BACTERIOPHAGE AS A POTENTIAL BIOLOGICAL CONTROL FOR BACTERIAL CANKER OF SWEET CHERRY

By

Lindsay E. Brown

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Plant Pathology – Master of Science

2023

ABSTRACT

Bacterial canker is a significant disease of sweet cherry in Michigan. While disease severity is highly variable between years, symptoms of the disease, especially blossom blast, can significantly reduce yield and requires control measures. Given the current lack of effective and economically viable treatments, bacteriophages are being explored as a potential control. Three bacteriophages were isolated from Michigan cherry orchard soil samples and characterized via *in vitro* and *in situ* experiments. This study had two primary objectives in developing effective field treatments: (1) determining if native Michigan bacteriophages are better able to control *Pseudomonas syringae* pv. syringae isolates from Michigan cherry orchards than a nonspecific commercial product and (2) investigating field treatment modifications designed to reduce bacteriophage UV exposure thereby improving the efficacy of the treatments. The Michigan bacteriophages were determined to lyse significantly more Michigan cherry orchard *P. s.* pv. syringae isolates than the commercial bacteriophage product *in vitro*. The field treatments designed to reduce UV exposure had a limited impact on the bacterial populations. However, significant reduction in *P. s.* pv. syringae populations was observed during a field trial conducted during a period of lower UV radiation, indicating that UV radiation exposure is a primary limiting factor in bacteriophage efficacy. This is supported by the significant decline in bacteriophage populations exposed to UV-C *in vitro*. These results indicate that if UV exposure is mitigated and the host range is well suited to the environment, bacteriophage could be a useful biocontrol.

This thesis is dedicated to the people who have made Michigan a home.

ACKNOWLEDGEMENTS

The author would like to thank committee members Dr. George Sundin, Dr. Timothy Miles, and Dr. Nikki Rothwell; fellow Sundin-ites both present and past including Erin Lauwers, Dr. Kristi Gdanetz-MacCready, Dr. Xiaochen Yuan, Dr. Roshni Kharadi, Dr. Tyre Proffer, Cory Outwater, Katherine Olive, Madison Dobbins, Jared Zaporski, and Rose Kithan; the wonderful people at the NWMHRC for their support and resources; her loved ones for their constant support; and the following sources of funding – Michigan Cherry Committee and Project GREEEN.

TABLE OF CONTENTS

CHAPTER 1:

LITERATURE REVIEW OF BACTERIOPHAGE APPLICATION FOR BIOLOGICAL CONTROL OF PLANT DISEASES

1.1 Bacteriophages as a Biological Control

Bacteriophages are widely dispersed environmental viruses that utilize bacteria as their hosts. The literally termed "bacteria eaters" coexist with bacteria in all environments from hot springs to oceans to soil (Keen 2015). First discovered in 1915, bacteriophages are the most abundant lifeform on the planet at an estimated population of 10^{31} phage particles (Comeau et al. 2008). Bacteriophages were initially investigated as a therapeutic control measure for bacteria. In the 1920s, phytobacteriology work conducted on cabbage rot caused by *Xanthomonas campestris* pv. campestris and potato blackleg disease caused by *Pectobacterium carotova* subsp. *atroseptica* showed promising results (Villalpando-Aguilar et al. 2023). This line of research was swiftly overtaken in the West by the rise of antibiotics in the twentieth century (Keen 2015). As antibiotic resistance has become a more prevalent issue in medicine and in agriculture, there has been a resurgence of interest in bacteriophages as a therapeutic alternative for control of bacteria in medical and agricultural settings (Sundin & Wang 2018; Svircev, Roach & Castle 2018). With the richness in abundance and diversity of bacteriophages in the environment, these viruses represent a potential resource that needs further exploration.

Bacteriophages present unique opportunities and challenges as a biological control agent. The lytic life cycle of bacteriophages is crucial as these bacteriophages can both reduce bacterial populations and replicate to maintain an effective population (Abedon 2018). Additionally, bacteriophage strains have high specificity to their bacterial hosts; this narrow host range prevents off target impacts on the environmental microbiome (Hyman & Abedon 2010). This

restricted host range can also prevent bacteriophages from effectively engaging with the diversity of pathogenic bacteria in an agricultural setting. Additionally, bacteriophages are particularly sensitive to environmental degradation caused by UV exposure, temperature and pH extremes, and desiccation. In an agricultural context, bacteriophages are most frequently applied to the phyllosphere which is highly exposed to these environmental stressors (Born et al. 2015; Iriarte et al. 2007). Understanding and addressing these challenges is necessary to take full advantage of the potential bacteriophages offer.

1.2 Bacteriophage Life Cycle

Bacteriophages are specifically of interest for biological control due to their ability to lyse their bacterial hosts and therefore reduce bacterial populations while increasing their own population. However, this lysis is not a ubiquitous step in all bacteriophage life cycles. Bacteriophage life cycles largely can be divided into two categories: lytic and lysogenic. Lytic life cycles are a required aspect of a successful biological control agent. Within a lytic life cycle, a bacteriophage will attach to a susceptible bacterium, insert genetic material, replicate within the host, assemble new virions, and lyse the host cell to release the mature bacteriophages. In lysogenic life cycles, the genetic material of the bacteriophage can be integrated into the chromosome of the host bacterium. The bacteriophage is then passively replicated with the bacterium as a prophage. While this lysogenic stage can switch to a lytic cycle and cause cell death, this is undependable and prevents lysogenic bacteriophages from being utilized as a biological control (Hobbs & Abedon 2016). Additionally, lysogenic bacteriophages can transduce mobile genetic elements and virulence factors into their hosts. Prophages can directly introduce novel genetics as they integrate into bacterial chromosomes (Abedon & LeJeune 2005). For example, biosynthetic genes encoding toxins, including botulism toxin and Shiga

toxin, are encoded within prophage genomes; these toxins are virulence factors of note as they can have direct repercussions on human health (Brüssow et al. 2004). Limiting the spread of these virulence factors, especially toxins, is crucial in developing a biological control agent for phytobacteriology applications. As such, lysogenic bacteriophages are not suited for this use.

The bacteriophage lytic capacity can be evaluated *in vitro* by plating host bacteria with a bacteriophage isolate on agar plates. As the bacteriophages lyse their host cells, they generate small, circular zones free of bacteria called plaques. These plaques can range from <0.5 mm to 7 mm depending on the bacteriophage morphology and the bacterial host interaction (Jurczak-Kurek et al. 2016). Completely clear plaques are associated with lytic bacteriophage isolates whereas opaque plaques indicate the presence of living bacteria within the plaque. Bacteriophage isolates selected from environmental samples for biological control applications must produce clear lytic plaques (Abedon 2018).

1.3 Host Range

The host range is defined as the bacterial strains a given bacteriophage isolate can productively infect, typically assessed by counting plaques (Hyman & Abedon 2010). Bacteriophages usually have a quite narrow host range, even within a particular bacterial species or pathovar. While the narrow host range does limit the potential for off target environmental impacts associated with field application, it can constrain the ability of bacteriophage treatments to successfully interact with the bacterial diversity found in a field setting. This limitation makes a wide host range a required component of a successful biological control. There are two primary steps that can be taken to overcome this restriction: geographic locality of isolation and cocktail development. The host range of bacteriophages has been shown to be associated with their isolation locations (Gayder et al. 2019). This indicates that lytic bacteriophage isolates from a

specific region are better suited to interact with and lyse bacterial strains from that same region. Utilizing multiple bacteriophage isolates with different host ranges in a cocktail formulation can also expand the overall host range and improve the efficacy of the bacteriophage treatment (Kering et al. 2019). Moreover, the use of multiple bacteriophage isolates in a cocktail can limit the development of bacteriophage-resistant bacterial populations which allows for the continued usage of bacteriophage treatments in agricultural systems (Kering et al. 2019).

1.4 Environmental Degradation

Environmental degradation is a primary barrier to the efficacy of bacteriophages in biological control applications. Bacteriophages must maintain a high titer after application to effectively control the target bacteria in the phyllosphere. This poses a unique challenge given that the canopy has a highly variable environment with fluctuating moisture, temperature, pH, and UV exposure (Xu et al. 2022). Bacteriophages are very sensitive to these environmental variables, particularly UV exposure which damages the bacteriophage genetic material (Born et al. 2015; Feng et al. 2003; Iriarte et al. 2007).

There are two predominant strategies that have been utilized to mitigate UV degradation: application formulation and application timing. Applying bacteriophage treatments in the evening can reduce initial UV exposure compared to midday bacteriophage application (Jones et al. 2007; Balogh et al. 2003). Protective additives can provide a physical barrier to mitigate UV exposure; experimental formulations include vegetable extracts, kaolin clay, casein, pregelatinized corn flour, and peptone (Balogh et al. 2003; Born et al. 2015).

Another recent strategy to increase bacteriophage populations *in vivo* and to overcome environmental degradation is the use of carrier bacteria in field applications. These carrier bacteria are nonpathogenic epiphytic indigenous bacterial strains within the host range of the

bacteriophage isolates that are being utilized for biocontrol. The use of carrier bacteria both ensures that the bacteriophage have more consistent access to host bacteria and limits initial environmental exposure, thus supporting bacteriophage population stability (Gayder et al. 2020). This strategy has been investigated in two studies by Agriculture and Agri-Food Canada. Gayder et al. (2020) combined bacteriophage isolates with *Pantoea agglomerans*, a nonpathogenic bacterial species commonly found in the apple phyllosphere, to control for *Erwinia amylovora* – the causal pathogen of fire blight. This carrier bacteria approach is specifically useful in the fire blight pathosystem because *P. agglomerans* is antagonistic to *E. amylovora* and was even used as an active ingredient in the commercial biocontrol Bloomtime (Pusey et al. 2011). As such, the carrier bacteria can serve the two-fold purpose of benefitting bacteriophage populations and reducing pathogenic bacterial populations. Boulé et al. (2011) also investigated a combination of bacteriophage isolates and *P. agglomerans* for the control of *E. amylovora*. Two bacteriophage isolates applied with the carrier bacteria to detached pear flowers reduced disease severity by 84 % and 96 %, respectively, when compared to a phosphate buffer control (Boulé et al. 2011). Application of the carrier bacterial strain independently of bacteriophage also resulted in a significant decrease in disease severity. These results were corroborated in a greenhouse experiment where disease severity was reduced by 56 % on bacteriophage-treated apple flowers on potted trees. (Boulé et al 2011). These findings support the usage of carrier bacteria in phytobacteriology applications. While further work in field settings is necessary to confirm the impact of carrier bacteria on the environmental degradation of bacteriophages, it presents a strong direction for bacteriophage research.

1.5 Field Research with Bacteriophage

The development of practical bacteriophage treatments is dependent on field experiments that can directly evaluate the strategies discussed above. However, the majority of phytobacteriology centered bacteriophage work has been conducted exclusively in a lab setting. There is a frequent schism in bacteriophage research wherein bacteriophage isolates that seem highly effective in a lab setting do not produce significant results when evaluated in a field setting. To bridge that divide and move towards the development of better bacteriophage treatments for phytobacteriology needs, the work that has been conducted in a field setting must be considered as a basis for future experimental design.

In 1970, populations of *Xanthomonas pruni,* causal agent of bacteria spot of peach, were significantly reduced on peach foliage with bacteriophage treatments (Civerolo 1970). Further work in this pathosystem was conducted in 1993, with reduced disease incidence observed in all three orchards treated with bacteriophage, but only one had a significant reduction (Saccardi et al. 1993). This article acknowledged the lack of current information on bacteriophage survival in a field setting; an issue that was revisited by the University of Florida phytobacteriology laboratory.

Balogh et al. (2003) examined protective formulations to enhance bacteriophage survival in the phyllosphere. Their pathosystem of interest was *X. campetris* pv. vesicatoria on tomato plants. They specifically investigated casecrete, skim milk, and pregelatinized corn flour additions to the bacteriophage mixtures and found that these additives significantly increased both bacteriophage population longevity and treatment efficacy in a field setting. This study also identified that application of bacteriophage treatments in the evening resulted in better control than morning treatments (Balogh et al. 2003). Despite the ability of skim milk to contribute to

bacteriophage survival in the phyllosphere, it was shown to increase the disease severity of *X. axonopodis* pv. citri and pv. citrumelo on *Citrus sinensis* cv. 'Valencia'. This may be due to the skim milk breaking surface tension on the leaves and aiding pathogen motility or acting as a nutrient source for the pathogen (Balogh et al. 2008). Thus, the identification of protective formulations that both benefit bacteriophage field longevity and do not positively impact pathogen survival is critical to the development of practical bacteriophage treatment options.

Another strategy for bacteriophage treatments in a phytobacteriology context is pretreatment of plant material. McKenna et al. (2001) treated *Streptomyces scabies* infested seed potatoes with a 24-hr bacteriophage soak prior to planting and observed a significant reduction in infected tubers from 23 % to 1.2 % infection. An additional study on potato focused on *Dickeya solani* indicated that bacteriophage-treated tubers had a 13 % increase in yield compared to the infected control and significantly less rotted tissue per tuber (Adriaenssens et al. 2012). Of note, the bacteriophage-treated plant material was immersed in soil in these studies. When in soil, bacteriophages have reduced sun exposure and can adsorb to the soil particles which can also serve to benefit their population stability (Bitton 1975; Iriarte et al. 2007). In 2016, Rombouts et al. investigated bacteriophage pretreatments for the control of the foliar pathogen *P. syringae* pv. porri on leeks. This study had variable results with one field trial associated with symptom reduction (Rombouts et al. 2016). While further studies on this are required, pretreatment of plant material is a promising avenue of research for phytobacteriology applications. Specifically, bacteriophage seed treatments are of current interest in several pathosystems (Rahimi-Midani et al. 2020; Tarakanov et al. 2022; Kimmelshue, Goggi, & Cademartiri 2019; Cemen et al. 2018; Adachi et al. 2012). However, these studies are lab based and must be furthered in a field context to fully understand the potentials associated with bacteriophage seed treatments.

1.6 Bacteriophages and Bacterial Canker Control

Pseudomonas syringae is a globally distributed, economically significant bacterial plant pathogen. There is a diverse array of pathovars within *P. syringae* adapted to different hosts and climatic factors. While each pathovar has a distinct host range, collectively, they form an overlapping continuum that can infect hundreds of species of herbaceous and woody plants (Kennelly et al. 2007). Currently, more than 60 pathovars of *P. syringae* have been identified (Young 2010). The *P. syringae* species complex can cause a variety of symptoms on host plants, including devastating cankers on susceptible woody plant species such as horse chestnut, kiwi, and cherry.

Pseudomonas syringae infections can damage leaves, flowers, and fruit in addition to the namesake cankers. While the damage to reproductive structures and leaves can reduce annual yields and photosynthetic capacity, cankers can cause permanent harm to the trees. The cankers and its associated gummosis damage the vasculature of the trees and cause girdling. This will effectively limit any movement of nutrients and kill portions of the tree acropetal to the cankers. If cankers form around tree trunks, this can cause outright tree death.

Horse chestnut bleeding canker, caused by *Pseudomonas syringae* pv. aesculi, is a developing epidemic in Northwestern Europe that is decimating horse chestnut populations. The primary treatment for diseased trees is currently eradication. Bacteriophage isolates associated with *P. s.* pv. aesculi have been identified for further assessment (James et al. 2020). As such, bacteriophage treatments have the potential to become a tool for bleeding canker management.

Pseudomonas syringae pv. actinidiae, the causal agent of bacterial canker of kiwifruit, is a globally distributed pathogen that has been a particularly devastating issue in New Zealand. The outbreak in New Zealand was exacerbated by copper-resistant bacterial populations which

limited chemical control options. While the New Zealand industry has moved towards a disease tolerant variety of kiwifruit, more tools to control *P. s.* pv. actinidiae are needed globally (Vanneste 2017). This has led to a focus on the development of novel bacteriophage treatments. Several studies have identified bacteriophage isolates for further research in controlling *P. s.* pv. actinidiae (Song et al. 2021; Flores et al. 2020; Pinheiro et al. 2020; Liu et al. 2021; Martino et al. 2021; Di Lallo et al. 2014; Yu et al. 2016; Park et al. 2018; Frampton et al. 2014; Ni et al. 2021; Ni et al. 2020; Bai et al. 2021). However, these studies were conducted *in vitro*. Expansion into field experiments is necessary to fully evaluate the selected bacteriophage isolates.

P. s. pv. syringae is the primary causal agent of bacterial canker in sweet cherry globally. In addition to canker development, this bacterial pathogen causes blossom blast which can devastate annual yields. Blossom blast also creates an opportunity for the bacteria to become endophytic and potentially cause cankers. As such, controlling bacterial growth on the flowers is critical. This pathosystem is well suited for bacteriophage treatments. As the primary window of control is during bloom, the bacteriophages do not need to persist long term in the field which is beneficial to the overall success of the treatments. *P. s.* pv. syringae is also a well dispersed epiphyte throughout the canopy which allows the bacteriophages being applied to the phyllosphere to have better odds of interacting with host bacteria (Xin et al. 2018). While this disease has historically been controlled with copper in Michigan, the development of copper-resistant bacterial populations has complicated disease management practices (Renick et al. 2008). Kasugamycin, an antibiotic able to control *P. s.* pv. syringae, has more recently been approved for use on cherries in the U.S.A. and is highly effective at reducing blossom blast symptoms (Lillrose et al. 2017). However, regular use of antibiotics can lead to the development

of antimicrobial resistance. This has led to a research focus on bacteriophage treatments specific to *P. s.* pv. syringae on sweet cherry (*Prunus avium* L.).

Akbaba & Ozaktan (2021) investigated bacteriophage isolates specific to this pathosystem in Turkey. The study showed that four of six total bacteriophage treatments reduced over 50 % of disease incidence on micropropagated cherry plantlets in a growth chamber setting (Akbaba & Ozaktan 2021). Based on this preliminary work with plant tissue, another study focused on field treatments can confirm the efficacy of these bacteriophage isolates.

In another study focused on this pathosystem, Rabiey et al. (2020) isolated and examined 70 bacteriophage samples and further tested the bacterial control capacity of 13 individual isolates as well as two cocktails on plant tissues. This study investigated the relationship between bacteriophages and *P. s.* pv. syringae on cherry leaves and cherry twigs. The bacteriophage isolates and cocktails significantly reduced bacterial populations on the cherry leaves for five weeks. Similarly, the bacteriophage treatments significantly reduced bacterial populations in the cherry twigs. These experiments were conducted in a growth chamber setting (Rabiey et al. 2020). Further work in this area can include field experiments and flower specific experiments to better assess the potential of bacteriophage treatments in this pathosystem. That specific area of research will be expanded upon in Chapter Three. For more information on *P. s.* pv. syringae and its interaction with *Prunus* spp., please refer to Chapter Two.

1.7 Future Work

Bacteriophages present a strong potential alternative to current phytobacteriology control methods like antibiotics and copper. That possibility is driving a revitalization of agricultural-focused bacteriophage research. To realize this potential, lytic bacteriophage treatments with an appropriately wide host range to control for the indigenous bacterial genetic diversity must be

generated. Additionally, these treatments must be formulated to promote bacteriophage population endurance. By prioritizing these factors, the challenges associated with translating bacteriophage treatments to the field setting can be addressed.

CHAPTER 2:

BACTERIAL CANKER OF CHERRY: DIAGNOSTIC GUIDE

2.1 Characterization of Disease

Hosts. The cherry bacterial canker disease complex is composed of six *Pseudomonas* spp. clades. Five of these *Pseudomonas* spp. are specific to fruit trees: *Pseudomonas amygdali* pv. morsprunorum, *Pseudomonas avellanae* pv. morsprunorum, *Pseudomonas syringae* pv. avii, *Pseudomonas syringae* pv. cerasicola, and *Pseudomonas cerasi* (Ménard et al. 2003; Kamiunten et al. 2000; Kałużna et al. 2016b). Contrastingly, *Pseudomonas syringae* pv. syringae has an exceptionally broad host range of over 180 plant species spanning diverse regions and growth habits (Bradbury 1986).

Disease. *Pseudomonas* spp. infections of *Prunus* spp. cause bacterial canker. This disease is also commonly known as blossom blast, spur blight, twig blight, or dieback depending on the apparent symptoms (Heimann & Hudelson 2004).

Pathogen. *Pseudomonas syringae* pv. syringae was first described as a lilac pathogen in 1902 by Van Hall (Young 2010). *Pseudomonas syringae* pv. morsprunorum was classified as a pathovar of *Pseudomonas syringae* in 1931 by Wormald (Young 1978). After the discovery of a variant *Pseudomonas syringae* pv. morsprunorum in 1975, the pathovar was divided into two races. Race 1 aligns with the original description of the pathovar and race 2 is associated with the variant (Freigoun & Crosse 1975). More recently, *Pseudomonas syringae* pv. morsprunorum race 1 has been reclassified as *Pseudomonas amygdali* pv. morsprunorum and *Pseudomonas syringae* pv. morsprunorum race 2 has been reclassified as *Pseudomonas avellanae* pv. morsprunorum (Bull et al. 2010). *Pseudomonas cerasi* Griffin was originally described in 1911, later grouped into *P. s.* pv. syringae in 1975, and finally redefined as a unique species in 2016 as

P. cerasi non Griffin (Kałużna et al. 2016b; Iličić et al. 2021). *P. s.* pv. cerasicola was designated as a causal agent of cherry bacterial canker in 2000 (Kamiunten et al. 2000). *P. s.* pv. avii was found to be pathogenic in wild cherry and some sweet cherry cultivars in 2003 (Ménard et al. 2003). *P. s.* pv. syringae, *Pseudomonas amygdali* pv. morsprunorum, and *Pseudomonas avellanae* pv. morsprunorum are the three most common pathovars.

Taxonomy. Kingdom Bacteria, phylum Proteobacteria, class Gammaproteobacteria, order Pseudomonadales, family Pseudomonadaceae, genus *Pseudomonas*, species *Pseudomonas* spp. Updated taxonomy can be found at the NCBI Taxonomy database (Schoch et al. 2020).

Symptoms and Signs. Epiphytic populations of *Pseudomonas* spp. fluctuate throughout the year; bacterial growth is favored by the cooler, wet weather during the spring and fall and decreases in the summer (Sundin, Jones, & Olson 1988; Latorre et al. 1985). Leaf drop in the fall provides an infection court for the elevated bacterial populations to enter the vasculature and infect adjacent buds. Cold temperatures during leaf fall are associated with successful endophytic colonization in *P. avium*. (Iličić et al. 2021). The overwintering endophytic population can cause cankers in the branches, dead buds/spurs, and generate primary inoculum on asymptomatic blooms the following spring (Renick et al. 2008).

The primary inoculum can then cause blossom blast (Figure 2.1) which both reduces the fruit cropping capacity of the tree and provides another opening for epiphytic populations to migrate into the trees. Blossom blast is particularly exacerbated in cool, wet spring weather (Kennelly et al. 2007). Other symptoms of bacterial canker include fruit and leaf lesions. Lesions on the fruit begin with a water-soaked appearance before transitioning into a dried chocolate brown color with the lesion encompassing the fruitlet and extending up the peduncle. This disease progression is pictured in Figure 2.3. Leaf lesions begin as small chlorotic regions on the

leaf which become defined necrotic lesions that drop out of the leaf leaving behind the characteristic “shot hole” appearance (Latorre & Jones 1979) (Figure 2.2).

Endophytic colonization of the vasculature can lead to dark, sunken cankers on scaffold branches and even the main leader. These cankers can range from small lesions which reduce the productive capacity of the tree to large, girdling lesions that can significantly impact acropetal tree health and result in tree death (Kennelly et al. 2007). Canker formation is often associated with gummosis. Gummosis is an orange-brown, polysaccharide-based exudate produced as a phytohormone-mediated stress response to numerous tree stressors including bacterial infection (Saniewski et al. 2006). Even though gummosis is a nonspecific stress symptom, it is seen as a typic symptom of *Pseudomonas* spp. infection when present in conjunction with cankers. While cankers can form because of fruit or leaf lesions spreading into the vasculature as shown in Figure 2.4, cankers can also form after infection via frost damage, pruning wounds, or insect damage (Young 1987).

Figure 2.1. Blossom blast symptoms on *P. avium* L. cv. ‘Ulster’. Petal browning and floral necrosis associated with *P. s.* pv. syringae infections. (Lauwers 2022).

Figure 2.2. *P. s.* pv. syringae associated leaf lesion symptoms on *P. avium* L. cv. 'Ulster'. Symptom development shown includes initial area of necrosis, the infected region dropping out of the leaf, and the resulting 'shot hole' symptom. Imaged with Keyence VHX-600.

Figure 2.3. Range of Pseudomonas spp. symptoms on *P. avium* L. cv. 'Ulster' fruitlets. Lesions begin as small, dark, and water soaked before expanding across the fruitlet resulting in dry, brown lesions encompassing the fruitlet. Imaged with Keyence VHX-600 at 50x.

Figure 2.4. Bacterial canker on a *P. avium* L. cv. 'Ulster' twig. Cankers are associated with sunken and shredded bark as well as black discoloration. Dried gummosis is also visible in this figure. Imaged with Keyence VHX-600.

The diversity of bacterial canker symptoms intersects with other disease symptoms associated with *Prunus* spp. These potential overlaps should be monitored during pathogen identification. Table 2.1 is a nonexhaustive list of these similar symptoms associated with *Prunus* spp. In addition to the pathogens below, blossom blight can appear similar to frost damage (Kennelly et al. 2007).

Table 2.1. Alternative causes of bacterial canker of *Prunus* spp. symptoms.

Bacterial Canker Symptoms	Alternative Causal Pathogen	Citation
Blossom Blight	*Monolinia spp.*	(Oliveira Lino et al. 2016)
	Xanthomonas arboricola pv. pruni	(Garita-Cambronero et al. 2018)
Leaf Lesions	*Xanthomonas arboricola pv. pruni*	(Garita-Cambronero et al. 2018)
	Blumeriella jaapii	(Andersen et al. 2018)
Fruit Lesions	*Xanthomonas arboricola pv. pruni*	(Garita-Cambronero et al. 2018)
Spur Dieback	*Monolinia spp.*	(Oliveira Lino et al. 2016)
	Xanthomonas arboricola pv. pruni	(Garita-Cambronero et al. 2018)
Canker	*Xanthomonas arboricola pv. pruni*	(Garita-Cambronero et al. 2018)
	Leucocytospora spp.	(Spotts et al. 1990)

Disease Cycle. Photographic representation of the annual disease stages (Figure 2.5).

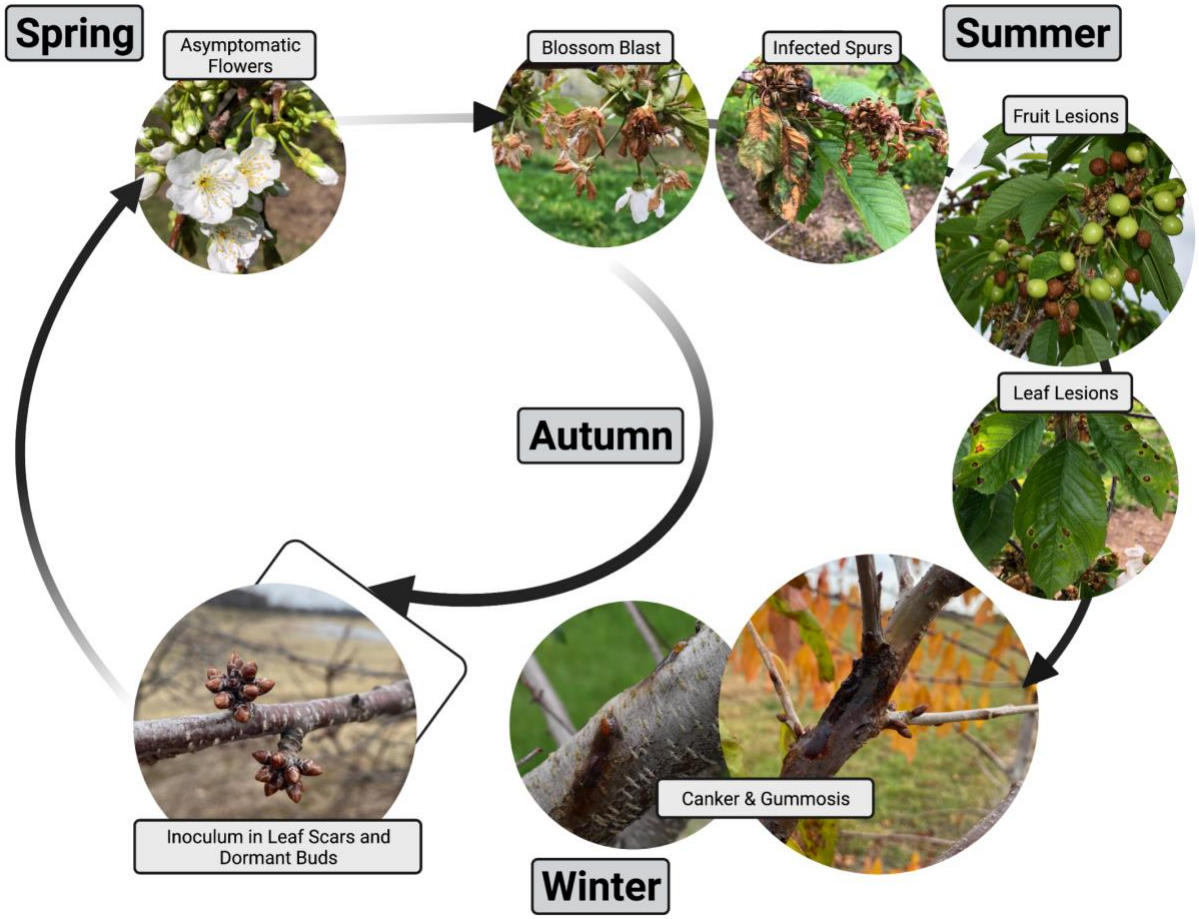

Figure 2.5. Disease cycle of bacterial canker of cherry. Overwintering inoculum in the canopy can result in infections of flowers, spurs, fruit, and leaves under conducive conditions. This allows for endophytic colonization resulting in the development of cankers. Images from Lindsay Brown, George Sundin, Erin Lauwers, and Katherine Olive. Created with Biorender. Primarily based on Kennelly et al. 2007.

Host Range. Six clades of *Pseudomonas* spp. are known to infect *Prunus* spp. with each pathovar showing specificity to differing cherry species. *P. s.* pv. syringae primarily infects *P. avium* L. (Jones 1971). *Pseudomonas amygdali* pv. morsprunorum and *Pseudomonas avellanae* pv. morsprunorum are most closely associated with *Prunus cerasus* L. in Michigan (Tart cherry) (Latorre & Jones 1979). *P. cerasi* has primarily been found on *P. cerasus* L. cultivars, although it has also been associated with *P. avium* L. (Kałużna et al. 2016a). *P. s.* pv. avii was found in wild cherry populations as well as *P. avium* L. (Ménard et al. 2003). *P. s.* pv. cerasicola has been shown to infect the ornamental *Prunus* × *yedoensis* (Kamiunten et al. 2000). While these associations between *Pseudomonas* spp. and *Prunus* spp. do not preclude other infections, they have been demonstrated as general trends.

Geographic Distribution. Bacterial canker is a globally distributed disease of cherry. This disease has been described in North America, South America, Europe, Africa, Asia, and Oceania. The putative *Pseudomonas* spp. associated with bacterial canker of cherry by country is below (Table 2.2).

Table 2.2. Countries with confirmed *Pseudomonas* spp. isolates infecting cherry.

Country	***Pseudomonas* spp.**	**Citation**
Australia	*P.s.* pv. syringae	(Wimalajeewa & Flett 1985)
Belgium	*P.s.* pv. syringae *Pseudomonas amygdali* pv. morsprunorum *Pseudomonas avellanae* pv. morsprunorum	(Gilbert et al. 2009)
Canada	*P.s.* pv. syringae *Pseudomonas amygdali* pv. morsprunorum	(Allen & Dirks 1978)
Chile	*P.s.* pv. syringae *Pseudomonas amygdali* pv. morsprunorum	(Beltrán et al. 2021) (Garcia et al. 2021)
China	*P.s.* pv. syringae	(Zeller et al. 1997)
England	*P.s.* pv. syringae *Pseudomonas amygdali* pv. morsprunorum *Pseudomonas avellanae* pv. morsprunorum	(Vicente et al. 2004)
France	*P.s.* pv. avii, *P.s.* pv. syringae *Pseudomonas amygdali* pv. morsprunorum *Pseudomonas avellanae* pv. morsprunorum	(Ménard et al. 2003)
Germany	*P.s.* pv. syringae	(Zeller et al. 1997)
Greece	*P.s.* pv. syringae	(Thomidis & Exadaktylou 2008)
Iran	*P.s.* pv. syringae	(Abbasi et al. 2013)
Japan	*P.s.* pv. cerasicola	(Kamiunten et al. 2000)
New Zealand	*P.s.* pv. syringae	(Young 1987)
Poland	*P.s.* pv. syringae *Pseudomonas amygdali* pv. morsprunorum *Pseudomonas avellanae* pv. morsprunorum *Pseudomonas cerasi*	(Kałużna et al. 2016a)
Russia	*P.s.* pv. syringae	(Maslova et al, 2020)
Serbia	*P.s.* pv. syringae *Pseudomonas amygdali* pv. morsprunorum	(Balaž et al., 2016)
South Africa	*P.s.* pv. syringae *Pseudomonas amygdali* pv. morsprunorum *Pseudomonas avellanae* pv. morsprunorum	(Roos & Hattingh 1987)
Turkey	*P.s.* pv. syringae	(Oksel et al. 2022)
USA	*P.s.* pv. syringae *Pseudomonas amygdali* pv. morsprunorum *Pseudomonas avellanae* pv. morsprunorum	(Jones 1971)

Pathogen Isolation and Storage. While *Pseudomonas* spp. is widely distributed throughout the plant, it is most frequently isolated from the blossoms. Asymptomatic blooms are stored on ice following collection, quickly submerged in phosphate buffer, sonicated for seven minutes, serial diluted, and pipetted onto KB agar. *Pseudomonas* spp. can also be readily isolated from cankers (Renick et al. 2008). Once plated, suspected *Pseudomonas* spp. colonies are purified based on morphology and biochemical assays. These desired colonies should be cream to yellow in color, fluorescent on KB agar, gram negative, and mucoid (Gormez et al. 2013). Purified isolates are stored in 15 % glycerol and maintained at -80 °C (Renick et al. 2008).

2.2 Pathogen Identification

Biochemical Characterization. After isolating suspected *Pseudomonas* spp. colonies, biochemical assays can be employed for isolate classification. LOPAT tests (Levan production, oxidase reaction, pectolytic activity, arginine hydrolase, and tobacco hypersensitivity) are employed to confirm the genus (Group 1a, +---+) and GATTa tests are used to differentiate pathovars of *Pseudomonas* spp. (Scheck et al. 1997). GATTa testing results by *Pseudomonas* spp. are listed Table 2.3.

Table 2.3. GATTa comparison of *Pseudomonas* spp. impacting cherry.

	Gelatin liquefication	**Aesculin hydrolysis**	**Tyrosinase activity**	**L (+) Tartrate utilization**
P.s. pv. syringae[1]	+	+	-	-
Pseudomonas amygdali pv. morsprunorum[1]	-	-	+	+
Pseudomonas avellanae pv. morsprunorum[1]	+	±	-	-
P.s. pv. avii[1]	+	-	-	-
P. cerasi[2]	+	-	+	-
P.s. pv. cerasicola[3]	-	*	*	*

*Information not available, ±Variable results, [1](Janse 2010), [2](Kałużna et al. 2016a), and [3](Kamiunten et al. 2000)

PCR Confirmation. The biochemical process of identifying the *Pseudomonas* spp. isolates can be confirmed via PCR. The pathogen identification can be PCR confirmed for syringomycin production with the *syrB* or *syrD* gene. Syringomycin is a virulence factor of *P. s.* pv. syringae. These primers include (*syrB* F: 5′-CTTTCCGTGGTCTTGATGAGG-3′), (*syrB* R: 5′-TCGATTTTGCCGTGATGAGTC-3′), (*syrD* F: 5′-AAACCAAGCAAGAGAAGAAGG-3′) and (*syrD* R: 5′-GGCAATACCGAACAGGAACAC-3′) (Sorenson et al. 1998). *Pseudomonas amygdali* pv. morsprunorum can be PCR confirmed for coronatine toxin production (Hulin et al. 2018a; Janse 2010). The primers associated with coronatine are (*cfl* F: 5'-GGCGCTCCCTCGCACTT-3') and (*cfl* R:5'GGTARRGGCGGGGGTGC-3') (Bereswill et al. 1994). *Pseudomonas avellanae* pv. morsprunorum and *P. s.* pv. avii can be PCR confirmed for yersiniabactin (Janse 2010). The primers for yersiniabactin are (*ybtS* F: 5'-CCTCTTTCGCCTTATTATGCTC-3') and (*ybtS* R: 5'- CGCTCGTTTATGTTCCGTC-3') (Heffernan et al. 2023).

Ice Nucleation Activity. Ice nucleation activity can also be used as an indicator for pathovar specificity. *P. s.* pv. syringae, *Pseudomonas amygdali* pv. morsprunorum, and *P. s.* pv. avii strains were all shown to have the complete set of genes for ice nucleation activity where *Pseudomonas avellanae* pv. morsprunorum strains do not (Hulin et al. 2018a). The ice nucleation proteins facilitate ice formation at temperatures higher than freezing (Burke & Lindow 1990). This activity can be assessed by monitoring ice formation in isolates during a controlled decline in temperature as described in Renick et al. (2008). In the field, ice nucleation activity can exacerbate frost events and provide brief infection courts in the frost-wounded tissues.

Pathogenicity Tests. *Pseudomonas syringae* isolate pathogenicity can be tested on green cherry fruitlets. After the fruitlets are surface sterilized, the peduncles are submerged in water and

fruitlets are stab-wounded on two sides with a sterile 16-gauge needle. Stab-wounded fruitlets are inoculated with a single isolate colony on both sides, kept in an incubated, dark, humidity chamber, assessed 48-hrs later, and compared to known pathogenic strains and a water control (Renick et al. 2008).

2.3 Disease Management

Cultural Control. Pruning must be timed correctly to prevent further infection. Pruning directly prior to or during tree dormancy in the late fall and winter can result in increased disease susceptibility. Similarly, pruning in the late summer leaves the newly induced shoot growth more susceptible to infection as the tissues cannot harden off prior to leaf fall (Young 1987). Summer pruning coincides with the lower *Pseudomonas* spp. summer populations. Pruning in dry, hot conditions shortly after harvest with frequent sterilization of loppers is optimal for limiting infection (Spotts et al. 2010). While pruning to remove inoculum is a generally accepted IPM practice, pruning to remove cankers does not necessarily limit the spread of infection as the bacterial populations are well established within the tree by the time cankers are visible (Young 1987). Copper or phosphite applications to pruning wounds have no significant impact on infection rates. However, stub pruning can limit cankers on the leader by physically distancing new cankers from the trunk (Carroll et al. 2010).

While no cherry cultivar is completely resistant to bacterial canker, several studies have investigated the innate resistance of different cultivars as a disease management strategy. Resistance to bacterial canker varies between cultivars and is dependent on rootstock, environmental conditions, bacterial strain, and inoculation method (Hulin et al. 2021; Spotts et al. 2010; Crosse 1963; Bedford et al. 2003; Mgbechi-Ezeri et al. 2018; Garrett 1986). These factors reduce the consistency of cultivar resistance studies. For example, *P. avium* L. cv.

‘Rainier’ was found to be more resistant by Spotts et al. (2010) and to be more susceptible by Mgbechi-Ezeri et al. (2018).

Table 2.4. *P. avium* L. resistance by cultivar.

Cultivar		**Resistance**	**Citation**
Prunus avium L.	Bing	Susceptible	(Spotts et al. 2010)
Prunus avium L.	JI 14039	Resistant	(Spotts et al. 2010)
Prunus avium L.	Lapins	Susceptible	(Bedford et al. 2003)
Prunus avium L.	Merchant	Resistant	(Bedford et al. 2003)
Prunus avium L.	Merpet	Resistant	(Bedford et al. 2003)
Prunus avium L.	Moreau	Susceptible	(Mgbechi-Ezeri et al. 2018)
Prunus avium L.	Napoleon	Susceptible	(Crosse 1963)
Prunus avium L.	Rainier	Resistant	(Spotts et al. 2010)
		Susceptible	(Mgbechi-Ezeri et al. 2018)
Prunus avium L.	Regina	Resistant	(Spotts et al. 2010)
		Resistant	(Mgbechi-Ezeri et al. 2018)
Prunus avium L.	Roundel	Resistant	(Crosse 1963)
Prunus avium L.	Royal Ann	Susceptible	(Bedford et al. 2003)
Prunus avium L.	Sweetheart	Susceptible	(Bedford et al. 2003)
Rootstock	Colt	Resistant	(Garret 1986)
Rootstock	F12/1	Susceptible	(Garret 1986)
Rootstock	Gisela 6	Susceptible	(Spotts et al. 2010)

Both nutrient deficiencies and surpluses can increase stress on the trees and predispose them to bacterial canker. Proper fertilization, specifically of nitrogen and calcium, reduces bacterial canker severity. Balanced time-release fertilizer applications along with copper applications significantly reduced disease incidence more than copper alone in *Prunus domestica* L. cv. 'French' (Sayler & Kirkpatrick 2003). However, increased foliar nitrogen levels and bark calcium levels were also associated with heightened incidence of bacterial canker lesions following calcium nitrate applications (Cao et al. 2013). This balance is dependent upon site history and competing disease pressures.

Chemical Control. Bloom and leaf fall are two critical junctures for chemical disease control to both prevent blossom blast and the associated crop loss and to limit overwintering inoculum in leaf scars (Young 1987). While copper applications can cause phytotoxicity, particularly after budbreak and during bloom, they are commonly used to reduce leaf scar inoculum in the autumn. However, copper resistance is an increasing issue with 31 % of pathogenic *Pseudomonas syringae* isolates tested showing resistance to copper in Michigan (Renick et al. 2008). Streptomycin has also been employed for bacterial canker control in woody plant nurseries. Swift development of resistance after usage has limited its efficacy (Sundin & Bender 1993; Scheck, Pscheidt, & Moore 1996). More recently, kasugamycin (Kasumin) has been labeled for bacterial canker control in cherry. Kasumin reduced blossom cluster infections by 90 % (Lillrose et al. 2017) and continues to be an effective treatment for blossom blast. However, given that it is an antibiotic, it has the potential to develop resistant bacterial populations after repeated usage like streptomycin.

Biological Control. While currently no biological control products have shown significant decreases in bacterial canker disease incidence on par with chemical controls like copper and Kasumin, several classes are being investigated including plant resistance inducers, biocontrol products with nonpathogenic epiphytic bacteria, and bacteriophage formulations (Lillrose et al. 2017; Rabiey et al. 2020). These products have the potential to fill the management gap left by burgeoning antimicrobial resistant *Pseudomonas* spp. populations.

CHAPTER 3:

IN VITRO AND *IN VIVO* STUDIES OF BACTERIOPHAGE ISOLATES AS A POTENTIAL BIOCONTROL FOR *PSEUDOMONAS SYRINGAE* PATHOVAR SYRINGAE INFECTING *PRUNUS AVIUM* L.

3.1 Abstract

Pseudomonas syringae pv. syringae, the primary causal pathogen of bacterial canker of sweet cherry, currently lacks effective control measures in Michigan. This study identified and characterized three bacteriophage isolates specific to Michigan cherry orchards as potential biological control agents; these isolates were named SunCherry1, 2, and 3. The SunCherry isolates were characterized *in vitro* prior to developing cocktails for field treatments. The SunCherry isolates were directly compared to a commercial bacteriophage isolate, REC1, from Omnilytics throughout this study (OmniLytics, Sandy, UT). The main goal of this study was adapting bacteriophages for field applications. The two major obstacles to the success of bacteriophages as a biological control agent are limited host ranges and environmental degradation. Bacteriophage isolates were isolated from the target environments so they might have a broader host range and better control of the diversity of indigenous bacterial populations in an orchard setting. The SunCherry isolates lysed 92 % of tested *P. s.* pv. syringae strains from Michigan cherry orchards. Bacteriophages are sensitive to environmental degradation, particularly UV exposure (Iriarte et al. 2007). This UV sensitivity was confirmed *in vitro*; all the SunCherry isolates' titer significantly declined following exposure to 180 $J \bullet m^{-2}$ of UV-C radiation. In four field experiments spanning 2022 and 2023, the SunCherry isolates were tested on *P. s.* pv. syringae-inoculated sweet cherry flowers with different formulations and timings designed to reduce UV radiation exposure. Specifically, morning and evening treatments were

compared as well as the impact of 0.75 % skim milk and 5 % peptone on treatment efficacy. The hypothesis being that, in mitigating UV exposure, the efficacy of the bacteriophage treatments would improve in a field setting. This study investigates the impact of these two factors on the success of bacteriophage treatments in a phytobacteriology field setting.

3.2 Introduction

Bacterial canker, caused by the pathogen *P. s.* pv. syringae, is an economically significant disease of *P. avium* L. First formally identified in 1891 in Europe, this disease has become a global issue for sweet cherry production (Cameron 1962). Bacterial canker of sweet cherry was reported in Michigan in 1971 and has become an ongoing concern for sweet cherry growers in the state (Jones 1971). Bacterial canker is associated with a variety of symptoms including leaf and fruit lesions, blossom blast, twig dieback and the namesake cankers (Kennelly et al. 2007). These various symptoms can reduce annual yields and tree vigor, but the cankers are especially damaging as they can girdle branches and the central leader resulting in tree death.

The severity of bacterial canker symptoms is highly variable between years. The disease can be particularly damaging if temperatures approach freezing during bloom; the pathogen's ice nucleation activity can exacerbate freezing events and cause blossom blast (Hirano & Upper 2000). During severe years of blossom blast, like 2021, crop yields are significantly reduced. As such, bacterial canker of sweet cherry must be carefully managed – especially during bloom.

Currently, there is not an effective disease control measure available in Michigan for bacterial canker of sweet cherry. Copper resistant-*P. s.* pv. syringae populations prevent the continued use of copper materials (Renick et al. 2008). Kasugamycin, an antibiotic registered for this pathosystem in 2020, showed promising initial results in blossom blast reduction (Lillrose et al. 2017). However, the use of kasugamycin is associated with the potential for antibiotic

resistant-*P. s.* pv. syringae population development and subsequent transfer to nontarget organisms, such as *Erwinia amylovora*, which would have substantial negative repercussions (McGhee & Sundin 2011). Furthermore, both usage of copper and kasugamycin have been associated with phytotoxic activity in sweet cherry (Renick et al. 2008; Lauwers 2022). As such, there is a continued effort to develop a new control for bacterial canker of sweet cherry.

This has led to an active interest in bacteriophages as a prospective biocontrol. Bacteriophages present a strong potential in this application. They are environmental viruses highly specific to their bacterial hosts that coexist with their hosts in diverse ecosystems throughout the world (Keen 2015). Despite this broad spectrum of bacterial interactions, individual bacteriophage isolates have a limited off target environmental impact due to their narrow host ranges (Morella et al. 2018). Bacteriophages also self-replicate in the environment through their lytic life cycle; they can increase their own populations while reducing host bacteria populations (Hyman & Abedon 2010). These two factors make bacteriophages a strong candidate for biological control applications.

However, these advantages are not without limits. The narrow host range of bacteriophage isolates can also present a challenge in adapting bacteriophages for biocontrol applications. The bacteriophages must be able to lyse the diversity of bacterial isolates indigenous to Michigan cherry orchards. As such, identifying bacteriophages with broad host ranges in the target ecosystem is a key part of developing an effective biocontrol. Combining bacteriophage isolates into a cocktail can increase the total lytic capacity of biological control treatments by expanding the potential host range (Gayder et al. 2019). Additionally, bacteriophages are sensitive to environmental degradation in the phyllosphere context (Balogh et al. 2003). The phyllosphere has near constant fluctuations in temperature, pH, moisture

availability, and UV radiation (Vacher et al. 2016). Exposure to this harsh environment, particularly to UV radiation, causes instability in bacteriophage populations soon after application (Iriarte et al. 2007; Born et al. 2015, Balogh et al. 2003). Identifying application amendments or timings to reduce this UV exposure is a necessary step in developing effective field treatments. These limitations must be addressed to fully take advantage of the potentials associated with bacteriophages in a biological control context.

In this study, these two key barriers (host range and environmental degradation) to adapting bacteriophages as a biological control in a field setting were explored. Field trials were conducted on mature sweet cherry trees in 2022 and 2023 in central and northwest Michigan both years. Flowers were inoculated with *P. s.* pv. syringae strains prior to being treated with bacteriophage applications and antibiotics. *P. s.* pv. syringae populations were then tracked over a 96-hr window (Lauwers 2022).

For the host range aspect, the impact of locality on host range capacity was examined. The hypothesis being that bacteriophage isolates indigenous to Michigan cherry orchards would lyse bacterial strains from that environment more efficiently than nonspecific commercial bacteriophages. The efficacy of Michigan bacteriophage isolates and commercial treatments was also compared in the field trials. Moreover, the Michigan bacteriophage isolates were characterized with multiple assays.

Methods for UV exposure reduction was tested both *in vitro* and via field trials. Two strategies were identified to reduce UV exposure: temporal and spatial. For the temporal-based UV protection, the efficacy of bacteriophage treatments applied in the morning and the evening were compared. The hypothesis being that bacteriophage treatments applied in the evening would have lower initial exposure to UV radiation and would therefore be more successful. In

2022, the impact of 0.75 % skim milk on bacteriophage treatment efficacy was examined (Balogh et al. 2003; Balogh et al. 2008). In 2023, a 5 % peptone amendment for UV mitigation was tested (Born et al. 2015). These treatments were selected based on prior studies on bacteriophage UV protectants (Balogh et al. 2003; Born et al. 2015).

This study seeks to provide insight into the development of bacteriophages for phytobacteriology applications. By addressing the specific issues discussed above, the barriers to the success of bacteriophages as a potential biological control can be reduced. Currently, most studies on bacteriophages in a phytobacteriology context are focused on *in vitro* tests and the few studies *in sensu* are not consistently successful (Rombouts et al. 2016; Balogh et al. 2008; Rabiey et al. 2020; Iriarte et al. 2007; Born et al. 2015; James et al. 2020; Akbaba & Ozaktan 2021; Song et al. 2021; Flores et al. 2020; Pinheiro et al. 2020; Liu et al. 2021; Martino et al. 2021; Di Lallo et al. 2014; Yu et al. 2016; Park et al. 2018; Frampton et al. 2014; Ni et al. 2021; Ni et al. 2020; Bai et al. 2021). Understanding the obstacles associated with bacteriophages in a field context is critical to translating that *in vitro* promise.

3.3 Materials and Methods

Bacterial strains and culture conditions. The bacterial strains utilized in these experiments were sourced from two distinct field collections. BY-38, a pathogenic strain of *P. s.* pv. syringae, was isolated in 2019 from a Michigan sweet cherry orchard. The host range experiments utilized a 70 isolate subcollection of a 2021 *P. s.* pv. syringae collection across 13 Michigan sweet cherry orchards (Lauwers 2022) and five phylogenetically diverse *Pseudomonas* spp. isolates (R_12, V_14, BP_25, *Pseudomonas syringae* pv. tomato DC3000, and *Pseudomonas phaseolicola*).

The bacterial isolates were stored in 15 % glycerol at -80 ℃. Once removed from storage, bacterial isolates were grown on King's B (KB) medium and incubated at either 28 ℃ or

room temperature (King et al. 1954). Broth culture were prepared by combining 5 ml of liquid KB medium and a single bacterial colony in a 14 ml polystyrene, round-bottom tube and incubating overnight in an incubator-shaker at 28 ℃ and 220 rpm.

For field experiments, spontaneous rifampicin-resistant mutants of BY-38 and BP-25 were used. These mutants were selected by spreading ~10^8 bacterial cells on KB agar medium amended with 100 µg ml^{-1} of rifampicin (Lauwers 2022). Broth cultures containing this antibiotic-resistant mutant were amended with rifampicin as well. These spontaneous mutants used throughout the field experiments were generated by plating samples on KB agar medium amended with both 100 µg ml^{-1} of rifampicin and 50 µg ml^{-1} of cycloheximide to inhibit fungal growth. Given the light-sensitive property of rifampicin, antibiotic-amended medium was covered during incubation.

Bacteriophage enrichment, isolation, purification, and storage. This procedure utilized both *P. s.* pv. syringae isolates and soil samples from Michigan sweet cherry orchards to generate bacteriophage isolates specific to pathogenic bacteria in this environment. Soil samples were collected from 13 sweet cherry orchards in NW Michigan in 2022 and stored at 4 ℃ for three months prior to processing. Bacteriophages were isolated via the enrichment method by incubating soil with host bacteria in a liquid nutrient broth following the protocol outlined by Akbaba & Ozaktan (2021). 48 ml of liquid KB medium and 2 ml 0.1 M $CaCO_3$ were sterilized in a 250-ml flask and inoculated with 5 ml of the target bacterial isolate culture. The broth culture was prepared as above, resuspended in phosphate buffer (PB) after washing, and adjusted turbidimetrically (OD_{600}: 0.8) to 10^9 Colony Forming Units (CFU) ml^{-1} using a Tecan Spark plate reader (Männedorf, Switzerland). After mixing the bacterial culture into the liquid enrichment medium, 50 g of soil was added to each flask. The flasks were then placed in the

incubator-shaker for 24-hrs at 28 ℃ and 220 rpm (Akbaba & Ozaktan 2021). The target bacterial strain was BY-38 for six bacteriophage isolates and 9-10 from the 2021 PSS collection for one bacteriophage isolate.

Following this initial enrichment step, the bacteriophages were isolated with centrifugation to remove the bulk soil, and 10 % v/v chloroform followed by 0.22 µm filter sterilization to remove the bacteria. Bacteriophage lysates were isolated and purified via pipetting serial diluted aliquots on soft agar plates and repeated plaque selections (Akbaba & Ozaktan 2021). The dilution series were performed with filter sterilized phage buffer (10 ml 1 M Tris HCl; 10 ml 1 M $MgSO_4$; 4 g NaCl; 1 ml 0.1 M $CaCl_2$; 980 ml diH_2O) (Sullivan 2015). Soft agar plates were prepared by combining soft molten agar (1:1 KB agar medium and sterile diH_2O) and 100 ml of the host bacterial cell suspension (OD_{600}: 0.8) via inversion and pouring the mixture onto KB agar already set in a petri dish. The soft agar plate is dried in a laminar flow hood prior to spot plating the dilution series. The resulting plates are incubated at 28 ℃ overnight (Lauwers 2022). This isolation step resulted in a mixture of plaques of varying turbidity and size. The plaque mixture was purified by selecting the clearest plaques with a sterile toothpick and repeating the previous dilution series and soft agar overlay procedure until the resulting plates have clear plaques with uniform morphology. This purification step was repeated a minimum of three times (Akbaba & Ozaktan 2021).

Bacteriophage isolates were preserved by first combining a 100 µl aliquot of the undiluted phage solution with the soft agar overlay preparation to generate a dense lawn of plaques. 5 ml of phage buffer was added to the incubated soft agar overlay plates. The top agar layer was thoroughly disrupted with a sterile plastic inoculation loop and both the phage buffer and crushed agar were transferred to a 15 ml falcon tube. The mixture was briefly vortexed and

centrifuged for 2 minutes at 10,000 x g. Three 1 ml aliquots were removed from the resulting supernatant, sterile filtered with 0.22 µm filters into 1.5 ml Eppendorf tubes, and treated with 10 % v/v chloroform for 20 minutes. The sterilized supernatant was stored in 30 % glycerol in triplicate at -80 ℃ (Akbaba & Ozaktan 2021). This process resulted in five bacteriophage isolates. Three isolates continued through the characterization process, known hence forth as SunCherry1, SunCherry2, and SunCherry3. The bacterial host used to isolate SunCherry1 and SunCherry2 was BY-38 and the bacterial host used to isolate SunCherry3 was PSS9-10, from the 2021 NW Michigan culture collection. BY-38 was also utilized as the primary bacterial host for the commercial bacteriophage isolate, REC1, throughout the *in vitro* experiments.

Bacteriophage amplification. Bacteriophage isolates can be amplified from the stored samples to produce a working stock that can be utilized in lab experiments and field trials. Sterile 250-ml flasks with 100 ml of liquid KB medium were incubated with 100 µl of the host bacterial cell suspension (OD_{600}: 0.8) and 100 µl of 1 M $CaCl_2$ in the incubator-shaker at 28 ℃ and 220 rpm for a 3- to 6-hr period. 100 µl of a given stored bacteriophage isolate was then added to the flask and the mixture was kept in the incubator-shaker for 48-hrs. 10 % v/v chloroform was then added to the mixture, which was incubated in the incubator-shaker for 20 minutes prior to being centrifuged for 2 minutes at 8,000 rpm. The supernatant was filtered with a 0.22 µm filter. The resulting bacteriophage lysate was stored at 4 ℃ and covered to prevent UV-degradation for a maximum of two months (Adapted from Omnilytics).

Transmission Electron Microscopy preparation. The bacteriophage lysates were ultracentrifuged and resuspended in 10 % of the original volume with phage buffer to produce high titer samples. 10 µl of a given high titer bacteriophage lysate was placed on a carbon coated grid. The grids were washed with diH_2O to remove any remaining phosphates from the buffer

and stained with uranyl acetate prior to imaging. The prepared samples were imaged with the JEOL 1400 Flash TEM at the Center for Advanced Microscopy at Michigan State University.

Bacteriophage DNA extraction. Bacteriophage DNA was extracted from high titer bacteriophage lysate (minimum 10^8 Plaque Forming Units (PFU) ml $^{-1}$) with a Phage DNA Isolation Kit (Norgen Biotek, Thorold, Canada) in keeping with the manufacturers' instructions. The resulting DNA concentration was assessed with the Invitrogen Qubit fluorometer. Extracted samples were stored at 20 ℃ for two weeks prior to submission for sequencing.

Bacteriophage genome sequencing. Libraries were prepared by the Michigan State University Genomics Core using the Roche Kapa HyperPrep DNA Library Kit (Indianapolis, Indiana) with Unique Dual Index adapters following the manufacturers' instructions. The resulting libraries were quantified and quality checked with the Qubit dsDNA HS and Agilent 4200 TapeStation HS DNA1000. The libraries were pooled and loaded into 10% of a lane of an Illumina v1.5 S4 flowcell and sequenced with a NovaSeq 6000 v1.5 300 cycle reagent cartridge. BBtools (v37.62) was implemented to remove adapters and trim reads based on sequence quality (Q20) and length (100 bp minimum) (Bushnell 2017). Bacterial host reads were removed by mapping reads to the *P. s.* pv. syringae B728a genome (GenBank Accession number: ASM1224) with MiniMap2 (v2.26-r1175) (Li 2018). Reads coverage was normalized to 100x with BBnorm (BBtools). Unicycler (v0.5.0) was used to generate de novo bacteriophage genome assemblies (Wick et al. 2017). Resulting scaffolds were confirmed for bacteriophage identity via NCBI Blast. At this point, SC3 and SC4 were found to be contaminated and are being sequenced again to determine their identity. The assemblies were annotated with Prokka (v1.14.6) (Seemann 2014). Assemblies were searched for genes associated with antimicrobial resistance with ABRicate (v1.0.1) (Seemann 2016). Hypothetical protein coding sequences from Prokka were annotated

with NCBI Protein Blast with a max score cutoff of 50 and checked for association with lysogeny or toxins. The assemblies and associated annotations were formatted for display with the genoPlotR package (v0.8.11) (Guy, Kultima, & Andersson 2010).

Host range. This initial host range experiment investigated the ability of the bacteriophage to lyse bacterial isolates representative of the indigenous *P. s.* pv. syringae populations in Michigan cherry orchards. A 70-isolate subcollection of Lauwers' 2021 *P. s.* pv. syringae collection representing 13 Michigan cherry orchards were tested against SunCherry1-3 and REC1 (Lauwers 2022). Soft agar plates were prepared as described previously with the 70 bacterial isolates turbidimetrically adjusted to OD_{600}: 0.8. The bacteriophages were serial diluted and drop plated onto the respective soft agar plates. The resulting plates were incubated at 28 ℃ overnight. The presence and absence of plaques was noted and compared between bacteriophage isolates. All combinations of bacteriophage and bacterial isolates had three experimental replicates.

Efficiency of Plating (EOP). The host range assay was further investigated with an efficiency of plating experiment to assess the infective capacity of the SunCherry bacteriophages more thoroughly. The tested bacterial isolates included the 70-isolate Michigan *P. s.* pv. syringae subcollection as well as five diverse *Pseudomonas* isolates (R_12, V_14, BP_25, *Pseudomonas syringae* pv. tomato DC3000, and *Pseudomonas phaseolicola*). The EOP is a ratio that indicates the bacteriophage titer on a given bacterial isolate compared to the bacteriophage titer on the initial bacterial host (Mirzaei & Nilsson 2015). The EOP ratio is an index of each combination's robustness. The EOP procedure followed the same process as the host range experiment. The bacteriophage isolates were plated on the initial bacterial hosts (BY-38 for SunCherry1, SunCherry2, and REC1 and PSS9-10 for SunCherry3) each day and compared to the other plated

bacterial isolates with the EOP ratio. Three experimental replicates were conducted and averaged to determine the final EOP for each bacteriophage-bacterial isolate combination.

Killing Curve. A killing curve experiment was conducted to examine the impact of bacteriophage concentration on bacterial growth. 500 µl of the *P. s.* pv. syringae bacterial hosts set at (OD_{600}: 0.2) 1 x 10^8 CFU ml^{-1} and resuspended in KB broth was added to 12 wells of a clear bottomed 24-well plate (Rabiey et al. 2020). The bacteriophage isolates were added to the wells to achieve a Multiplicity of Infection (MOI) of 0.5, 1, or 2, respectively. Here, MOI refers to the ratio of bacteriophages to bacteria in a given solution and is used to standardize the number of virions added to each well across the four isolates. Phage buffer was then added to the wells to achieve a uniform final volume of 1.2 ml. The controls consisted of three wells of 1.2 ml phage buffer and three wells of 500 µl of the prepared bacteria and 700 µl of phage buffer. The 24-well plates were then sealed with Parafilm to maintain consistent humidity and placed in the Tecan Spark. The Tecan Spark was set to 27 ℃ and the OD_{600} was measured every 20 minutes over a 21-hr period. The plate was shaken for 10 seconds prior to each measurement. Three experimental replicates with three technical replicates each were conducted for each bacteriophage isolate (Protocol adapted from Rabiey et al. 2020).

Impact of ultraviolet radiation exposure on bacteriophage population stability. This assay investigated the impact of UV-C exposure on bacteriophage population stability under different protective conditions. These treatments include uncovered, covered, 0.75 % instant skim milk (Meijer, Grand Rapids, MI) and 5 % peptone (RPI, Mt. Prospect, IL). For the covered and uncovered treatments, two 60-mm glass petri dishes were filled with 4.95 ml phage buffer and 50 µl of a given bacteriophage isolate and gently swirled to mix. For the 0.75 % skim milk and 5 % peptone treatments, 4.95 ml of phage buffer amended to the specified concentrations of skim

milk and peptone and 50 µl of a given bacteriophage isolate were combined in the 60-mm glass petri dishes. 100 µl aliquots were removed from each sample prior to any UV-C exposure. Samples were then placed in the UV-C box 1.2 m below the lamp at a rate of exposure of 1.5 J m^{-2}•s^{-2}. Aliquots were removed from the samples at the 1 and 2 min timepoints. The aliquots from 0 min, 1 min, and 2 mins were serial diluted to the 10^{-5} dilution and pipetted in triplicate on soft agar plates as previously described (Lauwers 2022). Plates were incubated at 28 ℃ overnight and bacteriophage plaques were enumerated and recorded. Data points were averaged across three experimental replicates and three technical replicates.

Bacteriophage population stability under differing pH and temperature conditions. In this assay, SunCherry bacteriophage isolates and REC1 were exposed to pH 4, 7, or 10 for 48-hrs and evaluated for population stability. Each of bacteriophage isolates were diluted to 1 x 10^6 PFU ml^{-1} in 1.5 ml of the pH-adjusted phage buffer. After 48-hrs of incubation at 4 ℃, the samples were serial diluted to 10^{-5} dilution and pipetted in triplicate on soft agar plates as described above. Plates were incubated at 28 ℃ overnight and bacteriophage plaques were enumerated and recorded. Results from two experimental replicates and three technical replicates were averaged for each data point.

The temperature assay evaluated the impact of differing storage temperatures on bacteriophage populations over a 30-day incubation period. The three SunCherry isolates and REC1 were serial diluted and quantified with the drop plate method in triplicate on soft agar plates. One ml aliquots of the bacteriophages were then stored at 4 ℃, 25 ℃, and 37 ℃ for a 30-day period after which the plating procedure was repeated. The populations were compared between the initial and final samples to determine if the incubation conditions negatively

impacted the bacteriophage population stability. The plaque counts from two experimental replicates and three technical replicates were averaged to assess this experiment.

***Pseudomonas syringae* pv. syringae population dynamics on sweet cherry flowers *in situ* treated with bacteriophage isolates.** During the spring of 2022 and 2023, field trials were conducted at two locations each year: the Michigan State University Plant Pathology (MSU-PLP) farm (42.689167, -84.485278) and the Northwest Michigan Horticultural Research Center (NWMHRC) (44.881996, -85.675251). At MSU-PLP, trials were conducted on *P. avium* L. cv. 'Ulster'. Trials were conducted on *P. avium* L. cv. 'Benton' at NWMHRC. Three to four branches were flagged across different trees for each treatment to serve as replicates. An individual tree had maximum three branches flagged, equidistant around the canopy. At full bloom, any unopened flowers on flagged branches were removed and the remaining open flowers were inoculated with *P. s.* pv. syringae between 6:00 and 8:30 PM. The inoculum consisted of two rifampicin resistant strains of *P. s.* pv. syringae, BY-38 and BP-25, diluted to a density of 10^{6-7} CFU ml^{-1} in a 15.12-liter backpack sprayer.

Morning treatment applications began 12-hrs after inoculations between 8:00 and 10:00 AM. Treatments were applied with 15.12-liter backpack sprayers. In 2022, the morning treatments at both locations consisted of water, a SunCherry cocktail, a SunCherry cocktail with an addition of 0.75 % skim milk, and 0.75 % skim milk. The SunCherry cocktails were diluted to 8.6×10^6 PFU ml^{-1} in the backpack sprayer. The morning treatments in 2023 consisted of water, a SunCherry cocktail, a SunCherry cocktail with 5 % Peptone, 5 % Peptone, and AgriPhage Nut and Stone Fruit bacteriophage cocktail (OmniLytics, Sandy, UT). Both the SunCherry cocktail and the AgriPhage product were diluted to 7.6×10^8 PFU ml^{-1} in 2023.

Evening treatments began between 6:00 and 7:00 PM. The evening treatments in 2022 were water, a SunCherry cocktail, a SunCherry cocktail supplemented with a 48-hr reapplication, 5 ml l^{-1} Kasumin 2L (UPL, Cary, NC) + 1.24 ml l^{-1} Li-700 (Loveland Products, Loveland, CO), and AgriPhage Nut and Stone Fruit bacteriophage cocktail. Both the SunCherry cocktail and the AgriPhage product were diluted to 8.6 x 10^6 PFU ml^{-1} in the backpack sprayer. In 2023, the evening treatments were water, a SunCherry cocktail, a SunCherry cocktail supplemented with a 48-hr reapplication, and 5 ml l^{-1} Kasumin 2L + 1.24 ml l^{-1} Li-700. These SunCherry cocktails were diluted to 7.6 x 10^8 PFU ml^{-1}.

Table 3.1. Field treatment summary across morning and evening applications for 2022 and 2023.

		2022	**2023**
Experiment Timing	**AM**	Water	Water
		SunCherry Bacteriophage Cocktail	SunCherry Bacteriophage Cocktail
		SunCherry Bacteriophage Cocktail + Skim Milk (0.75%)	SunCherry Bacteriophage Cocktail + Peptone (5%)
		Skim Milk (0.75%)	Peptone (5%)
			Agriphage Nut and Stone Fruit Bacteriophage Cocktail
	PM	Water	Water
		SunCherry Bacteriophage Cocktail	SunCherry Bacteriophage Cocktail
		SunCherry Bacteriophage Cocktail + 48h reapplication	SunCherry Bacteriophage Cocktail + 48h reapplication
		Kasumin 2L + Li-700	Kasumin 2L + Li-700
		Agriphage Nut and Stone Fruit Bacteriophage Cocktail	

Initial samples were taken after *P. s.* pv. syringae inoculation and one-hr prior to treatment applications. In 2022, subsequent sampling was conducted 12-, 24-, 48-, 72-, and 96-hrs post-treatment. In 2023, sampling was conducted 24-hrs post treatment application and replicated every 24-hrs for 96-hrs. For sampling, four replicates were taken for each treatment at each timepoint. Six flowers were sampled per replicate. The flowers were sampled randomly throughout the treated branches. Flower samples were then stored on ice until further processing. Each bag of flowers was weighed with the mass of an average of 20 bags subtracted from the value to achieve the mass of the flowers alone. Using sterilized forceps, the flowers were placed into 20 ml of chilled phosphate buffer in sterile 60-ml glass test tubes kept on ice during processing. Once submerged, samples were sonicated for seven minutes (model 250T: VWR Scientific, Houston, TX). 100 µl aliquots were taken from each sample and serial diluted in 900 µl phosphate buffer blanks. The prepared serial dilutions were then pipetted in triplicate on KB plates amended with 75 µg ml^{-1} rifampicin and 50 µg ml^{-1} cyclohexamide. Plates were then covered and incubated at room temperature for 48-hrs prior to counting to identify the colony forming units per gram of flower mass.

Weather data. To assess their potential impact on the field trials, the mean daily temperature (°C), the total daily precipitation (mm), the mean daily relative humidity (%), and the daily light integral ($mol•m^{-2}•d^{-1}$) were downloaded from the Michigan State University Enviroweather database (https://enviroweather.msu.edu/) for weather stations closest to the field trial locations for the dates described previously. Weather station ‘MSUHORT’ was used to obtain data associated with the MSU-PLP farm trial; located 1.6 km from the trees used for the field trials. Weather station ‘NWMHRS’, 0.5 km from the trees used in this study, was used to acquired weather data associated with the NWMHRC site.

Data and Statistics. All data was evaluated with R Studio (2022.07.2). Statistical analyses for the killing curves, pH stability, and field experiments were performed with analysis of variance and Tukey's honest significant difference to directly compare experimental treatments. One-tailed, paired Student's t-tests were used to analyze differences in UV assays and temperature stability between time points. Error bars represent standard error. Statistical significance was associated with a p-value cutoff of 0.05. Graphs were generated using the cowplot (v1.1.1) (Wilke 2021) and ggplot2 (v3.4.2) (Wickham 2016), and heatmap3 (v1.1.9) (Zhao et al. 2014) packages.

3.4 Results

Transmission electron microscopy. Micrographs of the three SunCherry isolates and REC1 isolate, shown below, were taken with a JEOL 1400 Flash TEM at magnifications ranging from 40,000 to 80,000.

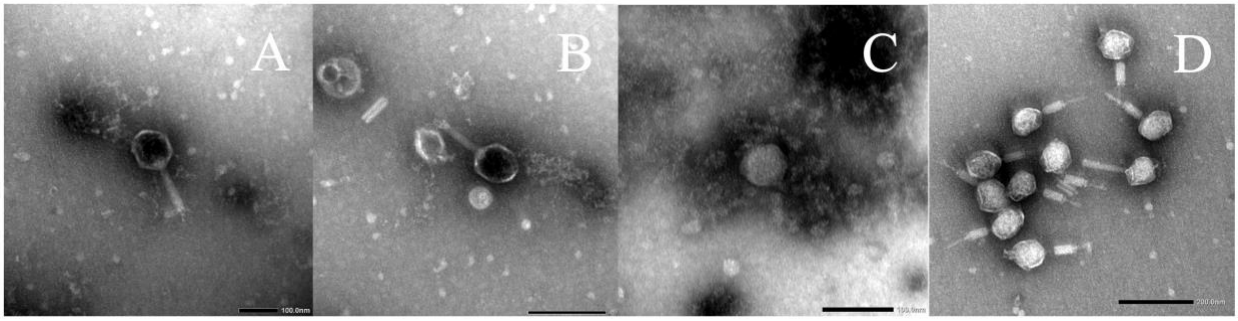

Figure 3.1. Transmission Electron Microscopy micrographs including A: SunCherry1, scale bar = 100.00 nm, B: SunCherry2, scale bar = 200.00 nm, C: SunCherry3, scale bar = 100.00 nm, D: REC1, scale bar = 200.00 nm.

Bacteriophage genome alignment and annotations. The SunCherry1, SunCherry2, and SunCherry3 genome assemblies were annotated, as shown below. The SunCherry1 assembly is 14,857 bp in length, the SunCherry2 assembly is 24,304 bp in length, and the SunCherry3 assembly is 50,733 bp in length. Annotated proteins associated with SunCherry1 and SunCherry2 and SunCherry3 were all from the class Caudovircetes. This designation was expected given that Caudovircetes accounts for 90 % of investigated bacteriophage isolates (Harper & Enright 2011). This result is in line with the TEM micrographs of the SunCherry isolates (Figure 3.1). The *Pseudomonas* bacteriophage REC1 utilized in this study has been sequenced and annotated (NCBI:txid2886030) (Schoch et al. 2020).

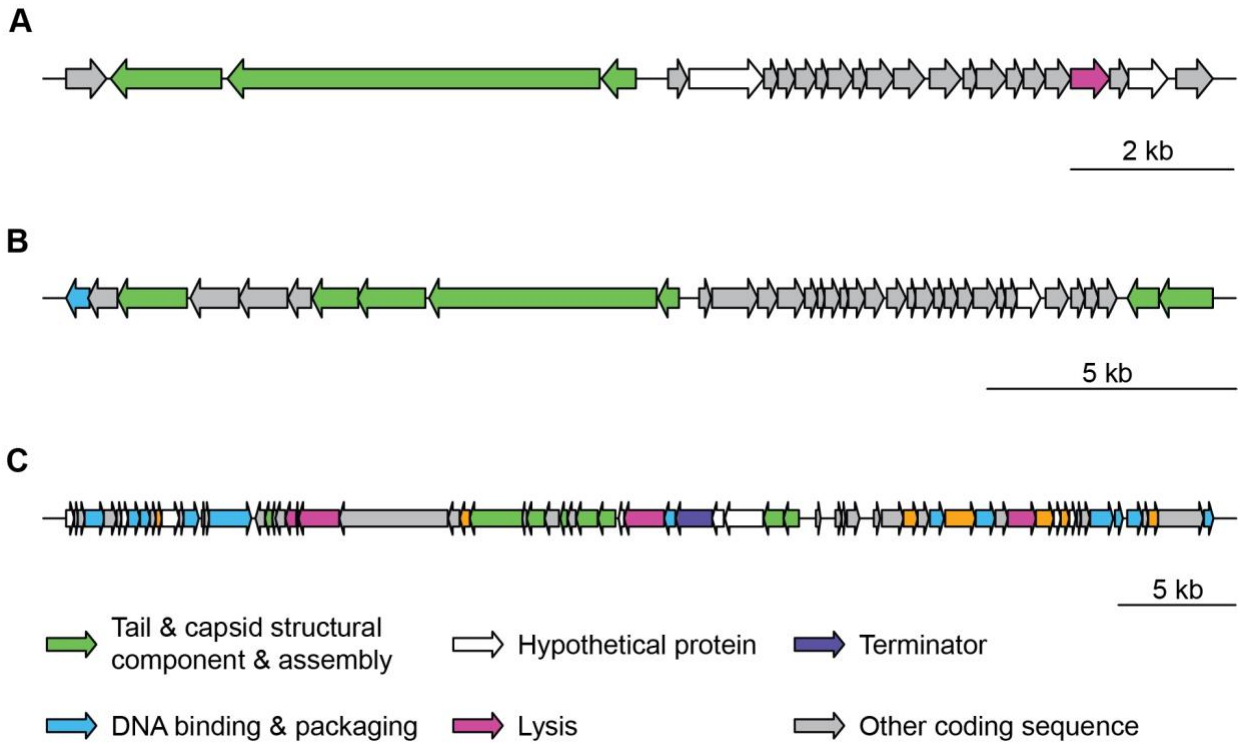

Figure 3.2. Genomic assemblies for SunCherry1 (A), SunCherry2 (B) and SunCherry3 (C). Annotations were performed with BlastP with a max score cutoff of 50. The assembly comparison was created with genoPlotR.

Comparative host range. Both the commercial bacteriophage and SunCherry isolates were tested against pathogenic *P. s.* pv. syringae isolates from Michigan cherry orchards. The commercial bacteriophage isolate, REC1, lysed 72 % (54/75) whereas the SunCherry isolates lysed 92 % (69/75) of the tested bacterial isolates (Figure 3.3). The SunCherry isolates lysed significantly more of the tested bacterial isolates than the commercial bacteriophage isolate ($p<0.05$).

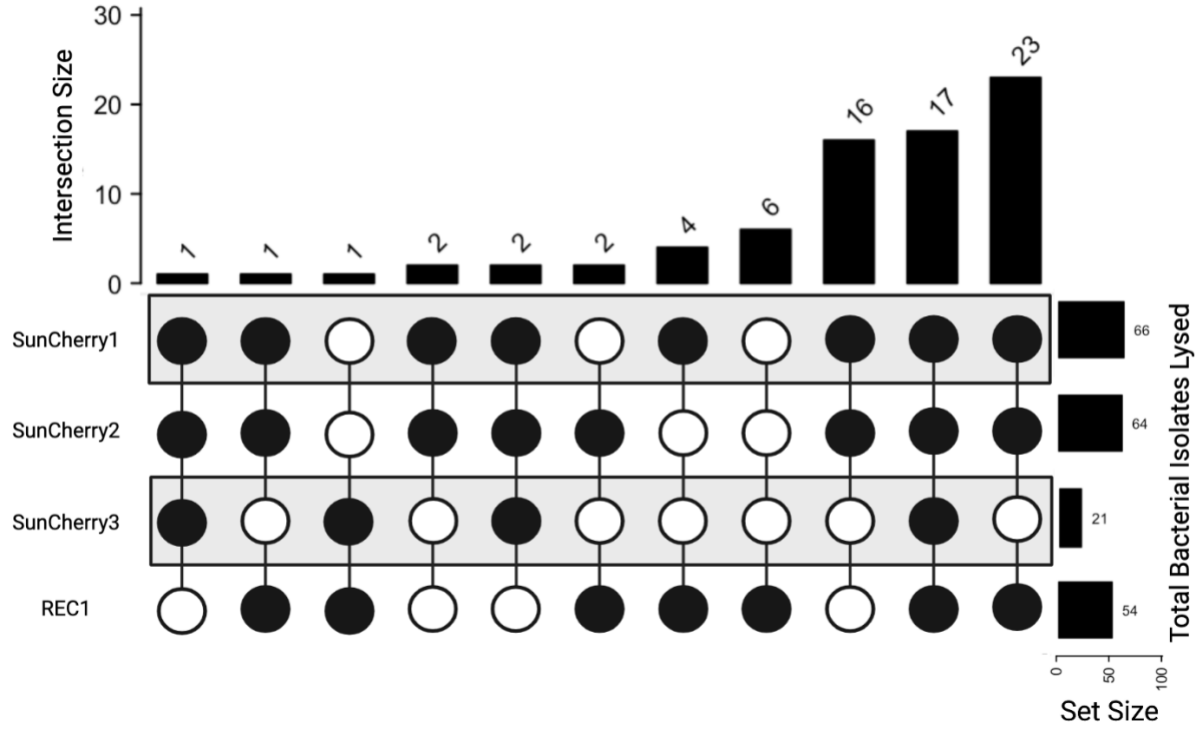

Figure 3.3. UpSet plot of commercial bacteriophage host range and SunCherry host range. Dark circles indicate lytic interactions and white circles indicate incompatible bacteriophage-host matches. The intersection size refers the number of bacterial isolates lysed by that combination of bacteriophage isolates. The set size refers to the total bacterial isolates lysed by each bacteriophage isolate.

Efficiency of plating (EOP). The bacteriophage isolates were serial diluted and pipetted onto double layer KB agar plates amended with 75 bacterial isolates. Bacterial strains 1-70 consisted of 5 or 6 strains from 13 sweet cherry orchards spanning northwest Michigan. The additional five bacterial strains were R_12, V_14, BP_25, *Pseudomonas syringae* pv. tomato DC3000, and *Pseudomonas phaseolicola*. After overnight incubation, the bacteriophage titer was calculated for each isolate-bacteriophage combination and compared to the bacteriophage titer associated with the reference host bacteria strain. This was averaged across three experimental replicates. The result of this experiment was a series of ratios demonstrating the efficacy of the interaction between each bacteriophage isolate and bacterial strain. At a ratio of 1:1, the bacteriophage was as effective at lysing a given bacterial strain as the host bacteria strain. Lower ratios indicate a less effective relationship. These EOP ratios are shown in Figure 3.4.

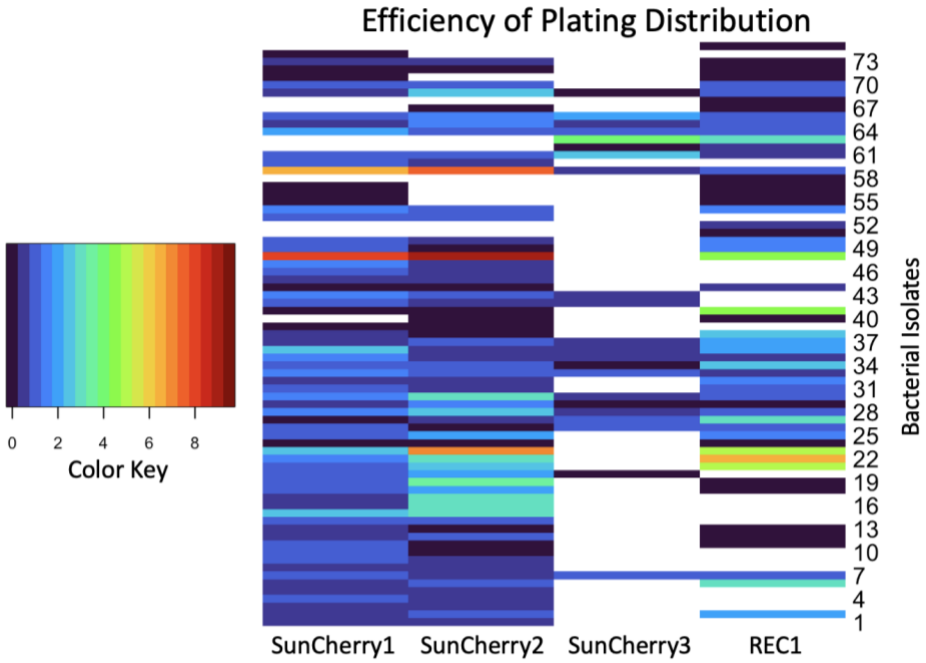

Figure 3.4. Distribution of EOP ratios across SunCherry isolates (SC1-3) and REC1. The EOP ratio indicates the bacteriophage titer on a given bacterial isolate divided by the bacteriophage titer on the host bacterial isolate. Higher EOP ratios are associated with more effective bacteriophage-host interactions. Each EOP ratio represents the average of three experimental replicates.

Evaluation of killing curves. For the killing curve assay, SunCherry1-3 and REC1 were added to their respective host bacteria strains at an MOI of 0.5, 1, and 2 in 24-well plates with a bacterial control and a buffer control. For SunCherry1, all bacteriophage-bacteria ratios were significantly different ($p<0.05$) besides MOI 0.5-MOI 1. All bacteriophage-bacteria ratios were significantly different for SunCherry2. For SunCherry3 and REC1, the reference strain was significantly higher than all MOIs as well as the buffer control. However, all MOIs were equal for these isolates, indicating that SunCherry3 and REC1 can more effectively lyse host bacteria at lower concentrations of bacteriophage than SunCherry1 and SunCherry2.

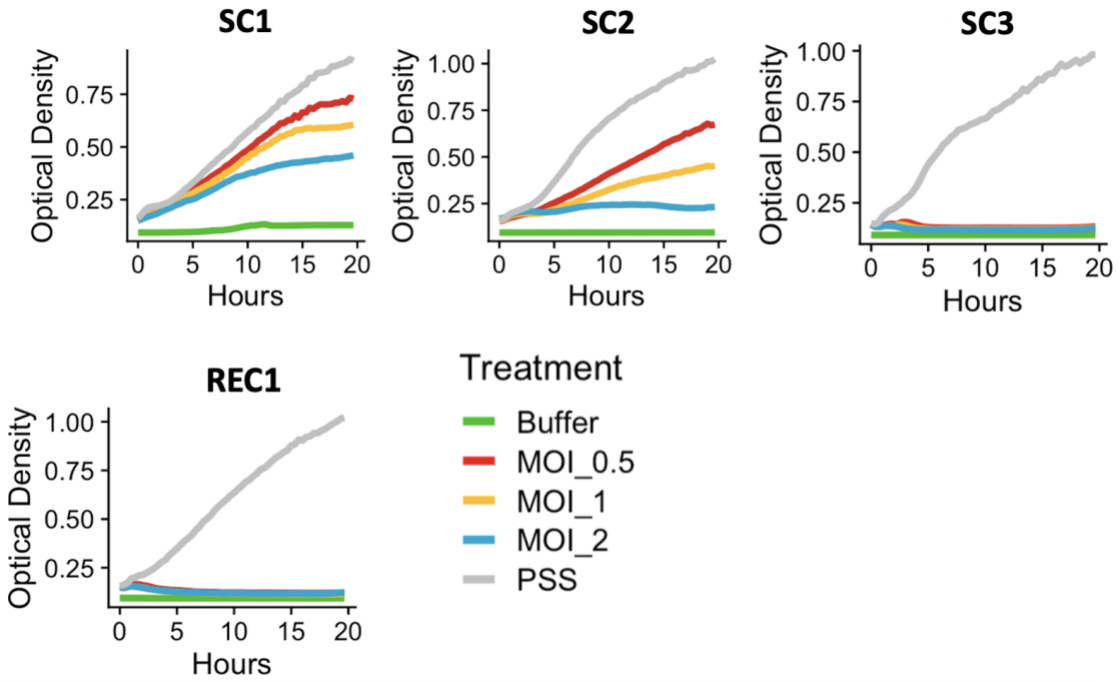

Figure 3.5. Killing curves for SunCherry isolates (SC1-3) and REC1. The killing curves reflect the impact of different concentrations of bacteriophage (MOI 0.1, 1, and 2) on *P. s.* syringae population survival. Each data point represents the average of three experimental replicates and three technical replicates.

Impact of ultraviolet radiation exposure on bacteriophage population stability. The SunCherry isolates and REC1 were exposed to UV-C radiation for two minutes at 1.5 J m^{-2}•s^{-2}. Aliquots were removed after zero minutes, one minute (90 J m^{-2}), and two minutes (180 J m^{-2}) of exposure. The titer of these aliquots was then determined as previously described. Treatments included exposed, covered, 0.75 % skim milk, and 5 % peptone.

Differences in titer by treatment and time were evaluated using paired t-tests ($p<0.05$). For all SunCherry isolates, the uncovered samples had significant decreases in titer between all three time points. The uncovered samples were also significantly less than the covered samples at both one minute and two minutes for all bacteriophage isolates. The 5 % peptone treatment was associated with stable bacteriophage titers for SunCherry2, SunCherry3, and REC1 whereas the titer significantly decreased between one and two minutes for SunCherry1. The 0.75 % skim milk treated bacteriophage samples had a significant decrease in titer for all SunCherry isolates by two minutes. For SunCherry1, 3, and REC1, the skim milk treatments were significantly less than the peptone treatments between one and two minutes. This difference was noted at two minutes for SunCherry2.

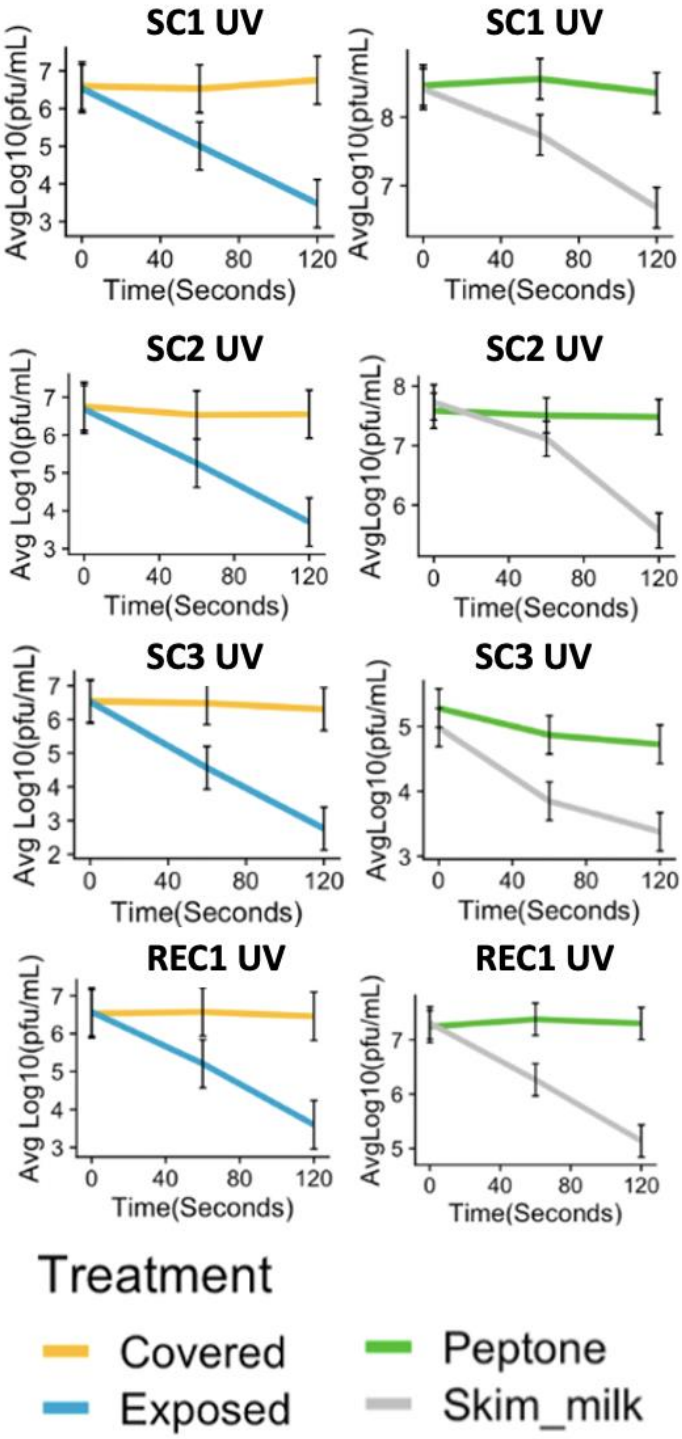

Figure 3.6. Impact of UV-C radiation exposure on the survivability of SunCherry isolates (SC1-3) and REC1. Treatments conditions included covered, exposed, 5 % peptone, and 0.75 % skim milk. Error bars indicate standard error ($p<0.05$). Each data point represents the average of three experimental replicates and three technical replicates.

Bacteriophage population stability under differing pH and temperature conditions. The SunCherry isolates and REC1 were diluted to 1 x 10^6 pfu ml^{-1} in 1.5 ml of phage buffer adjusted to pH 4, 7, and 10, respectively. The titer of the SunCherry isolates was ascertained after 48-hrs (Figure 3.7). SunCherry1 and SunCherry3 had significantly lower titers after 48-hrs of incubation at pH 4. REC1 had significantly lower titer after 48-hrs of incubation at pH 10. SunCherry2 remained stable at all three pH levels after 48-hrs.

For the differential temperature assay, the titers of the SunCherry isolates and REC1 were evaluated on day one with two experimental replicates and three technical replicates averaged together. Aliquots of the bacteriophage isolates were incubated at three temperatures (4 ^oC, 21 ^oC, 37 ^oC) for 30 days at which point the titer of each incubated sample was reevaluated (Figure 3.8). The bacteriophage titers before and after incubation at these three temperatures was evaluated with paired t-tests ($p<0.05$). The titers of SunCherry1 and SunCherry2 were unstable at 21 ^oC and 37 ^oC and significantly decreased between day 1 and 30. The titers of REC1 remained stable at all three temperatures. The titer of SunCherry3 significantly decreased at each temperature over the incubation period.

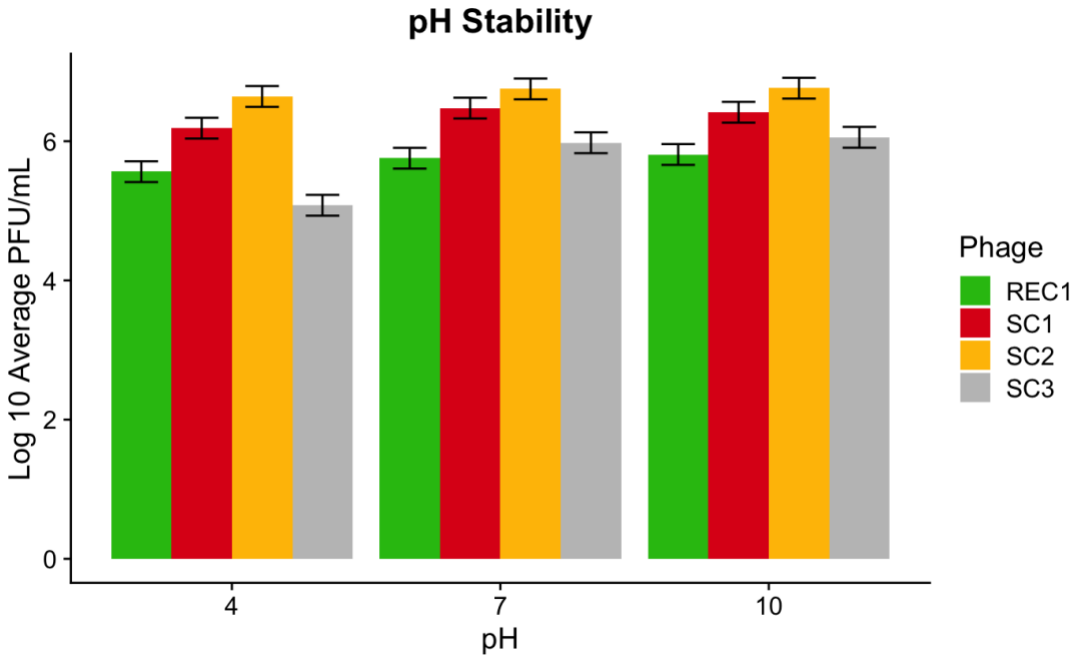

Figure 3.7. SunCherry and REC1 isolates titer stability after 48-hrs of exposure to pH 4, 7, and 10. Error bars indicate standard error ($p<0.05$). Each data point represents the average of two experimental replicates and three technical replicates.

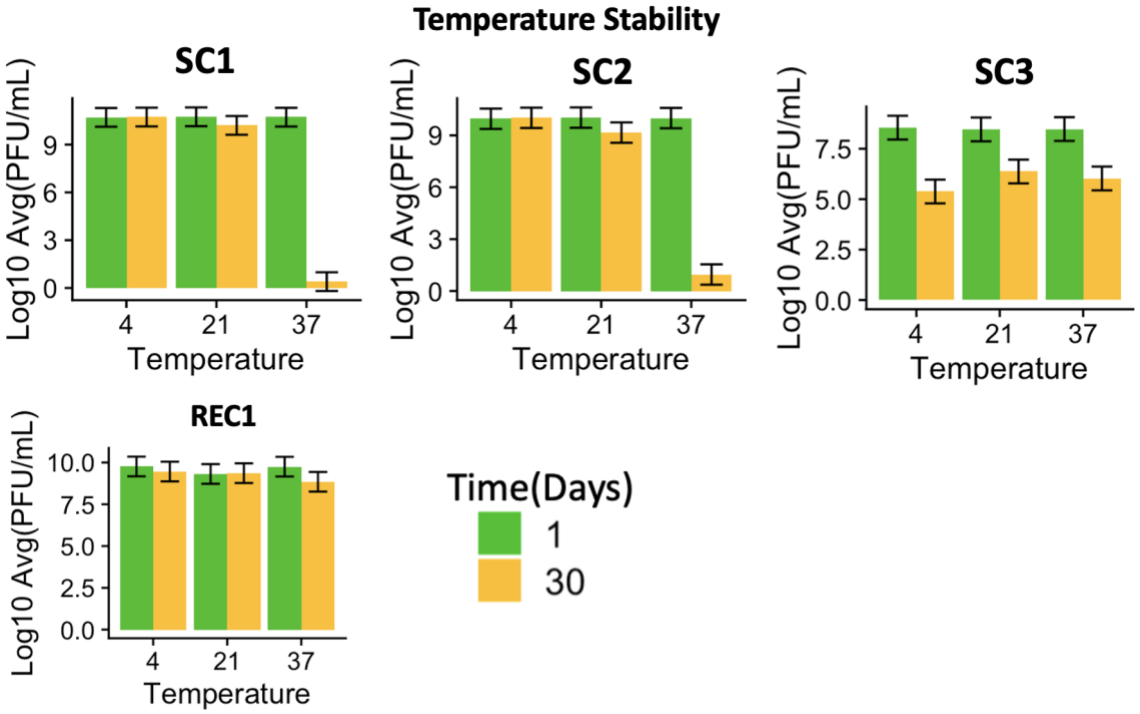

Figure 3.8. SunCherry and REC1 isolates titer stability after 30 days of incubation at 4 °C, 21 °C, 37 °C. Error bars indicate standard error ($p<0.05$). Each data point represents the average of two experimental replicates and three technical replicates.

***Pseudomonas syringae* pv. syringae population dynamics on sweet cherry flowers *in situ* treated with bacteriophage isolates.** The morning treatments tested two different formulations designed to reduce UV radiation exposure and increase the efficacy of the bacteriophage treatment. The 0.75 % skim milk was tested during 2022 and the 5 % peptone was tested during 2023. In keeping with the finding of Balogh et al. (2008), skim milk did not serve to bolster the bacteriophage treatments and instead was associated with an increase in bacterial populations, particularly in the MSU 2022 experiment. In contrast, the 5 % peptone control had a lower bacterial population at the 96-hr sampling time than either bacteriophage treatment in both the MSU and NWMHRC 2023 experiments. The morning SunCherry treatment was significantly lower than the water control in the MSU 2023 experiment at the 96-hr timepoint. This experiment also had unique weather. It had a significantly lower Daily Light Integral than the MSU 2022 and NWHRC 2023 experiments, higher relative humidity than the MSU 2022 experiments, and it was colder than all three other experiments. Of note, these conditions were also present during a different experiment following the same protocols conducted by Lauwers (2022) in which a bacteriophage treatment was lower than the water control at one timepoint.

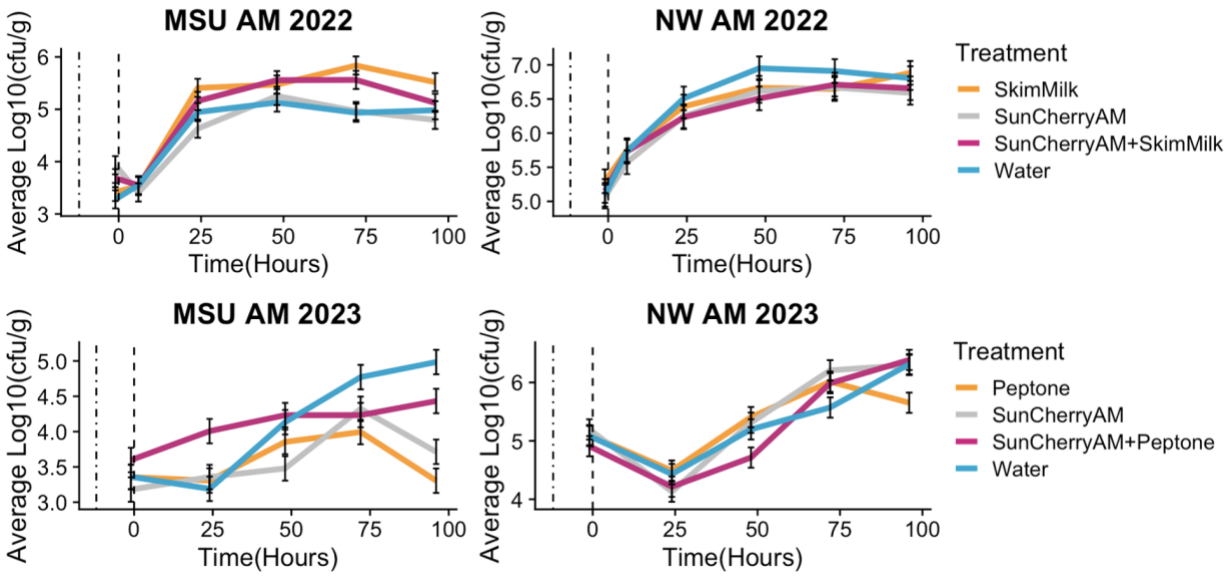

Figure 3.9. Population dynamics of *P. s.* pv. syringae applied to *P. avium* L. cv. 'Ulster' and cv. 'Benton' flowers and treated with a water control (blue), the SunCherry cocktail (grey), the SunCherry cocktail amended with 0.75 % skim milk or 5 % peptone (magenta), and 0.75 % skim milk or 5 % peptone (orange). Dashed-dot line (-12-hrs) indicates inoculation time and dashed line (0-hrs) indicates treatment application. MSU = MSU-PLP farm, NW = NWMHRC. Error bars indicate standard error ($p<0.05$). Each data point represents the average of four experimental replicates and three technical replicates.

An evening application of the SunCherry cocktail as well as the SunCherry cocktail with a 48-hr reapplication was tested to assess the impact of application timing on bacteriophage treatment efficacy (Figure 3.10). The evening application was not associated with a lower bacterial population as anticipated. However, this study did not yield the anticipated results. In three of the four experiments, the morning SunCherry treatment resulted in a lower bacterial population than the evening SunCherry treatments for at least one time point. Moreover, the SunCherry cocktail with a 48-hr reapplication had a reduction in bacterial population following the second treatment in only one experiment (MSU 2023). Both the 48-hr reapplication treatment and the morning and evening application comparison had unexpected results.

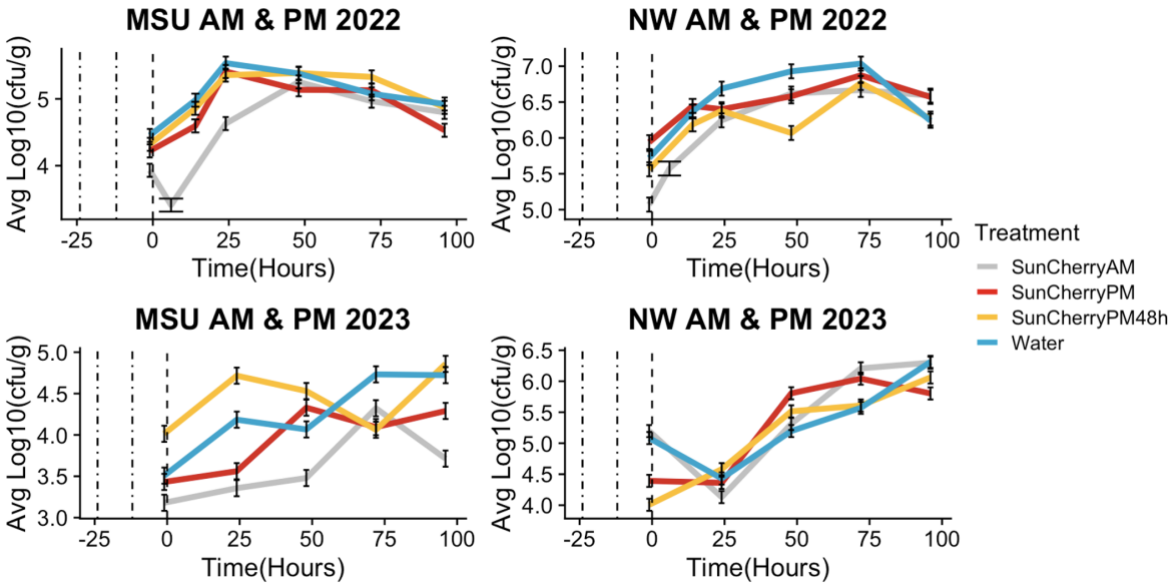

Figure 3.10. Population dynamics of *P. s.* pv. syringae applied to *P. avium* L. cv. 'Ulster' and cv. 'Benton' flowers and treated with a SunCherry cocktail in the evening (red) and morning (grey) as well as a SunCherry cocktail in the evening followed by a 48-hr reapplication (yellow) and a water control (blue). The left vertical dot-dash line (-25-hrs) on the graphs indicates the evening treatments' inoculation time. The right vertical dot-dash line (-12-hrs) shows the morning treatments' inoculation time. The dashed line (0-hrs) represents the application timing. MSU = MSU-PLP farm, NW = NWMHRC. Error bars indicate standard error ($p<0.05$). Each data point represents the average of four experimental replicates and three technical replicates.

The Michigan-specific SunCherry bacteriophages (Figure 3.10) were directly compared to the commercial Agriphage product designed for this pathosystem - the Nut and Stone Fruit bacteriophage cocktail (AgriPhage, Sandy, UT). This bacteriophage product was applied with the evening treatments in 2022 and with the morning treatments in 2023. The commercial product was diluted to match the titer of the SunCherry cocktail for all experiments. While the commercial cocktail had lower bacterial populations than the various treatments during the MSU 2022 experiment, this trend was not consistent across the four experiments. Moreover, the Agriphage treatment was significantly lower than the water control at the 0-hr (prior to treatment application) and 12-hr timepoints in the MSU 2022 experiment, which indicates that this difference could be associated with technical errors in inoculation rather than treatment efficacy.

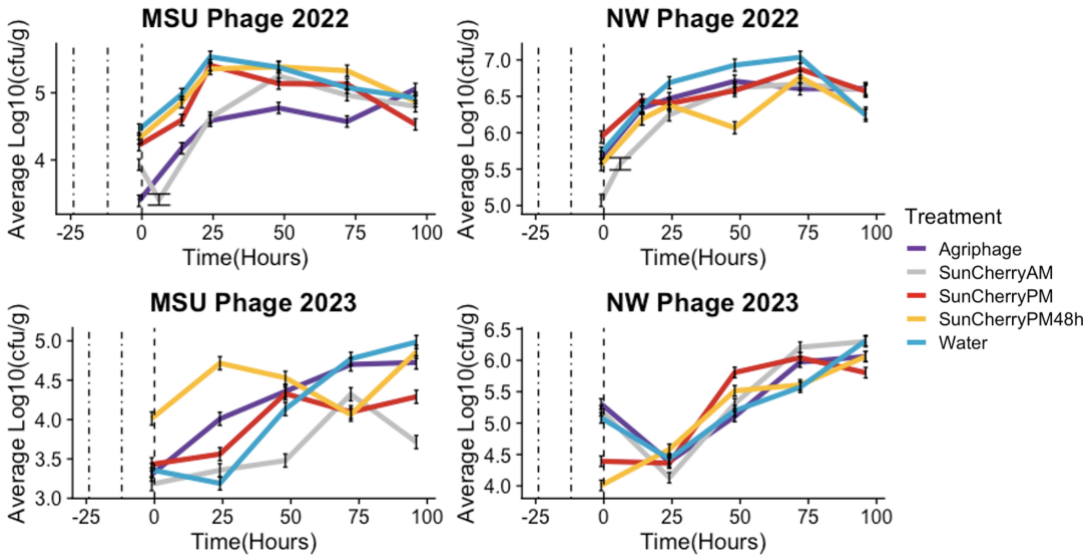

Figure 3.11. Population dynamics of *P. s.* pv. syringae applied to *P. avium* L. cv. 'Ulster' and cv. 'Benton' flowers and treated with a SunCherry cocktail in the evening (red) and morning (grey) as well as a SunCherry cocktail in the evening followed by a 48-hr reapplication (yellow) and a water control (blue). The Agriphage (purple) was applied in the evenings in 2022 experiments and in the morning in the 2023 experiments. The left vertical dot-dash line (-25-hrs) on the graphs indicates the evening treatments' inoculation time. The right vertical dot-dash line (-12-hrs) shows the morning treatments' inoculation time. The dashed line (0-hrs) represents the application timing. MSU = MSU-PLP farm, NW = NWMHRC. Error bars indicate standard error ($p<0.05$). Each data point represents the average of four experimental replicates and three technical replicates.

Kasugamycin, a currently available antibiotic for this pathosystem under the trade name Kasumin 2L, was also tested with the evening applications in these four experiments. This treatment consisted of 5 ml l^{-1} Kasumin 2L + 1.24 ml l^{-1} Li-700. The kasugamycin treatments were associated with a significant decline in the bacterial populations. However, the bacterial populations rebounded in 2022 by the last sampling time point at 96-hrs. This rebound was not observed in 2023. Additionally, the kasugamycin treated flowers had noticeable browning petals in 2022 which also was not observed in 2023.

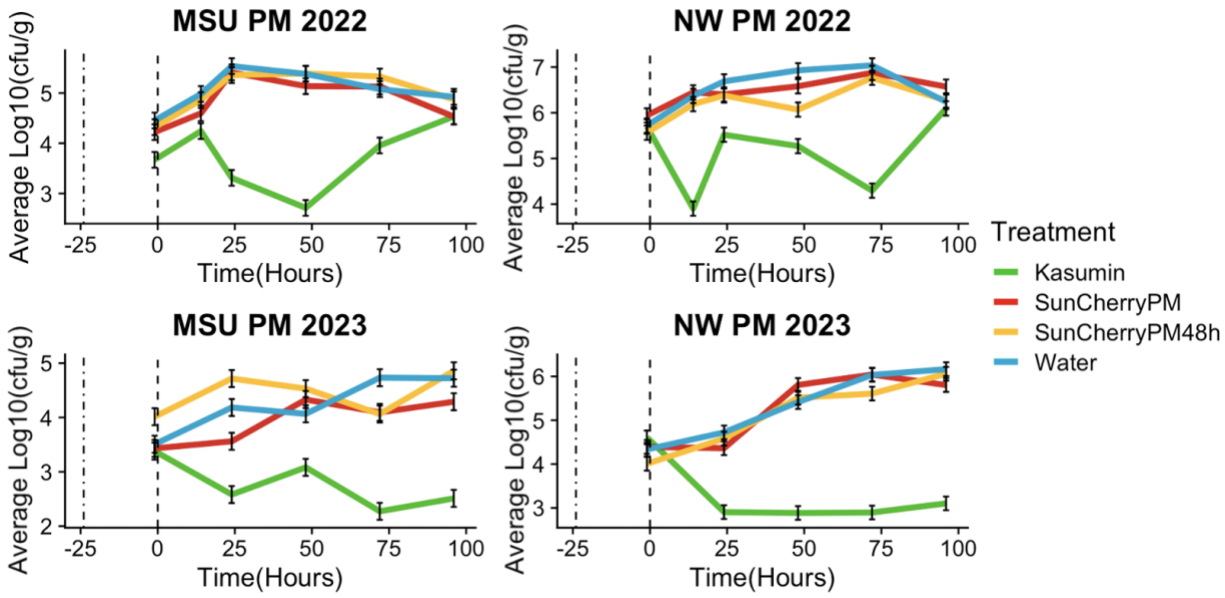

Figure 3.12. Population dynamics of *P. s.* pv. syringae applied to *P. avium* L. cv. 'Ulster' and cv. 'Benton' flowers and treated with kasugamycin (green), a SunCherry cocktail in the evening (red) and morning (grey) as well as a SunCherry cocktail in the evening followed by a 48-hr reapplication (yellow) and a water control (blue). The dot-dash line (-25-hrs) on the graphs indicates the evening treatments' inoculation time. The dashed line (0-hrs) represents the application timing. MSU = MSU-PLP farm, NW = NWMHRC. Error bars indicate standard error ($p<0.05$). Each data point represents the average of four experimental replicates and three technical replicates.

Weather data. Information regarding weather during field trials is displayed in Table 5. All experiments experienced an equivalently small volume of precipitation ($p>0.05$). MSU 2022 (55.9 %) and MSU 2023 (69.8 %) had a significantly different relative humidity ($p<0.05$). MSU 2023 was also significantly colder than the other three experiments with snow fall observed in the orchard during the experiment.

Table 3.2. Weather data for MSU Plant Pathology Farm and NWMHRC across all field experiment dates.

Location	Year	Time Point	Average Temperature (ºC)	Total Precipitation (mm)	Average Relative Humidity (%)	Daily Light Integral ($mol \cdot m^{-2} \cdot d^{-1}$)
MSU	2022	5/9	15.9	0	48.8	52
		5/10	20.4	0	51.5	31
		5/11	22.5	0	62.4	23
		5/12	21.0	0	47.8	50
		5/13	20.2	0	59.7	51
		5/14	22.4	5.33	65.4	37
NWMHRC	2022	5/12	24.2	0.25	61.7	27
		5/13	23.5	0	51.5	17
		5/14	19.3	0.25	63.9	38
		5/15	17.0	0	58	24
		5/16	13.1	0	58	21
		5/17	8.8	0	66	46
MSU	2023	4/20	13.1	0	70.7	26
		4/21	12.8	1.25	64.1	22
		4/22	5.8	4.06	74.5	17
		4/23	3.4	0	71.3	24
		4/24	3.9	0	63.5	22
		4/25	2.5	2.54	74.8	17
NWMHRC	2023	5/7	11.2	1.6	77.9	16
		5/8	12.9	0	68.4	36
		5/9	12.0	0	57.7	53
		5/10	14.6	0	62	53
		5/11	18.3	0	55.9	52
		5/12	19.0	0	58.1	39

3.5 Discussion

This study found that bacteriophage isolates local to Michigan cherry orchards were better able to lyse pathogenic *P. s.* pv. syringae isolates from the same geographic region than commercial bacteriophages, which were developed on strains from other regions of the United States of America. Gayder et al. (2019) demonstrated that locality can have a significant impact on the efficacy of a host-virus relationship wherein bacteriophages are better adapted to interact with bacteria from their region of origin. These findings support that relationship. The Michigan bacteriophage isolates were demonstrated to interact with significantly more Michigan pathogens of interest than a nonspecific commercial product. As such, commercial bacteriophage biological control treatments should be developed not only for a specific pathogen but also for the genetic diversity within geographic regions. This can improve the efficacy of a bacteriophage treatment to control for the diversity of bacterial pathogens in a given region which will in turn limit selection for bacteriophage resistant bacteria populations. Incorporating local bacteriophage isolates can improve the efficacy and longevity of treatment usage and should be made a priority for commercial bacteriophage development.

Reducing ultraviolet radiation is another primary hurdle in adapting bacteriophages for biological control applications. This study demonstrated that *in vitro* exposure to UV-C radiation significantly decreases the titer of all SunCherry isolates. The only SunCherry field treatment that was significantly lower than the water control (MSU 2023, SunCherryAM 96-hr) was associated with a significantly lower Daily Light Integral than the MSU 2022 and NWMHRC 2023 experiments. MSU 2023 had significantly colder temperatures than the other three field experiments as well. Of note, these lower Daily Light Integral conditions were also present during a different experiment conducted by Lauwers (2022) in which the bacterial population

associated with a bacteriophage treatment was lower than the water control at one timepoint. This confirms that reducing UV exposure is a critical factor in the success of bacteriophage treatments.

This study did not find that evening applications of bacteriophages controlled bacterial populations better than morning applications. However, the discrepancy between expectations and results could potentially be accounted for by the difference in time between inoculations and treatments. The morning treatments were applied 12- to 14-hrs post inoculation whereas evening treatments were applied 22- to 24-hrs post inoculation.

The treatment formulations designed to reduce UV exposure had similarly unexpected results. Balogh et al. (2003) found that skim milk additions can enhance bacteriophage longevity in a field setting. Further work by this author found that the skim milk formulation was associated with an increase in *Xanthomonas axonopodis* pv. citruli and pv. citrumelo populations (Balogh et al. 2008). In this study, 0.75 % skim milk did not prevent bacteriophage degradation following exposure to 180 J m^{-2} of UV-C radiation. The 0.75 % skim milk addition also contributed to bacterial growth in the 2022 field experiments. This phenomenon could be associated with the skim milk benefitting the *P. s.* pv. syringae population stability (Warburton & Pixton 1978; Balogh et al. 2008). *P. s.* pv. syringae strains have also been documented to hydrolyze casein; thus, skim milk could directly benefit the bacterial population (Goszczynska & Serfontein 1998). These potential confounding factors could have contributed to the bacterial growth observed in the 0.75 % skim milk formulations in the 2022 field experiments.

While the 0.75 % skim milk control had the highest bacterial populations in both 2022 morning experiments, the 5 % peptone control had the lowest bacterial populations in both 2023 morning experiments. Peptone is a primary ingredient in King's B medium, which is used

specifically to benefit bacterial growth. Most likely, the 5 % peptone-water control acted as a nonselective nutrient source for the microbial community in the phyllosphere, such as *Pantoea agglomerans* or *Pseudomonas fluorescens* (Torres et al. 2012; Makhzoum et al. 1995). This could have allowed for increased epiphytic competition or antibiosis and, consequently, a reduction in *P. s.* pv. syringae populations. Also of note, 5 % peptone was an effective UV-C protectant *in vitro* for the bacteriophage and could therefore serve a dual purpose in reducing bacterial populations. Peptone formulations for bacteriophage-based biological control treatments merits further investigation; for example, research can be conducted on the impact of different peptone-water concentrations on phyllosphere microbial community composition or the relationship between different peptone concentrations associated with bacteriophage treatments and pathogen populations.

This study also investigated two currently available commercial products: Kasumin 2L and the AgriPhage Nut and Stone Fruit bacteriophage cocktail. Kasumin 2L was the most consistently effective treatment for *P. s.* pv. syringae population reduction in a field setting. Despite this, bacterial populations rebounded in both 2022 field experiments and phytotoxicity was observed on Kasumin 2L-treated flowers in 2022. Additionally, Kasumin 2L has the underlying risk for the selecting for antibiotic resistance in the pathogen population. As such, further investigation into the efficacy of kasugamycin products is needed. The commercial bacteriophage product lysed 72 % of tested pathogenic *P. s.* pv. syringae isolates from Michigan cherry orchards, which could result in an increase in frequency of bacteria resistant to this bacteriophage product. Moreover, the current formulation of this cocktail did not significantly or consistently reduce bacterial populations in a field setting at the reduced rate (Figure 3.11) or at the full field rate (tested in 2023, not included here). These results indicate that an effective

control for bacterial canker of sweet cherry is still needed in Michigan. Further work in developing bacteriophage cocktails for field applications must focus on appropriate host ranges and environmental degradation mitigation. Overcoming these challenges will allow for effective phytobacteriology bacteriophage applications.

BIBLIOGRAPHY

Abbasi, V., Rahimian, H., & Tajick-Ghanbari, M. A. (2013). Genetic variability of Iranian strains of *Pseudomonas syringae* pv. syringae causing bacterial canker disease of stone fruits. *European Journal of Plant Pathology, 135*(2), 225–235. https://doi.org/10.1007/s10658-012-0095-1

Abedon, S. T. (2018). Detection of bacteriophages: Phage plaques. In D. R. Harper, S. T. Abedon, B. H. Burrowes, & M. L. McConville (Eds.), *Bacteriophages: Biology, Technology, Therapy* (pp. 1–32). Springer International Publishing. https://doi.org/10.1007/978-3-319-40598-8_16-1

Abedon, S. T., & LeJeune, J. T. (2005). Why bacteriophage encode exotoxins and other virulence factors. *Evolutionary Bioinformatics*, 1. https://doi.org/10.1177/117693430500100001

Adachi, N., Tsukamoto, S., Inoue, Y., & Azegami, K. (2012). Control of bacterial seedling rot and seedling blight of rice by bacteriophage. *Plant Disease, 96*(7), 1033–1036. https://doi.org/10.1094/PDIS-03-11-0232-RE

Adriaenssens, E. M., Van Vaerenbergh, J., Vandenheuvel, D., Dunon, V., Ceyssens, P.-J., De Proft, M., Kropinski, A. M., Noben, J.-P., Maes, M., & Lavigne, R. (2012). T4-Related bacteriophage LIMEstone isolates for the control of soft rot on potato caused by '*Dickeya solani*.' *PLoS ONE, 7*(3). https://doi.org/10.1371/journal.pone.0033227

Akbaba, M., & Ozaktan, H. (2021). Evaluation of bacteriophages in the biocontrol of *Pseudomonas syringae* pv. syringae isolated from cankers on sweet cherry (*Prunus avium* L.) in Turkey. *Egyptian Journal of Biological Pest Control, 31*(1), 35. https://doi.org/10.1186/s41938-021-00385-7

Allen, W. R., & Dirks, V. A. (1978). Bacterial canker of sweet cherry in the Niagara peninsula of Ontario: *Pseudomonas* species involved and cultivar susceptibilities. *Canadian Journal of Plant Science, 58*(2), 363–369. https://doi.org/10.4141/cjps78-057

Andersen, K. L., Sebolt, A. M., Sundin, G. W., & Iezzoni, A. F. (2018). Assessment of the inheritance of resistance and tolerance in cherry (*Prunus* sp.) to *Blumeriella jaapii*, the causal agent of cherry leaf spot. *Plant Pathology, 67*(3), 682–691. https://doi.org/10.1111/ppa.12765

Bae, J. Y., Wu, J., Lee, H. J., Jo, E. J., Murugaiyan, S., Chung, E., & Lee, S.-W. (2012). Biocontrol potential of a lytic bacteriophage PE204 against bacterial wilt of tomato. *Journal of Microbiology and Biotechnology, 22*(12), 1613–1620. https://doi.org/10.4014/jmb.1208.08072

Bai, J., Liu, Y., Liu, M., Luo, S., Cheng, Y., Li, G., Liu, C., Wen, S., Xia, M., He, X., & Jin, Y. (2022). Application of phage therapy against red-fleshed kiwifruit canker. *Biological Control*, 169, 104893. https://doi.org/10.1016/j.biocontrol.2022.104893

Balaž, J., Iličić, R., Ognjanov, V., Ivanović, Ž., & Popović, T. (2016). Etiology of bacterial canker on young sweet cherry trees in Serbia. *Journal of Plant Pathology*, *98*(2), 285–294.

Ballio, A., Barra, D., Bossa, F., Collina, A., Grgurina, I., Marino, G., Moneti, G., Paci, M., Pucci, P., Segre, A., & Simmaco, M. (1991). Syringopeptins, new phytotoxic lipodepsipeptides of *Pseudomonas syringae* pv. syringae. *FEBS Letters*, *291*(1), 109–112. https://doi.org/10.1016/0014-5793(91)81115-O

Balogh, B., Jones, J. B., Momol, M. T., Olson, S. M., Obradovic, A., King, P., & Jackson, L. E. (2003). Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. *Plant Disease*, *87*(8), 949–954. https://doi.org/10.1094/PDIS.2003.87.8.949

Balogh, B., Canteros, B. I., Stall, R. E., & Jones, J. B. (2008). Control of citrus canker and citrus bacterial spot with bacteriophages. *Plant Disease*, *92*(7), 1048–1052. https://doi.org/10.1094/PDIS-92-7-1048

Bedford, K. E., Sholberg, P. L., & Kappel, F. (2003). Use of a detached leaf bioassay for screening sweet cherry cultivars for bacterial canker resistance. *Acta Horticulturae*, 622, 365–368. https://doi.org/10.17660/ActaHortic.2003.622.37

Beltrán, M. F., Osorio, V., Lemus, G., Millas, P., France, A., Correa, F., Sagredo, B., Beltrán, M. F., Osorio, V., Lemus, G., Millas, P., France, A., Correa, F., & Sagredo, B. (2021). Bacterial community associated with canker disease from sweet cherry orchards of central valley of Chile presents high resistance to copper. *Chilean Journal of Agricultural Research*, *81*(3), 378–389. https://doi.org/10.4067/S0718-58392021000300378

Bender, C. L., & Scholz-Schroeder, B. K. 2004. New insights into the biosynthesis, mode of action, and regulation of syringomycin, syringopeptin, and coronatine. *Virulence and Gene Regulation*, 125–158. https://doi.org/10.1007/978-1-4419-9084-6_4

Bereswill, S., Bugert, P., Völksch, B., Ullrich, M., Bender, C. L., & Geider, K. (1994). Identification and relatedness of coronatine-producing *Pseudomonas syringae* pathovars by PCR analysis and sequence determination of the amplification products. *Applied and Environmental Microbiology*, *60*(8), 2924–2930. https://doi.org/10.1128/aem.60.8.2924-2930.1994

Bitton, G. (1975). Adsorption of viruses onto surfaces in soil and water. *Water Research*, *9*(5), 473–484. https://doi.org/10.1016/0043-1354(75)90071-8

Born, Y., Bosshard, L., Duffy, B., Loessner, M. J., & Fieseler, L. (2015). Protection of *Erwinia amylovora* bacteriophage Y2 from UV-induced damage by natural compounds. *Bacteriophage*, *5*(4), e1074330. https://doi.org/10.1080/21597081.2015.1074330

Bradbury, J. F. (1986). *Pseudomonas syringae* pv. syringae. In Guide to Plant Pathogenic Bacteria. (175-177). CAB International Mycological Institute, Kew, England.

Brüssow, H., Canchaya, C., & Hardt, W.D. (2004). Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiology and Molecular Biology Reviews*, *68*(3), 560–602. https://doi.org/10.1128/mmbr.68.3.560-602.2004

Boulé, J., Sholberg, P. L., Lehman, S. M., O'gorman, D. T., & Svircev, A. M. (2011). Isolation and characterization of eight bacteriophages infecting *Erwinia amylovora* and their potential as biological control agents in British Columbia, Canada. *Canadian Journal of Plant Pathology*, *33*(3), 308–317. https://doi.org/10.1080/07060661.2011.588250

Bull, C. T., De Boer, S. H., Denny, T. P., Firrao, G., Saux, M. F.-L., Saddler, G. S., Scortichini, M., Stead, D. E., & Takikawa, Y. (2010). Comprehensive list of names of plant pathogenic bacteria., 1980-2007. *Journal of Plant Pathology*, *92*(3), 551–592.

Bultreys, A., & Kaluzna, M. (2010). Bacterial cankers caused by *Pseudomonas syringae* on stone fruit species with special emphasis on the pathovars syringae and morsprunorum race 1 and race 2. *Journal of Plant Pathology*, 92, S21–S33.

Burke, M. J., & Lindow, S. E. (1990). Surface properties and size of the ice nucleation site in ice nucleation active bacteria: Theoretical considerations. *Cryobiology*, *27*(1), 80–84. https://doi.org/10.1016/0011-2240(90)90054-8

Bushnell, B. (2017). BBTools - sourceforge.net/projects/bbmap/.

Cao, T., Duncan, R. A., Kirkpatrick, B. C., Shackel, K. A., & DeJong, T. M. (2013). Effect of calcium and nitrogen fertilization on bacterial canker susceptibility in stone fruits. Fruits, *68*(3), 245–254. https://doi.org/10.1051/fruits/2013071

Cameron, H. R. (Herbert R. (1962). Diseases of deciduous fruit trees incited by *Pseudomonas syringae* van Hall: A review of the literature with additional data. *Technical Bulletin 66*.

Carroll, J., Robinson, T., Burr, T., Hoying, S., & Cox, K. (2010). Evaluation of pruning techniques and bactericides to manage bacterial canker of sweet cherry. *New York Fruit Quarterly*, *18*(1), 8.

Cemen, A., Saygili, H., Horuz, S., & Aysan, Y. (2021). Potential of bacteriophages to control bacterial speck of tomato (*Pseudomonas syringae* pv. tomato). *Fresenius Environmental Bulletin*, 27, 9366–9373.

Civerolo, E.L. (1970). Comparative relationships between two *Xanthomonas pruni* bacteriophages and their bacterial host. *Phytopathology*, 60, 1385-1388.

Comeau, A. M., Hatfull, G. F., Krisch, H. M., Lindell, D., Mann, N. H., & Prangishvili, D. (2008). Exploring the prokaryotic virosphere. *Research in Microbiology*, *159*(5), 306–313. https://doi.org/10.1016/j.resmic.2008.05.001

Crosse, J. E. (1963). Bacterial canker of stone-fruits. *Annals of Applied Biology*, *52*(1), 97–104. https://doi.org/10.1111/j.1744-7348.1963.tb03731.x

Di Lallo, G., Evangelisti, M., Mancuso, F., Ferrante, P., Marcelletti, S., Tinari, A., Superti, F., Migliore, L., D'Addabbo, P., Frezza, D., Scortichini, M., & Thaller, M. C. (2014). Isolation and partial characterization of bacteriophages infecting *Pseudomonas* syringae pv. actinidiae, causal agent of kiwifruit bacterial canker. *Journal of Basic Microbiology*, *54*(11), 1210–1221. https://doi.org/10.1002/jobm.201300951

Freigoun, S. O., & Crosse, J. E. (1975). Host relations and distribution of a physiological and pathological variant of *Pseudomonas morsprunorum*. *Annals of Applied Biology*, *81*(3), 317–330. https://doi.org/10.1111/j.1744-7348.1975.tb01647.x

Feng, Y. Y., Ong, S. L., Hu, J. Y., Tan, X. L., & Ng, W. J. (2003). Effects of pH and temperature on the survival of coliphages MS2 and Qß *Journal of Industrial Microbiology and Biotechnology*, *30*(9), 549–552. https://doi.org/10.1007/s10295-003-0080-y

Flores, O., Retamales, J., Núñez, M., León, M., Salinas, P., Besoain, X., Yañez, C., & Bastías, R. (2020). Characterization of bacteriophages against *Pseudomonas syringae* pv. actinidiae with potential use as natural antimicrobials in kiwifruit plants. *Microorganisms*, *8*(7). https://doi.org/10.3390/microorganisms8070974

Frampton, R. A., Taylor, C., Holguín Moreno, A. V., Visnovsky, S. B., Petty, N. K., Pitman, A. R., & Fineran, P. C. (2014). Identification of bacteriophages for biocontrol of the kiwifruit canker phytopathogen *Pseudomonas syringae* pv. actinidiae. *Applied and Environmental Microbiology*, *80*(7), 2216–2228. https://doi.org/10.1128/AEM.00062-14

García, H., Miranda, E., Lopez, M., Parra, S., Rubilar, C., Silva-Moreno, E., Rubio, J., & Ramos, C. (2021). First report of bacterial canker caused by *Pseudomonas syringae* pv. morsprunorum race 1 on sweet cherry in Chile. *Disease Note.* https://doi.org/10.1094/PDIS-11-20-2524-PDN

Garcia, R., Piazza, G., Wen, Z., Pyle, D., & Solaiman, D. (2009). The non-nutritional performance characteristics of peptones made from rendered protein. *Journal of Industrial Microbiology & Biotechnology*, 37, 95–102. https://doi.org/10.1007/s10295-009-0652-6

Garita-Cambronero, J., Palacio-Bielsa, A., & Cubero, J. (2018). *Xanthomonas arboricola* pv. pruni, causal agent of bacterial spot of stone fruits and almond: Its genomic and phenotypic characteristics in the *X. arboricola* species context. *Molecular Plant Pathology*, *19*(9), 2053–2065. https://doi.org/10.1111/mpp.12679

Garrett, C. M. E. (1986). Influence of rootstock on the susceptibility of sweet cherry scions to bacterial canker, caused by *Pseudomonas syringae* pvs. morsprunorum and syringae. *Plant Pathology, 35*(1), 114–119. https://doi.org/10.1111/j.1365-3059.1986.tb01989.x

Gayder, S., Parcey, M., Castle, A. J., & Svircev, A. M. (2019). Host range of bacteriophages against a world-wide collection of *Erwinia amylovora* determined using a quantitative PCR assay. *Viruses*, *11*(10). https://doi.org/10.3390/v11100910

Gayder, S., Parcey, M., Nesbitt, D., Castle, A. J., & Svircev, A. M. (2020). Population dynamics between *Erwinia amylovora*, *Pantoea agglomerans* and bacteriophages: Exploiting synergy and competition to improve phage cocktail efficacy. *Microorganisms*, *8*(9). https://doi.org/10.3390/microorganisms8091449

Gilbert, V., Legros, F., Maraite, H., & Bultreys, A. (2009). Genetic analyses *of Pseudomonas syringae* isolates from Belgian fruit orchards reveal genetic variability and isolate-host relationships within the pathovar syringae and help identify both races of the pathovar morsprunorum. *European Journal of Plant Pathology*, *124*(2), 199–218. https://doi.org/10.1007/s10658-008-9406-y

Gormez, A., Sahin, F., Gulluce, M., & Aslan, I. (2013). Identification and characterization of *Pseudomonas syringae* isolated from apricot trees in the Erzurum province of Turkey and evaluation of cultivar reaction. *Journal of Plant Pathology*, *95*(3), 525–532.

Goszczynska, T., & Serfontein, J. J. (1998). Milk–Tween agar, a semiselective medium for isolation and differentiation of *Pseudomonas syringae* pv. syringae, *Pseudomonas syringae* pv. phaseolicola and *Xanthomonas axonopodis* pv. phaseoli. *Journal of Microbiological Methods*, *32*(1), 65–72. https://doi.org/10.1016/S0167-7012(98)00005-0

Guy, L., Kultima, J., & Andersson, S. (2010). genoPlotR: comparative gene and genome visualization in R. *Bioinformatics*, *26*(18), 2334-2335.

Harper, D., & Enright, M. (2011). Bacteriophages for the treatment of *Pseudomonas aeruginosa* infections. *Journal of Applied Microbiology*, *111*(1), 1–7. https://doi.org/10.1111/j.1365-2672.2011.05003.x

Heffernan, J. R., Katumba, G. L., McCoy, W. H., & Henderson, J. P. (2023). Yersiniabactin is a quorum sensing autoinducer and siderophore in uropathogenic *Escherichia coli*. *bioRxiv.* https://doi.org/10.1101/2023.02.09.527953

Heimann, M., & Hudelson, B. (2004) Bacterial Canker. Wisconsin Horticulture. Retrieved June 16, 2022, from https://hort.extension.wisc.edu/articles/bacterial-canker

Hirano, S. S., & Upper, C. D. (2000). Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*-a pathogen, ice nucleus, and epiphyte. *Microbiology and Molecular Biology Reviews: MMBR*, *64*(3), 624–653. https://doi.org/10.1128/MMBR.64.3.624-653.2000

Hobbs, Z., & Abedon, S. T. (2016). Diversity of phage infection types and associated terminology: The problem with 'Lytic or lysogenic.' *FEMS Microbiology Letters*, *363*(7). https://doi.org/10.1093/femsle/fnw047

Hulin, M. T., Armitage, A. D., Vicente, J. G., Holub, E. B., Baxter, L., Bates, H. J., Mansfield, J. W., Jackson, R. W., & Harrison, R. J. (2018). Comparative genomics of *Pseudomonas syringae* reveals convergent gene gain and loss associated with specialization onto cherry (*Prunus avium*). *New Phytologist*, *219*(2), 672–696. https://doi.org/10.1111/nph.15182

Hulin, M. T., Jackson, R. W., Harrison, R. J., & Mansfield, J. W. (2020). Cherry picking by pseudomonads: After a century of research on canker, genomics provides insights into the evolution of pathogenicity towards stone fruits. *Plant Pathology*, *69*(6), 962–978. https://doi.org/10.1111/ppa.13189

Hulin, M. T., Mansfield, J. W., Brain, P., Xu, X., Jackson, R. W., & Harrison, R. J. (2018). Characterization of the pathogenicity of strains of *Pseudomonas syringae* towards cherry and plum. *Plant Pathology*, *67*(5), 1177–1193. https://doi.org/10.1111/ppa.12834

Hulin, M. T., Vadillo Dieguez, A., Cossu, F., Lynn, S., Russell, K., Neale, H. C., Jackson, R. W., Arnold, D. L., Mansfield, J. W., & Harrison, R. J. (2021). Identifying resistance in wild and ornamental cherry towards bacterial canker caused by Pseudomonas syringae. *Plant Pathology*. https://doi.org/10.1111/ppa.13513

Hutchison, M. L., & Gross, D. C. (1997). Lipopeptide phytotoxins produced by *Pseudomonas syringae* pv. syringae: Comparison of the biosurfactant and ion channel-forming activities of syringopeptin and syringomycin. *MPMI*, 10, 347–354.

Hyman, P., & Abedon, S. T. (2010). Bacteriophage host range and bacterial resistance. *Advances in Applied Microbiology*, 70, 217–248. https://doi.org/10.1016/S0065-2164(10)70007-1

Iličić, R., Balaž, J., Ognjanov, V., & Popović, T. (2021). Epidemiology studies of *Pseudomonas syringae* pathovars associated with bacterial canker on the sweet cherry in Serbia. *Plant Protection Science*, 57, 196–205. https://doi.org/10.17221/140/2020-PPS

Iriarte, F. B., Balogh, B., Momol, M. T., Smith, L. M., Wilson, M., & Jones, J. B. (2007). Factors affecting survival of bacteriophage on tomato leaf surfaces. *Applied and Environmental Microbiology*, *73*(6), 1704–1711. https://doi.org/10.1128/AEM.02118-06

James, S. L., Rabiey, M., Neuman, B. W., Percival, G., & Jackson, R. W. (2020). Isolation, Characterization and experimental evolution of phage that infect the horse chestnut tree pathogen, *Pseudomonas syringae* pv. aesculi. *Current Microbiology*, *77*(8), 1438–1447. https://doi.org/10.1007/s00284-020-01952-1

Janse, J. D. (2010). Diagnostic methods for phytopathogenic bacteria of stone fruits and nuts in COST 873. *EPPO Bulletin*, *40*(1), 68–85. https://doi.org/10.1111/j.1365-2338.2009.02356.x

Jones, A. (1971). Bacterial canker of sweet cherry in Michigan. *Plant Disease Reporter*, *55*(11), 961-965.

Jones, J. B., Jackson, L. E., Balogh, B., Obradovic, A., Iriarte, F. B., & Momol, M. T. (2007). Bacteriophages for plant disease control. *Annual Review of Phytopathology*, *45*(1), 245–262. https://doi.org/10.1146/annurev.phyto.45.062806.094411

Jurczak-Kurek, A., Gąsior, T., Nejman-Faleńczyk, B., Bloch, S., Dydecka, A., Topka, G., Necel, A., Jakubowska-Deredas, M., Narajczyk, M., Richert, M., Mieszkowska, A., Wróbel, B., Węgrzyn, G., & Węgrzyn, A. (2016). Biodiversity of bacteriophages: Morphological and biological properties of a large group of phages isolated from urban sewage. *Scientific Reports*, *6*(1). https://doi.org/10.1038/srep34338

Kałużna, M., Willems, A., Pothier, J. F., Ruinelli, M., Sobiczewski, P., & Puławska, J. (2016). Characterization and genetic diversity of causal agent of stone fruit bacterial canker *Pseudomonas cerasi*, a new pathogen of cherry. *Acta Horticulturae*, 1149, 9–14. https://doi.org/10.17660/ActaHortic.2016.1149.2

Kałużna, M., Willems, A., Pothier, J. F., Ruinelli, M., Sobiczewski, P., & Puławska, J. (2016). *Pseudomonas cerasi* sp. Nov. (Non Griffin, 1911) isolated from diseased tissue of cherry. *Systematic and Applied Microbiology*, *39*(6), 370–377. https://doi.org/10.1016/j.syapm.2016.05.005

Kamiunten, H., Nakao, T., & Oshida, S. (2000). *Pseudomonas syringae* pv. cerasicola, pv. nov., the causal agent of bacterial gall of cherry tree. *Journal of General Plant Pathology*, *66*(3), 219–224. https://doi.org/10.1007/PL00012949

Keen, E. C. (2015). A century of phage research: Bacteriophages and the shaping of modern biology. *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology*, *37*(1), 6–9. https://doi.org/10.1002/bies.201400152

Kennelly, M., Cazorla, F., di Vicente, A., Ramos, C., & Sundin, G. (2007). *Pseudomonas syringae* diseases of fruit trees: Progress toward understanding and control. *Plant Disease*, *91*(1), 4-17. https://doi.org/10.1094/PD-91-0004

Kering, K. K., Kibii, B. J., & Wei, H. (2019). Biocontrol of phytobacteria with bacteriophage cocktails. *Pest Management Science*, *75*(7), 1775–1781. https://doi.org/10.1002/ps.5324

Kimmelshue, C., Goggi, A. S., & Cademartiri, R. (2019). The use of biological seed coatings based on bacteriophages and polymers against *Clavibacter michiganensis* subsp. *Nebraskensis* in maize seeds. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-54068-3

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, 301–307.

Latorre, B. A., González, J. A., Cox, J. E., & Vial, F. (1985). Isolation of *Pseudomonas syringae* pv. syringae from cankers and effect of free moisture on its epiphytic populations on sweet cherry trees. *Plant Disease*, *69*(5), 409–412.

Latorre, B. A., & Jones, A. L. (1979). Evaluation of weeds and plant refuse as potential sources of inoculum of *Pseudomonas syringae* in bacterial canker of cherry. *Phytopathology*, *69*(10), 1122–1125.

Lauwers, Erin M. (2022). Investigation of bacteriophage as a biological control for bacterial canker of sweet cherry. [Master's thesis, Michigan State University]. https://doi.org/10.25335/Z00N-5S13

Li, H. (2018). Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics, 34:3094-3100. doi:10.1093/bioinformatics/bty191

Lillrose, T., Lang, G. A., & Sundin, G. W. (2017). Strategies to minimize bacterial canker in high density sweet cherry orchards. *Acta Horticulturae*, 1161, 457–462. https://doi.org/10.17660/ActaHortic.2017.1161.73

Liu, Y., Liu, M., Hu, R., Bai, J., He, X., & Jin, Y. (2021). Isolation of the novel phage PHB09 and its potential use against the plant pathogen *Pseudomonas syringae* pv. actinidiae. *Viruses*, *13*(11). https://doi.org/10.3390/v13112275

Makhzoum, A., Knapp, J. S., & Owusu, R. K. (1995). Factors affecting growth and extracelluar lipase production by *Pseudomonas fluorescens* 2D. *Food Microbiology*, 12, 277–290. https://doi.org/10.1016/S0740-0020(95)80108-1.

Martino, G., Holtappels, D., Vallino, M., Chiapello, M., Turina, M., Lavigne, R., Wagemans, J., & Ciuffo, M. (2021). Molecular characterization and taxonomic assignment of three phage isolates from a collection infecting *Pseudomonas syringae* pv. actinidiae and *P. syringae* pv. phaseolicola from northern Italy. *Viruses*, *13*(10), Article 10. https://doi.org/10.3390/v13102083

Maslova, M., Grosheva, E., Shamshin, I., Kuznetsova, A., & Fedorenko, A. (2020). The impact of *Pseudomonas syringae* bacteria on the plant pathogenic fungi and cherry plants. *BIO Web of Conferences*, 21. https://doi.org/10.1051/bioconf/20202100019

McGhee, G. C., & Sundin, G. W. (2011). Evaluation of kasugamycin for fire blight management, effect on nontarget bacteria, and assessment of kasugamycin resistance potential in *Erwinia amylovora*. *Phytopathology*, *101*(2), 192–204. https://doi.org/10.1094/PHYTO-04-10-0128

McKenna, F., El-Tarabily, K. A., Hardy, G. E. St. J., & Dell, B. (2001). Novel in vivo use of a polyvalent *Streptomyces* phage to disinfest *Streptomyces scabies*-infected seed potatoes. *Plant Pathology*, *50*(6), 666–675. https://doi.org/10.1046/j.1365-3059.2001.00648.x

Ménard, M., Sutra, L., Luisetti, J., Prunier, J. P., & Gardan, L. (2003). *Pseudomonas syringae* pv. avii (pv. nov.), the causal agent of bacterial canker of wild cherries (*Prunus avium*) in France. *European Journal of Plant Pathology*, *109*(6), 565–576. https://doi.org/10.1023/A:1024786201793

Mgbechi-Ezeri, J., Johnson, K., Porter, L., & Oraguzie, N. (2018). Development of a protocol to phenotype sweet cherry (*Prunus avium* L.) for resistance to bacterial canker. *Crop Protection*, *112*, 246-251. https://doi.org/10.1016/j.cropro.2018.06.009

Mirzaei, M. K., & Nilsson, A. S. (2015). Isolation of phages for phage therapy: A comparison of spot tests and efficiency of plating analyses for determination of host range and efficacy. *PLOS ONE*, *10*(3). https://doi.org/10.1371/journal.pone.0118557

Mo, Y. Y., Geibel, M., Bonsall, R. F., & Gross, D. C. (1995). Analysis of sweet cherry (*Prunus avium* L.) leaves for plant signal molecules that activate the *syr*B gene required for synthesis of the phytotoxin, syringomycin, by *Pseudomonas syringae* pv. syringae. *Plant Physiology*, 107, 603–612.

Morella, N. M., Gomez, A. L., Wang, G., Leung, M. S., & Koskella, B. (2018). The impact of bacteriophages on phyllosphere bacterial abundance and composition. *Molecular Ecology*, *27*(8), 2025–2038. https://doi.org/10.1111/mec.14542

Ni, P., Wang, L., Deng, B., Jiu, S., Ma, C., Zhang, C., Almeida, A., Wang, D., Xu, W., & Wang, S. (2020). Combined application of bacteriophages and carvacrol in the control of *Pseudomonas syringae* pv. actinidiae planktonic and biofilm forms. *Microorganisms*, *8*(6). https://doi.org/10.3390/microorganisms8060837

Ni, P., Wang, L., Deng, B., Jiu, S., Ma, C., Zhang, C., Almeida, A., Wang, D., Xu, W., & Wang, S. (2021). Characterization of a lytic bacteriophage against *Pseudomonas syringae* pv. actinidiae and its endolysin. *Viruses*, *13*(4). https://doi.org/10.3390/v13040631

Oksel, C., Avin, F. A., Mirik, M., & Baysal-Gurel, F. (2022). Identification and genetic characterization of *Pseudomonas syringae* pv. syringae from sweet cherry in Turkey. *Plant Disease*, *106*(4), 1253–1261. https://doi.org/10.1094/PDIS-10-21-2241-RE

Oliveira Lino, L., Pacheco, I., Mercier, V., Faoro, F., Bassi, D., Bornard, I., & Quilot-Turion, B. (2016). Brown rot strikes *Prunus* fruit: An ancient fight almost always lost. *Journal of Agricultural and Food Chemistry*, *64*(20), 4029–4047. https://doi.org/10.1021/acs.jafc.6b00104

Park, J., Lim, J.-A., Yu, J.-G., & Oh, C.-S. (2018). Genomic features and lytic activity of the bacteriophage PPPL-1 effective against *Pseudomonas syringae* pv. actinidiae, a cause of bacterial canker in kiwifruit. *28*(9), 1542–1546. https://doi.org/10.4014/jmb.1807.06055

Pinheiro, L. A. M., Pereira, C., Frazão, C., Balcão, V. M., & Almeida, A. (2019). Efficiency of phage φ6 for biocontrol of *Pseudomonas syringae* pv. syringae: An in vitro preliminary study. *Microorganisms, 7*(9), 286. https://doi.org/10.3390/microorganisms7090286

Pusey, P. L., Stockwell, V. O., Reardon, C. L., Smits, T. H. M., & Duffy, B. (2011). Antibiosis activity of *Pantoea agglomerans* biocontrol strain E325 against *Erwinia amylovora* on apple flower stigmas. *Phytopathology*, *101*(10), 1234–1241. https://doi.org/10.1094/PHYTO-09-10-0253

Rabiey, M., Roy, S. R., Holtappels, D., Franceschetti, L., Quilty, B. J., Creeth, R., Sundin, G. W., Wagemans, J., Lavigne, R., & Jackson, R. W. (2020). Phage biocontrol to combat *Pseudomonas syringae* pathogens causing disease in cherry. *Microbial Biotechnology*, *13*(5), 1428–1445. https://doi.org/10.1111/1751-7915.13585

Rahimi-Midani, A., Kim, J.-O., Kim, J. H., Lim, J., Ryu, J.-G., Kim, M.-K., & Choi, T.-J. (2020). Potential use of newly isolated bacteriophage as a biocontrol against *Acidovorax citrulli*. *Archives of Microbiology*, *202*(2), 377–389. https://doi.org/10.1007/s00203-019-01754-5

Renick, L. J., Cogal, A. G., & Sundin, G. W. (2008). Phenotypic and genetic analysis of epiphytic *Pseudomonas syringae* populations from sweet cherry in Michigan. *Plant Disease*, *92*(3), 372–378. https://doi.org/10.1094/PDIS-92-3-0372

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., & Lavigne, R. (2016). Characterization of novel bacteriophages for biocontrol of bacterial blight in leek caused by *Pseudomonas syringae* pv. porri. *Frontiers in Microbiology*, 7, 279. https://doi.org/10.3389/fmicb.2016.00279

Roos, I. M. M., & Hattingh, M. J. (1987). Pathogenicity and numerical analysis of phenotypic features of *Pseudomonas syringae* strains isolated from deciduous fruit trees. *Phytopathology, 77*(6), 900–908.

Saccardi, A., Gambin, E., Zaccardelli, M., Barone, G., & Mazzucchi, U. (1993). *Xanthomonas campestris* pv. pruni control trials with phage treatments on peaches in the orchard. *Phytopathologia Mediterranea, 32*(3), 206–210.

Saniewski, M., Ueda, J., Miyamoto, K., Horbowicz, M., & Puchalski, J. (2006). Hormonal control of gummosis in Rosaceae. *Journal of Fruit and Ornamental Plant Research*, 14, 137–144.

Sayler, R. J., & Kirkpatrick, B. C. (2003). The effect of copper sprays and fertilization on bacterial canker in French prune. *Canadian Journal of Plant Pathology*, *25*(4), 406–410. https://doi.org/10.1080/07060660309507097

Scheck, H. J., Canfield, M. L., Pscheidt, J. W., & Moore, L. W. (1997). Rapid evaluation of pathogenicity in *Pseudomonas syringae* pv. syringae with a lilac tissue culture bioassay and syringomycin DNA probes. *Plant Disease, 81*(8), 905–910. https://doi.org/10.1094/PDIS.1997.81.8.905

Scheck, H. J., Pscheidt, J. W., & Moore, L. W. (1996). Copper and streptomycin resistance in strains of *Pseudomonas syringae* from Pacific Northwest nurseries. *Plant Disease (USA)*.

Schellenberg, B., Ramel, C., & Dudler, R. (2010). *Pseudomonas syringae* virulence factor syringolin A counteracts stomatal immunity by proteasome inhibition. *Molecular Plant-Microbe Interactions®*, *23*(10), 1287–1293. https://doi.org/10.1094/MPMI-04-10-0094

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., Leipe, D., Mcveigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., Sullivan, J. P., Sun, L., Turner, S., & Karsch-Mizrachi, I. (2020). NCBI Taxonomy: A comprehensive update on curation, resources, and tools. https://doi.org/10.1093/database/baaa062

Scholz-Schroeder, B. K., Soule, J. D., & Gross, D. C. (2003). The sypA, sypB, and sypC synthetase genes encode twenty-two modules involved in the nonribosomal peptide synthesis of syringopeptin by *Pseudomonas syringae* pv. syringae B301D. *Molecular Plant-Microbe Interactions®*, *16*(4), 271–280. https://doi.org/10.1094/MPMI.2003.16.4.271

Scholz-Schroeder, B. K., Soule, J. D., Lu, S. E., Grgurina, I., & Gross, D. C. 2001. A physical map of the syringomycin and syringopeptin gene clusters localized to an approximately 145-kb DNA region of *Pseudomonas syringae* pv. syringae strain B301D. *Mol Plant Microbe Interact*, 14, 1426–1435.

Seemann, T. (2014). Prokka: Rapid prokaryotic genome annotation. *Bioinformatics (Oxford, England)*, *30*(14), 2068–2069. https://doi.org/10.1093/bioinformatics/btu153

Seemann, T. (2016). ABRicate: mass screening of contigs for antibiotic resistance genes. https://github.com/tseemann/abricate

Song, Y.-R., Vu, N. T., Park, J., Hwang, I. S., Jeong, H.-J., Cho, Y.-S., & Oh, C.-S. (2021). Phage PPPL-1, A new biological agent to control bacterial canker caused by *Pseudomonas syringae* pv. actinidiae in kiwifruit. *Antibiotics*, *10*(5). https://doi.org/10.3390/antibiotics10050554

Sorensen, K. N., Kim, K.-H., & Takemoto, J. Y. (1998). PCR detection of cyclic lipodepsinonapeptide-producing *Pseudomonas syringae* pv. syringae and similarity of strains. *Applied and Environmental Microbiology*, *64*(1), 226–230.

Spotts, R. A., Facteau, T. J., Cervantes, L. A., & Chestnut, N. E. (1990). Incidence and control of *Cytospora* canker and bacterial canker in a young sweet cherry orchard in Oregon. *Plant Disease*, *74*(8), 577–580.

Spotts, R. A., Wallis, K. M., Serdani, M., & Azarenko, A. N. (2010). Bacterial canker of sweet cherry in Oregon—Infection of horticultural and natural wounds, and resistance of cultivar and rootstock combinations. *Plant Disease*, *94*(3), 345–350. https://doi.org/10.1094/PDIS-94-3-0345

Sullivan, M. (2015). *Phage buffer*. https://www.protocols.io/view/Phage-Buffer-c5ey3d

Sundin, G. W., Jones, A. L., & Olson, B. D. (1988). Overwintering and population dynamics of *Pseudomonas syringae* pv. syringae and *P.s.* pv. morsprunorum on sweet and sour cherry trees. *Canadian Journal of Plant Pathology*, *10*(4), 281–288. https://doi.org/10.1080/07060668809501701

Sundin, G. W., & Bender, C. L. (1993). Ecological and genetic analysis of copper and streptomycin resistance in *Pseudomonas syringae* pv. syringae. *Applied and Environmental Microbiology*, *59*(4), 1018–1024. https://doi.org/10.1128/aem.59.4.1018-1024.1993

Sundin, G. W., & Wang, N. (2018). Antibiotic resistance in plant-pathogenic bacteria. *Annual Review of Phytopathology*, 56, 161–180. https://doi.org/10.1146/annurev-phyto-080417-045946

Svircev, A., Roach, D., & Castle, A. (2018). Framing the future with bacteriophages in agriculture. *Viruses*, *10*(5), 218. https://doi.org/10.3390/v10050218

Tarakanov, R. I., Lukianova, A. A., Evseev, P. V., Toshchakov, S. V., Kulikov, E. E., Ignatov, A. N., Miroshnikov, K. A., & Dzhalilov, F. S.-U. (2022). Bacteriophage control of *Pseudomonas savastanoi* pv. glycinea in soybean. *Plants*, *11*(7). https://doi.org/10.3390/plants11070938

Thomidis, T., & Exadaktylou, E. (2008). Susceptibility of 30 cherry (*Prunus avium*) genotypes to the bacterium *Pseudomonas syringae* pv. syringae. *New Zealand Journal of Crop and Horticultural Science*, *36*(3), 215–220. https://doi.org/10.1080/01140670809510237

Torres, R., Viñas, I., Usall, J., Remón, D., & Teixidó, N. (2012). Influence of diluent and sample processing methods on the recovery of the biocontrol agent *Pantoea agglomerans* CPA-2 from different fruit surfaces. *International Journal of Food Microbiology*, *158*(1), 85–88. https://doi.org/10.1016/j.ijfoodmicro.2012.06.019

Warburton, S., & Pixton, S. W. (1978). The moisture relations of spray dried skimmed milk. *Journal of Stored Products Research*, *14*(2), 143–158. https://doi.org/10.1016/0022-474X(78)90009-7

Wick, R. R., Judd, L. M., Gorrie, C. L., & Holt, K. E. (2017). Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLOS Computational Biology*, *13*(6), e1005595. https://doi.org/10.1371/journal.pcbi.1005595

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. https://ggplot2.tidyverse.org.

Wilke, C. (2021). cowplot: Streamlined plot theme and plot annotations for ggplot2. https://github.com/wilkelab/cowplot/

Wimalajeewa, D. L. S., & Flett, J. D. (1985). A study of populations of *Pseudomonas syringae* pv. syringae on stonefruits in Victoria. *Plant Pathology*, *34*(2), 248–254. https://doi.org/10.1111/j.1365-3059.1985.tb01356.x

Vacher, C., Hampe, A., Porté, A. J., Sauer, U., Compant, S., & Morris, C. E. (2016). The phyllosphere: microbial jungle at the plant–climate interface. *Annual Review of Ecology, Evolution, and Systematics*, *47*(1), 1–24. https://doi.org/10.1146/annurev-ecolsys-121415-032238

Vanneste, J. L. (2017). The Scientific, Economic, and Social Impacts of the New Zealand Outbreak of Bacterial Canker of Kiwifruit (*Pseudomonas syringae* pv. actinidiae). *Annual Review of Phytopathology*, *55*(1), 377–399. https://doi.org/10.1146/annurev-phyto-080516-035530

Vicente, J. G., Alves, J. P., Russell, K., & Roberts, S. J. (2004). Identification and discrimination of *Pseudomonas syringae* isolates from wild cherry in England. *European Journal of Plant Pathology*, *110*(4), 337–351. https://doi.org/10.1023/B:EJPP.0000021060.15901.33

Villalpando-Aguilar, J. L., Matos-Pech, G., López-Rosas, I., Castelán-Sánchez, H. G., & Alatorre-Cobos, F. (2023). Phage therapy for crops: Concepts, experimental and bioinformatics approaches to direct its application. *International Journal of Molecular Sciences*, *24*(1), Article 1. https://doi.org/10.3390/ijms24010325

Xin, X.-F., Kvitko, B., & He, S. Y. (2018). *Pseudomonas syringae*: What it takes to be a pathogen. *Nature Reviews. Microbiology*, *16*(5), 316–328. https://doi.org/10.1038/nrmicro.2018.17

Xu, N., Zhao, Q., Zhang, Z., Zhang, Q., Wang, Y., Qin, G., Ke, M., Qiu, D., Peijnenburg, W. J. G. M., Lu, T., & Qian, H. (2022). Phyllosphere microorganisms: Sources, drivers, and their interactions with plant hosts. *Journal of Agricultural and Food Chemistry*, *70*(16), 4860–4870. https://doi.org/10.1021/acs.jafc.2c01113

Young, J. M. (1987). Orchard management and bacterial diseases of stone fruit. *New Zealand Journal of Experimental Agriculture*, *15*(2), 257–266. https://doi.org/10.1080/03015521.1987.10425568

Young, J. M. (2010). Taxonomy of Pseudomonas Syringae. *Journal of Plant Pathology*, 92.

Yu, J.-G., Lim, J.-A., Song, Y.-R., Heu, S., Kim, G. H., Koh, Y. J., & Oh, C.-S. (2016). Isolation and characterization of bacteriophages against *Pseudomonas syringae* pv. actinidiae causing bacterial canker disease in kiwifruit. *Journal of Microbiology and Biotechnology*. *26*(2), 385–393. https://doi.org/10.4014/jmb.1509.09012

Zeller, W., Xie, Y., Bereswill, S., & Geider, K. (1997). Taxonomy and virulence of bacterial blight (*Pseudomonas syringae* pv. syringae) from pome fruit and stone fruit trees. *Developments of Plant Pathology*. (pp. 465–469). https://doi.org/10.1007/978-94-011-5472-7_83

Zhao, S., Guo, Y., Sheng, Q., & Shyr, Y. (2014). Heatmap3: An improved heatmap package with more powerful and convenient features. *BMC Bioinformatics*, *15*(10), 16. https://doi.org/10.1186/1471-2105-15-S10-P16