et al. 2015). The efficacy of these products in those studies compared to the lack of efficacy in the field trials in this study may reflect a number of factors, including the timing of BCA applications relative to pathogen inoculations, inoculation methods, the specific strains used, and field conditions. In this study, inoculation with *Psa* occurred several days after applications of BCAs and copper products, with the timing of bactericide applications and *Psa* inoculation dependent on weather conditions, i.e., *Psa* inoculations did not occur at the same time as bactericide applications in each trial, primarily because most bactericides are

preventative, not curative (e.g., du Toit and Derie 2008; Lamichhane et al. 2018). In contrast, Nikolić et al. (2019) inoculated beet plants with *B. amyloliquefaciens* strains concurrently with inoculation of a pathogenic strain of *Psa* by injecting bacterial suspensions into plants using a syringe. *B. amyloliquefaciens* is the active ingredient in Double Nickel, which was evaluated in the 2019, 2020, and 2021 trials in this study. Using spray applications of BCAs may be less efficacious compared to injection on plants on a small scale, because injecting both the BCA and pathogen together into plant tissue ensures the BCA is in direct contact with the pathogen. Unfortunately, injection of BCAs is infeasible for full-scale crop production. Fousia et al. (2015) applied the pathogen *P. syringae* pv. *tomato* and a BCA *B. subtilis* QST 713 by spraying plants with the pathogen one day after the BCA application, ensuring direct contact between the BCA and the pathogen. The gap of several days between bactericide applications and pathogen inoculation in this study may have affected efficacy of the treatments compared to concurrent applications of treatments with *Psa* inoculation, since BCAs may not persist very long on plant surfaces (Jones et al. 2012). However, using BCAs for disease management in growers' field conditions cannot be timed precisely based on when plants become colonized by a pathogen. If the BCAs are only effective when applied concurrently with a pathogen, or within a short period before or after colonization of plants by a pathogen, this severely limits the viability of BCAs for disease control, particularly given the latent nature of infection of plants by most plant pathogens and the reality that plants in crops may be exposed to multiple rounds of colonization by pathogens (Lamicchane et al. 2018).

Another significant difference between previous research and this study on BLS is that plants were inoculated at early stages of development and indoors, i.e., Nikolić et al. (2019) inoculated 28-day-old beet plants, and Fousia et al. (2015) inoculated tomato plants at the 3- to 4-leaf stage, whereas plants in this study were inoculated 1 to 2 months after transplanting vernalized seedlings. It can be more difficult to achieve complete coverage of the canopy when applying treatments to larger plants in

fields, so applications of BCAs to younger plants likely results in more thorough coverage than achieved with the applications in the Swiss chard field trials in this study. In addition, environmental conditions in the field trials (with direct sun exposure, wind, rain, and the presence of other microflora or pests on the canopy) may have affected survival of microorganisms in the BCAs on the plant surfaces, making it more difficult to ensure the BCA remained viable on the plant surface. Nonetheless, most of the BCA products evaluated in this study are marketed for use in both greenhouse and field situations.

B. amyloliquefaciens strain *SS*-12.6 evaluated by Nikolić et al. (2019), and various *Bacillus* strains evaluated by Mora et al. (2015) using in vitro agar plate assays may have been more effective antagonists of *Psa* than the *Bacillus* strains in several of the BCA products evaluated in this study, i.e., Double Nickel, Lifegard, Serenade Aso, and Serenade Opti. However, far more studies have demonstrated efficacy of BCAs in vitro vs. studies evaluating BCAs in vivo, because of the many factors discussed previously that can affect survival and efficacy of BCAs in field conditions. Further research is needed on potential optimization of applications of BCAs, e.g., those containing *Bacillus* strains, for more effective control of BLS.

Regalia, which contains an extract of the giant knotweed, *R. sachalinensis*, has shown efficacy at controlling some fungal diseases of plants, such as gray mold (*Botrytis cinerea*) and powdery mildew (*Leveillula taurica*) on tomato (Esquivel-Cervantes et al. 2022), powdery mildew (*Blumeria graminis* f. sp. *tritici*) on wheat (Vechet et al. 2009), and powdery mildew (*Sphaerotheca fuliginea*) on English cucumber (Bokshi et al. 2008; Daayf et al. 1995; Daayf et al. 2000). However, despite Regalia being registered for control of various *Pseudomonas* and *Xanthomonas* pathogens, there is very little research evaluating the efficacy of this product for control of bacterial pathogens (Esquivel-Cervantes et al. 2022; Trueman 2015). Schneider and Ullrich (1994) found that applications of *R. sachalinensis* extracts resulted in less severe bacterial speck on tobacco plants inoculated with *P. syringae* pv. *tobaci* and *P. syringae* pv. *pisi*. In this study on BLS of beet and chard, Regalia was not effective at reducing BLS severity or *Psa*

infection of the harvested seed. The lack of efficacy may have been influenced by the timing of applications relative to inoculation of the pathogen, or due to beet and chard plants having a reduced systemic resistance response when treated with the product compared to tobacco and tomato plants.

The bactericide treatments evaluated in this study were also examined for the effects on seed quantity and quality, including TSW in the 2017, 2018, and 2020 trials, and seed yield and germination in the 2020 and 2021 trials. There was a significant effect of bactericide treatments on seed yield in the 2020 trial, but not in the 2021 trial. However, the effect in 2020 was because plots treated with Serenade Opti had reduced seed yield compared to the non-treated control plots. A different formulation of Serenade, Serenade Aso, was used in the 2021 trial, which may not have been as phytotoxic as the Serenade Opti treatment in the 2020 trial. Both products contain the same active ingredient, *B. subtilis* strain QST 713, but Serenade Opti is formulated as a wettable powder with 26.2% active ingredient, and Serenade Aso is a suspension concentrate with 1.34% active ingredient. Research is needed to determine if these BCAs have negative effects on yield of seed crops in different environments. The bactericide treatments had no effect on TSW and seed germination in any of the field trials. Inoculation with *Psa* also had no effect on seed germination or TSW, although TSW was only recorded in one of the two trials in which inoculation was included as a main factor in a factorial design with bactericide treatments.

In conclusion, none of the bactericide treatments evaluated in these field trials proved to be effective consistently at reducing severity of BLS in Swiss chard seed crops or reducing the amount of *Psa* infection of the harvested seed. Although the copper bactericide ManKocide looked promising in the 2017 trial, this product did not prove efficacious in the 2018 trial, and no other copper treatment was efficacious in the other three trials. Differences in disease severity across trials may have affected consistency in efficacy of ManKocide. The current recommendation to use copper bactericides for control of BLS in beet or Swiss chard seed crops was not supported by the results of this research.

Further research could address some of the limitations of these trials, e.g., changes in bactericide application timing based on crop growth stage, and evaluation of other BCAs for control of *Ps*a. In addition, isolates of *Psa* used in such trials should be screened for tolerance to copper, since copper products currently are used for control of BLS (Nampijja et al. 2023). Other management avenues being explored include methods of disinfecting seed lots and other seed treatments to reduce seed infection levels and the risk of seed transmission of *Psa*, removing sources of inoculum besides infected seed, and modifying production practices to reduce conduciveness of field conditions for development of BLS (Bull 2019).

3.5 REFERENCES

Arabiat, S., Chanda, A. K., Chittem, K., and Khan, M. F. R. 2016. First report of *Pseudomonas syringae* pv. *aptata* causing bacterial blight of sugar beet *(Beta vulgaris*) in Oregon. Plant Dis. 100(11):2334.

Association of Official Seed Analysts. 2022. AOSA rules for testing seeds. https://analyzeseeds.com (accessed 10 Oct. 2023).

Badge SC. Environmental Protection Agency Reg. No. 10163-396. Gowan Company LLC, Yuma, AZ.

Bokshi, A., Jobling, J., McConchie, R. 2008. A single application of Milsana® followed by Bion® assists in the control of powdery mildew in cucumber and helps overcome yield losses. J. Hortic. Sci. Biotechnol. 83:701-706.

Bonaterra, A., Badosa, E., Daranas, N., Francés, J., Roselló, G., and Montesinos, E. 2022. Bacteria as biological control agents of plant diseases. Microorganisms 10(9):1759.

Brown, N. A., and Jamieson, C. O. 1913. A bacterium causing a disease of sugar beet and nasturtium leaves. J. Agric. Res. 1:189-210.

Bull, C. T. 2019. Integrated management of emerging seedborne bacterial diseases of cucurbits and chenopods (IMDCC). United States Department of Agriculture National Institute of Food and Agriculture Specialty Crops Research Initiative Project No. 2019-51181-30019. https://www.pseudosonseed.org/ (accessed 10 Oct. 2023).

Cueva Fungicide Concentrate. Environmental Protection Agency Reg. No. 67702-2-70051. Certis USA LLC, Columbia, MD.

Daayf, F., Ongena, M., Boulanger, R., El Hadrami, I., Bélanger, R. R. 2000. Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew-infected plants with extracts of *Reynoutria sachalinensis*. J. Chem. Ecol. 26:1579-1593.

Daayf, F., Schmitt, A., and Belanger, R. 1995. The effects of plant extracts of *Reynoutria sachalinensis* on powdery mildew development and leaf physiology of long English cucumber. Plant Dis. 79(6):577-580.

Double Nickel 55 Biofungicide. Environmental Protection Agency Reg. No. 70051-108. Certis USA LLC, Columbia, MD.

du Toit, L. J., and Derie, M. L. 2008. Effect of inoculum concentration and timing of ManKocide application on control of bacterial blight of carrot, 2007. Plant Dis. Manage. Rep. 2:V008.

Derie, M. L., Holmes, B. J., Safni, I., Bull, C. T, and du Toit, L. J. 2016. Seedborne inoculum thresholds of *Pseudomonas syringae* pv. *aptata*, causal agent of bacterial leaf spot, in 'baby leaf' Swiss chard crops. Phytopathology 106:S4.142 (Abstract).

du Toit, L. 2007. Crop profile for table beet seed in Washington. U.S. Department of Agriculture National Pest Management Regional IPM Center. Davis, CA. https://ipmdata.ipmcenters.org/documents/cropprofiles/WAbeetseed2007.pdf (accessed 10 Oct. 2023).

Dutta, B., Ingram, T., Gitaitis, R. D., Langston, D. B., Brenneman, T., Webster, T. M., and Davis, R. F. 2014. First report of bacterial blight of sugar beet caused by *Pseudomonas syringae* pv. *aptata* in Georgia, USA. Plant Dis. 98(10):1423.

Esquivel-Cervantes, L. F., Tlapal-Bolaños, B., Tovar-Pedraza, J. M., Pérez-Hernández, O., Leyva-Mir, S. G., and Camacho-Tapia, M. 2022. Efficacy of biorational products for managing diseases of tomato in greenhouse production. Plants 11(13):1638.

Fousia, S., Paplomatas, E. J., and Tjamos, S. E. 2015. *Bacillus subtilis* QST 713 confers protection to tomato plants against *Pseudomonas syringae* pv. *tomato* and induces plant defence-related genes. J. Phytopathol. 164(4):264-270.

Gitaitis, R., and Walcott, R. 2007. The epidemiology and management of seedborne bacterial diseases. Annu. Rev. Phytopathol. 45:371-397.

Grahn, C., Benedict, C., Thornton, T., and Miles, C. 2015. Baby-leaf salad green production guide for western Washington. Washington State University Extension Bulletin EM095E.

Griffin, K., Gambley, C., Brown, P., and Li, Y. 2017. Copper-tolerance in *Pseudomonas syringae* pv. *tomato* and *Xanthomonas* spp. and the control of diseases associated with these pathogens in tomato and pepper. A systematic literature review. Crop Prot. 96:144-150.

Harveson, R. M., Hanson, L. E., and Hein, G. L. 2009. Compendium of Beet Diseases and Pests, 2nd Ed. American Phytopathological Society, St. Paul.

Husseini, A., and Akköprü, A. 2020. The possible mechanisms of copper resistance in the pathogen *Pseudomonas syringae* pathovars in stone fruit trees. Phytoparasitica 48:705-718.

Ignjatov, M., Gvozdanović-Varga, J., Milošević, D., Nikolić, Z., Ivanović, Ž., and Popović, T. 2015. First report of bacterial leaf spot of chard (*Beta vulgaris* subsp. *cicla*) caused by *Pseudomonas syringae* pv. *syringae* in Serbia. Plant Dis. 99(5):723.

Jacobsen, J. 2009. Bacterial leaf spot. Pp. 59-60, In: Compendium of Beet Diseases and Pests, 2nd Ed. R. M. Harveson, L. E. Hanson, and G. L. Hein, eds. American Phytopathological Society, St. Paul.

Jones, J. B., Jackson, L. E., Balogh, B., Obradovic, A., Iriarte, F. B., and Momol, M. T. 2007. Bacteriophages for plant disease control. Annu. Rev. Phytopathol. 45:245-262.

Jones, J. B., Vallad, G. E., Iriarte, F. B., Obradović, A., Wernsing, M. H., Jackson, L. E., Balogh, B., Hong, J. C., and Momol, M. T. 2012. Considerations for using bacteriophages for plant disease control. Bacteriophage 2(4):208-214.

KleenGrow. Environmental Protection Agency Registration No. 81820-2. Pace 49, Inc., Delta, BC.

Koike, S. T., Azad, H. R., and Cooksey, D. C. 2002. First report of bacterial leaf spot of spinach caused by a *Pseudomonas syringae* pathovar in California. Plant Dis. 86(8):921.

Koike, S. T., Henderson, D. M., Bull, C. T., Goldman P. H., and Lewellen, R. T. 2003. First report of bacterial leaf spot of Swiss chard caused by *Pseudomonas syringae* pv. *aptata* in California. Plant Dis. 87(11):1397.

Lamichhane, J. R., Messéan, A., and Morris, C. E. 2015. Insights into epidemiology and control of diseases of annual plants caused by the *Pseudomonas syringae* species complex. J. Gen. Plant Pathol. 81(5):331-350.

Lamichhane, J. R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J. B., and Aubertot, J. N. 2018. Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. Agron. Sustain. Develop. 38(3):1-18.

Lifegard WG Biological Plant Activator. Environmental Protection Agency Reg. No. 70051-119. Certis USA LLC, Columbia, MD.

ManKocide Fungicide/Bactericide. Environmental Protection Agency Reg. No. 91411-7-70051. Certis USA LLC, Columbia, MD.

Monteil, C. L., Guilbaud, C., Glaux, C., Lafolie, F., Soubeyrand, S., and Morris, C. E. 2012. Emigration of the plant pathogen *Pseudomonas syringae* from leaf litter contributes to its population dynamics in alpine snowpack. Environ. Microbiol. 14(8):2099-2112.

Mora, I., Cabrefiga, J., and Montesinos, E. 2015. Cyclic lipopeptide biosynthetic genes and products, and inhibitory activity of plant-associated *Bacillus* against phytopathogenic bacteria. PLoS One 10(5):e0127738.

Nampijja, M., Derie, M. L., and du Toit, L. J. 2021. First report of bacterial leaf spot caused by *Pseudomonas syringae* pv. *aptata* on Swiss chard, *Beta vulgaris* subsp. *vulgaris*, in Arizona. Plant Dis. 105:3738.

Nampijja, M., Crane, S., du Toit, L., and Ocamb, C. 2023. Beet, Red (*Beta vulgaris*)-Bacterial Leaf Spot. Pacific Northwest Plant Disease Management Handbook, Oregon State University, Corvallis.

https://pnwhandbooks.org/plantdisease/host-disease/beet-red-beta-vulgaris-bacterial-leaf-spot (accessed 10 Oct. 2023).

Navazio, J., Colley, M., and Zyskowski, J. 2010. Principles and Practices of Organic Beet Seed Production in the Pacific Northwest. Organic Seed Alliance, Port Townsend, WA. https://seedalliance.org/publications/principles-practices-organic-beet-seed-production-pacific-northwest/ (accessed 10 Oct. 2023).

Nikolić, I., Berić, T., Dimkić, I., Popović, T., Lozo, J., Fira, D., and Stanković, S. 2019. Biological control of *Pseudomonas syringae* pv. *aptata* on sugar beet with *Bacillus pumilus* SS-10.7 and *Bacillus amyloliquefaciens* (SS-12.6 and SS-38.4) strains. J. Appl. Microbiol. 126(1):165-176.

Nordox 75 WG. Environmental Protection Agency Reg. No. 48142-4. Monterey Chemical Co., Fresno, CA.

Nottingham, S. 2004. Beetroot. Archived from the original (e-book) on 2 Sep. 2012.

O'Brien, R. G., and Sparshott, P. E. 1999. Bacterial blight in Queensland beetroot crops. Austral. Plant Pathol. 28(2):182.

Organic Seed Disinfection ProBio gopure. (10 August 2023). Germains Seed Technology. https://germains.com/us/product/probio-gopure-organic-seed-disinfection/ (accessed 23 Sep. 2023).

Pethybridge, S. J., Kikkert, J. R., Hanson, L. E., and Nelson, S. C. 2018. Challenges and prospects for building resilient disease management strategies and tactics for the New York table beet industry. Agronomy 8(7):112.

Regalia Biofungicide. Environmental Protection Agency Reg. No. 84059-3. Marrone Bioinnovations, Davis, CA.

Rotondo, F., Vrisman, C. M., Rani, R., Testen, A. L., Deblais, L., and Miller, S. A. 2020. First report of *Pseudomonas syringae* pv. *aptata* causing bacterial leaf spot on common beet (*Beta vulgaris*) in Ohio. Plant Dis. 104(2):561.

Safni, I., Ramos-Sepulveda, L., Goldman, P., Derie, M., du Toit, L., Koike, S., Stockwell, V., and Bull, C. 2016. Genetic diversity of *Pseudomonas syringae* causing bacterial leaf spot on table beet (*Beta vulgaris*) and Swiss chard (*Beta vulgaris* subsp. *cicla*). Phytopathology 106:S4.143.

Scheck, H. J., and Pscheidt, J. W. 1998. Effect of copper bactericides on copper-resistant and -sensitive strains of *Pseudomonas syringae* pv. *syringae*. Plant Dis. 82(4):397-406.

Schneider, S., and Ullrich, W. R. 1994. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. Physiol. Mol. Plant Pathol. 45(4):291-304.

Schrader, W. L., and Mayberry, S. K. 2003. Beet and Swiss chard production in California. University of California Division of Agriculture and Natural Resources Publication 8096.

Serenade Opti. Environmental Protection Agency Reg. No. 264-1160. Bayer CropScience LP, St. Louis, MO.

Serenade Aso. Environmental Protection Agency Reg. No. 264-1152. Bayer CropScience LP, St. Louis, MO.

Sundin, G. W., Castiblanco, L. F., Yuan X., Zeng, Q., and Yang, C-H. 2016. Bacterial disease management: challenges, experience, innovation and future prospects. Challenges in bacterial molecular plant pathology. Mol. Plant Pathol. 17(9):1506-1518.

Trueman, C. L. 2015. Copper alternatives for management of bacterial spot (*Xanthomonas gardneri*) and bacterial speck (*Pseudomonas syringae* pv. *tomato*) in processing tomatoes. ActaHort. 1069:243-249.

United States Department of Agriculture National Agricultural Statistics Service. 2022. https://quickstats.nass.usda.gov/ (accessed 1 Sep. 2022).

Upper, C. D., Hirano, S. S., Dodd, K. K., and Clayton, M. K. 2003. Factors that affect spread of *Pseudomonas syringae* in the phyllosphere. Phytopathology 93:1082-1092.

Vechet, L., Burketova, L., and Sindelarova, M. 2009. A comparative study of the efficiency of several sources of induced resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat under field conditions. Crop Prot. 28(2):151-154.

Zhang, S., Fu, Y., Mersha, Z., and Pernezny, K. 2017. Assessment of copper resistance in *Pseudomonas syringae* pv. *phaseolicola*, the pathogen of halo blight on snap bean. Crop Prot. 98:8-15.

Table 3.1. Bactericide treatments used in a Swiss chard seed crop field trial each year from 2017 to 2021 to evaluate the efficacy of spray applications at reducing the amount of *Pseudomonas syringae* pv. *aptata* (*Psa*) detected on harvested seed

	Trial				
Treatment	**2017**	**2018**	**2019**	**2020**	**2021**
1	No chemical	No chemical	No chemical	No chemical	No chemical
2	ManKocide	ManKocide	Badge	Badge	Badge
3	Nordox 75WG	Nordox 75WG	Nordox 75WG	Nordox 75WG	Nordox 75WG
4	Cueva	Serenade Opti	Serenade Opti	Serenade Opti	Serenade Aso
5	KleenGrow + Cueva	Regalia	Regalia	Regalia	Regalia
6			Double Nickel LC	Double Nickel LC	Double Nickel LC
7					Lifegard WG
Action[a]	**Date completed**[a]				
Transplant	24 April	2 April	9 April	9 April	13 April
1st application[b]	19 May	29 May	14 May	19 May	29 May
2nd application	5 June	July[d]	20 May	26 May	7 June
3rd application	---	---	13 June	8 Jun	16 June
Psa inoculation[c]	22 June	1 June	28 May	1 Jun	18 June
Seed harvest	September[d]	7 September	11 September	1 September	17 September

[a] Dates that vernalized plants were transplanted into the field, chemical applications applied, *Psa* inoculations, and seed harvests.

[b] First application of a bactericide treatment via handpump backpack sprayer or CO_2-pressurized backpack sprayer.

[c] *Psa* inoculation using a Washington strain demonstrated to be highly virulent on beet seedlings.

[d] Exact date unknown.

Table 3.2. Bactericide treatments evaluated for control of *Pseudomonas syringae* pv. *aptata* (*Psa*) in Swiss chard seed crop field trials over five years for reducing the severity of bacterial leaf spot and the amount of *Psa* infection of the harvested seed

Product	Active ingredient	Formulation	FRAC code[a]	Mode of action[a]	Rate of product/ha
Badge[b]	Copper oxychloride 16.81% Copper hydroxide 15.36%	Suspension concentrate	M01	Multi-site contact activity; disrupt cellular proteins	2.24 kg
Cueva	Copper octanoate (copper soap) 10.0%	Flowable liquid	M01	Multi-site contact activity; disrupt cellular proteins	14.04 liters
Double Nickel LC	*Bacillus amyloliquefaciens* D747 25%	Liquid concentrate	BM02	Anti-fungal, anti-bacterial, growth promotion, host plant defense induction, competitive exclusion	2.47 liters
KleenGrow + Cueva	Didecyldimethylammonium chloride 7.5% + copper octanoate 10.0%	Not classified	N/A M01	Membrane disruption + multi-site contact activity; disrupt cellular proteins	1.83 liters + 14.04 liters
Lifegard WG	*Bacillus mycoides* Isolate J (*BmJ*) 40.0%	Water dispersible granules	P06	Host plant defense induction	0.316 kg
ManKocide	Manganese 3.0%, Zinc 0.4%, ethylenebisdithiocarbamate ion ($C_4H_6N_2S_4$) 11.6%, copper hydroxide 46.1%	Dry flowable	M01 M03	Multi-site contact activity; disrupt cellular proteins	2.79 kg
Nordox 75WG[b]	Cuprous oxide 83.9% (75% metallic copper)	Wettable granule	M01	Multi-site contact activity; disrupt cellular proteins	2.24 kg[c] 1.12 kg[d]
Regalia[b]	Extract of *Reynoutria sachalinensis* 5%	Micro-emulsion concentrate	P05	Host plant defense induction	1.17 liters
Serenade Aso	*Bacillus subtilis* strain QST 713 (1.34%)	Suspension concentrate	BM02	Stops fungal spores from germinating, disrupts cell membrane growth, inhibits attachment of pathogen to the leaf	9.34 kg
Serenade Opti	*Bacillus subtilis* strain QST 713 (26.2%)	Wettable powder	BM02	Stops fungal spores from germinating, disrupts cell membrane growth, inhibits attachment of pathogen to the leaf	1.12 kg

[a] FRAC code = Fungicide Resistance Action Committee code used to identify active ingredients which affect the same target site (https://www.frac.info/home).

[b] NuFilm 17 (spreader sticker) was added to three treatments at 1.169 liters/ha (1.4 ml/liter water).

[c] Rate used in 2017 and 2018 trials.

[d] Rate used in 2019, 2020, and 2021 trials.

Table 3.3. Analysis of variance (ANOVA) for bacterial leaf spot (BLS) severity, area under the disease progress curve (AUDPC), yield of harvested seed, thousand seed weight (TSW), seed germination, and colony forming units (CFU) of *Pseudomonas syringae* pv. *aptata* (*Psa*) per gram of harvested seed for a bactericide field trial conducted in a Swiss chard seed crop in western Washington each year from 2017 to 2021

Trial and dependent variable[a]	Normality test[b]	Test of variances[c]	R^2 [d]	CV[e]	F-test probability				Transformation[h]
					Replication	Bactericide treatment[f]	Inoculation treatment[g]	Inoculation x bactericide	
2017									
TSW[i]	0.9707	0.2767	0.42	7.07	0.8445	0.1364			
Psa CFU/g seed	0.7723	0.3480	0.62	23.83	0.4623	**0.0131**[k]			Square-root
2018									
TSW	0.8009	0.2557	0.77	10.74	0.0001	0.2290			
Psa CFU/g seed	0.6796	0.0253	0.72	17.54	0.0005	0.2467			Square-root
2019									
Psa CFU/g seed	0.2164	0.2905	0.56	5.89	0.0734	**0.0716**			
2020									
BLS severity[j]	0.1579	0.7073	0.41	21.03	0.0117	0.8144	0.9612	0.1852	
Seed yield	0.3283	0.5447	0.53	13.10	0.2103	**0.0309**	0.5121	**0.0094**	
TSW	0.2900	0.9220	0.37	8.75	0.0175	0.4665	0.4815	0.7216	
Germination	0.8872	0.9556	0.40	4.90	0.0216	0.8174	0.3855	0.1954	
Psa CFU/g seed	0.2748	0.2054	0.45	8.36	0.7454	**0.0764**	0.1308	**0.0507**	
2021									
BLS 22 June[j]	0.1307	0.9108	0.28	56.86	0.8110	0.5812	**0.0427**	0.5684	Square-root
BLS 30 June	0.2577	0.0730	0.39	86.64	0.2206	0.2653	**0.0512**	0.2586	
BLS 14 July	0.3279	0.0224	0.21	37.56	0.5726	0.5052	0.5264	0.8498	Log_{10}
BLS 27 July	0.3352	0.0881	0.35	47.22	0.0075	0.5020	0.6291	0.9561	
AUDPC	0.2334	0.0199	0.24	40.86	0.1129	0.8981	0.4229	0.8200	
Seed yield	0.0960	0.2095	0.39	28.58	0.0447	0.3795	0.1056	0.3758	
Germination	0.5808	0.6116	0.38	3.37	0.0340	0.7949	0.1408	0.2241	
Psa CFU/g seed	0.5248	0.6075	0.41	84.35	0.4031	0.6899	**0.0043**	**0.1048**	

[a] Each trial consisted of a randomized complete block design with four replications.

[b] Shapiro-Wilk test for normality of residuals, *P*-value.
[c] Levene's test for homogeneity of variances, *P*-value.
[d] R^2 = Coefficient of determination for the ANOVA.
[e] CV = Coefficient of variation for the ANOVA.
[f] Treatment = Bactericide treatments.
[g] Inoculation = Inoculation or no inoculation with a Washington strain of *Psa*.
[h] Transformation used to fulfill assumptions for parametric data analysis.
[I] Thousand seed weight.
[j] Severity of BLS symptoms, based on percentage of the canopy with symptoms.
[k] Bolded values represent statistically significant or marginally significant data.

Table 3.4. Bactericide field trials completed in 2017, 2018, and 2019 to evaluate the efficacy of foliar treatments to control bacterial leaf spot caused by *Pseudomonas syringae* pv. *aptata* (*Psa*) in Swiss chard seed crops

		Log_{10}CFU Psa/g seed[a]			Thousand seed weight (g)[b]		
Year	**Treatment[c]**	**Mean**	**LSD[d]**	***P*-value[e]**	**Mean**	**LSD**	***P*-value**
2017	Non-inoculated control	4.15 ab	5.81[f]	**0.0131**[k]	14.80 a	1.60	0.1364
	Inoculated control	4.48 b			14.92 a		
	Cueva	4.33 b			16.22 a		
	KleenGrow + Cueva	4.23 ab			14.02 a		
	ManKocide	2.93 a			15.56 a		
	Nordox	3.55 ab			14.79 a		
2018	Non-inoculated control	6.10 a	362.05[g]	0.2467	11.23 a	1.70	0.2290
	Inoculated control	6.25 a			9.88 a		
	ManKocide	6.22 a			10.23 a		
	Nordox	6.21 a			11.56 a		
	Regalia	6.25 a			10.26 a		
	Serenade	6.40 a			9.93 a		
2019	Non-inoculated control	4.75 a	0.46	**0.0716**			
	Inoculated control	5.51 b					
	Badge	5.12 ab					
	Double Nickel	5.18 ab					
	Nordox	5.28 ab					
	Regalia	5.35 ab					
	Serenade	5.25 ab					

[a] Colony forming units of *Psa* detected/g of seed harvested.

[b] Thousand seed weight (g) of the harvested seed.

[c] Foliar treatments used in each bactericide field trial.

[d] LSD = least significant difference, with separation based on the transformed data.

[e] *P*-value = probability that the given results would be seen if the null hypothesis were true.

[f] Means comparison was calculated using square root-transformed log_{10}*Psa* CFU/g seed, but original means are shown.

[g] Means comparison was calculated using the square root-transformed raw data for *Psa* CFU/g seed, but original means are shown.

[k] Bolded values represent statistically significant or marginally significant data.

Table 3.5. Bactericide field trial completed in 2020 to evaluate the efficacy of foliar treatments to control bacterial leaf spot (BLS) caused by *Pseudomonas syringae* pv. *aptata* (*Psa*) and to reduce the amount of *Psa* infection on the harvested seed in a Swiss chard seed crop[a]

Inoculation treatment[b]	Bactericide treatment[c]	Mean BLS severity (%)[d]	Seed yield (g/plant)	Thousand seed weight (g)	Seed germination (%)	Log_{10} CFU *Psa*/g seed[e]
Inoculation main effect						
Inoculated		28.04	207.3	12.67	91.1	5.67
Non-inoculated		27.96	202.2	12.44	89.9	5.46
	LSD	3.46	15.8	0.65	2.6	0.27
	P-value	0.9612	0.5121	0.4815	0.3855	0.1308
Bactericide main effect						
	Control	26.63	222.7 a	12.93	90.6	5.17
	Badge	28.75	215.6 ab	12.12	89.2	5.69
	Double Nickel	29.13	200.6 ab	12.72	91.4	5.70
	Nordox	26.00	215.3 ab	12.66	90.3	5.86
	Regalia	28.13	194.0 ab	12.05	89.6	5.43
	Serenade Opti	29.38	180.4 b	12.84	91.9	5.54
	LSD	5.99	27.3	1.12	4.5	0.47
	P-value	0.8144	**0.0309**[f]	0.4665	0.8174	**0.0764**
Interaction						
Non-inoculated	Control	25.75	238.3 a	12.78	90.6	4.65 a
	Badge	33.75	188.3 ab	11.78	87.4	5.82 b
	Double Nickel	29.50	208.9 ab	12.72	88.1	5.38 ab
	Nordox	23.75	231.3 ab	13.04	91.9	5.88 b
	Regalia	26.25	209.0 ab	11.92	88.1	5.56 ab
	Serenade Opti	28.75	168.2 b	12.39	93.5	5.47 ab
Inoculated	Control	27.50	207.0 ab	13.08	90.6	5.68 ab
	Badge	23.75	242.9 a	12.46	91.0	5.56 ab
	Double Nickel	28.33	185.2 ab	12.57	95.2	6.01 b
	Nordox	28.25	199.4 ab	12.27	88.6	5.84 b
	Regalia	30.00	178.9 ab	12.17	91.1	5.30 ab
	Serenade Opti	30.00	192.7 ab	13.30	90.4	5.62 ab
	LSD	8.66	39.7	1.61	6.6	0.69
	P-value	0.1852	**0.0094**	0.7216	0.1954	**0.0507**

[a] The trial entailed a 6 x 2 factorial treatment design with inoculation treatments and bactericide treatments as the independent variables. Means are listed for each dependent variable by treatment, including the least significant difference (LSD) and P-value.

[b] Inoculation with a local, highly virulent strain of Psa.

[c] Bactericide treatments applied.

[d] BLS severity, measured as a percentage of the canopy with symptoms.

[e] Colony forming units (CFU) of Psa detected/g seed via a seed wash of a sample of seed harvested from each plot.

[f] Bolded values represent statistically significant or marginally significant data.

Table 3.6. Bactericide field trial completed in 2021 to evaluate the efficacy of foliar treatments to control bacterial leaf spot (BLS) caused by *Pseudomonas syringae* pv. *aptata* (*Psa*) and to reduce the amount of *Psa* infection on the harvested seed in a Swiss chard seed crop[a]

		Mean BLS severity ratings (%)[c]							
Inoculation treatment[b]	**Bactericide treatment**	**22 June**	**30 June**	**14 July**	**27 July**	**AUDPC[d]**	**Seed yield (g/plant)**	**Seed germination (%)[e]**	**Log_{10} CFU *Psa*/g seed[f]**
Inoculation main effect									
Non-inoculated		0.10 a	0.20 a	0.88	2.53	35.62	107.9	90.3	1.07 a
Inoculated		0.16 b	0.32 b	0.95	2.70	38.92	122.5	89.1	2.19 b
	LSD	0.07[g]	0.12	0.24[h]	0.67	8.23	17.8	1.6	0.74
	P-value	**0.0428**	**0.0512**	0.5265	0.6291	0.4229	0.1056	0.1408	**0.0043**
Bactericide main effect									
	Control	0.08	0.24 ab	1.06	3.00	39.38	103.8	90.6	1.89
	Badge	0.19	0.35 ab	0.88	2.00	35.98	136.2	90.8	0.99
	Double Nickel	0.08	0.14 a	1.13	3.00	39.13	108.4	88.7	2.11
	Lifegard WG	0.14	0.25 ab	1.00	3.06	41.52	126.7	90.1	1.90
	Nordox	0.15	0.41 b	0.88	2.50	38.45	107.4	89.1	1.42
	Regalia	0.15	0.24 ab	0.69	2.25	32.37	106.2	89.4	1.76
	Serenade Aso	0.14	0.20 ab	0.76	2.50	34.11	117.5	89.6	1.34
	LSD	0.18[g]	0.23	0.10[h]	1.25	15.40	33.3	3.1	1.39
	P-value	0.5812	0.2653	0.5056	0.5020	0.8981	0.3795	0.7949	0.6899
Interaction									
Non-inoculated	Control	0.05	0.28	1.25	3.50	44.60	109.0	93.4	1.66
	Badge	0.28	0.18	0.88	2.00	37.46	123.8	91.9	0.00
	Double Nickel	0.05	0.08	1.00	2.90	34.96	109.1	88.9	1.81
	Lifegard WG	0.10	0.30	0.88	3.00	38.51	95.7	91.3	2.58
	Nordox	0.10	0.30	0.88	2.50	35.26	102.8	89.6	0.55
	Regalia	0.08	0.08	0.63	2.25	26.81	96.0	88.9	0.91
	Serenade Aso	0.08	0.20	0.65	2.75	31.78	119.0	88.5	0.00
Inoculated	Control	0.10	0.20	0.88	2.50	34.16	98.7	87.8	2.11
	Badge	0.10	0.53	0.88	2.00	34.49	148.6	89.6	1.98
	Double Nickel	0.10	0.20	1.25	3.13	43.29	107.8	88.5	2.41
	Lifegard WG	0.18	0.20	1.13	3.13	44.53	157.8	88.9	1.22
	Nordox	0.20	0.53	0.88	2.50	41.64	112.0	93.4	2.29
	Regalia	0.23	0.40	0.75	2.25	37.93	116.5	89.9	2.61
	Serenade Aso	0.20	0.20	0.88	2.25	36.44	116.1	90.8	2.69

LSD	0.26[g]	0.32	0.15[h]	1.77	21.78	47.1	4.3	1.97
P-value	0.5682	0.2586	0.8499	0.9561	0.8200	0.3758	0.2241	**0.1048**

[a] The trial entailed a 7 x 2 factorial treatment design with inoculation treatments and bactericide treatments as the independent variables. Means are listed for each dependent variable by treatment, including the *P*-value.

[b] Inoculation with a local, highly virulent strain of *Psa.*

[c] Rating of BLS severity as a percentage of the canopy with symptoms.

[d] Area under the disease progress curve calculated based on the BLS ratings.

[e] Seed germination assay results: percentage of seeds that germinated normally.

[f] Colony forming units (CFU) of *Psa* detected/g seed via a seed wash of a sample of seed harvested from each plot.

[g] Means comparison was calculated using square root-transformed data, but original means are shown.

[h] Means comparison was calculated using $\log_{10}$ transformed data, but original means are shown.

Fig. 3.1. 2020 Field trial evaluating bactericide treatments for control of bacterial leaf spot (BLS) in a Swiss chard seed crop. **A)** Vernalized seedlings after transplanting. **B)** Visible herbicide carryover damage to plants of a male parent line (left) vs. female plants (right). **C-D)** Female plants setting seed. **E-F)** BLS symptoms on leaves.