OPTIMIZING TRELLISED CANOPY ARCHITECTURE FOR SWEET CHERRY: TREE ESTABLISHMENT AND PROTECTION FROM BACTERIAL CANKER INFECTION

By

Tiffany Lillrose Law

A THESIS

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

Horticulture – Master of Science

2017

ABSTRACT

OPTIMIZING TRELLISED CANOPY ARCHITECTURE FOR SWEET CHERRY: TREE ESTABLISHMENT AND PROTECTION FROM BACTERIAL CANKER INFECTION

By

Tiffany Lillrose Law

Sweet cherries (*Prunus avium* L.) are typically grown as self-supported central or multiple leader trees. The new trellised "Upright Fruiting Offshoots" (UFO) training system for sweet cherry provides a narrow planar canopy to maximize light and spray distribution and increase orchard labor efficiency. Research determined training techniques at plant establishment that optimize early canopy development and yield potential. Bacterial canker (caused by *Pseudomonas syringae* pv. *syringae*) can reduce yields, cause loss of limbs, and even tree death, especially of young trees. Trellis wire and pruning wounds create opportunities for bacterial canker infection during tree establishment. Research to reduce such potential infections is critical to the adoption of new trellised training systems. Testing of possible control products for canker often yield variable results in the field and reliable testing protocols are needed. Experiments that focus on the effects of temperature, inoculum load, and plant wounding were conducted under controlled conditions to assess their impact on infection incidence. Infection potential due to trellis wire abrasion also was evaluated to determine the suitability of different wire types for cherry trellis systems. These studies contribute to the evolving development of systematic canker management recommendations for growers considering adoption of new cherry training systems. Successful canker management likely will be based on integrating environmental factors, plant susceptibility factors, timing of orchard tasks, and potential spray products.

To my family,
Thank you for everything.

ACKNOWLEDGEMENTS

I would like to thank Dr. Paolo Sabbatini, Dr. George Sundin, and Dr. Nikki Rothwell for serving on my committee and for helping guide my research. I would also like to thank Dr. Greg Lang for serving as my major advisor and for all the help he provided. I would also like to thank Tammy Wilkinson for always being there to help with my data and experiments; thank you for making me smile and laugh. I would also like to thank my family for all their support and patience throughout this process.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

CFU	Colony forming units
FAA	Formalin-acidic acid-alcohol
Gi3	Gisela 3
Gi5	Gisela 5
Gi6	Gisela 6
hrp	Hypersensitive response and pathogenicity
INA	Ice nucleation active
KGB	Kym Green Bush
MBS	Modified Brunner-Spindle
PBS	Phosphate buffered saline
PDV	Prune Dwarf Virus
PNRSV	Prunus Necrotic Ring-Spot Virus
PS	*Pseudomonas syringae*
PSA	*Pseudomonas syringae* pv. *actinidiae*
PSM	*Pseudomonas syringae* pv. *morsprunorum*
PSS	*Pseudomonas syringae* pv. *syringae*
PV	Perpendicular V
RCBD	Randomized Complete Block Design
S	Spindle
SB	Spanish Bush
SL	Steep Leader

SS	Slender Spindle
SSA	Super Slender Axe
TCSA	Trunk cross sectional area
TSA	Tall Spindle Axe
TT	Tatura Trellis
T3SS	Type III secretion system
UFO	Upright Fruiting Offshoots
Vase	Vase-Shaped
VCL	Vogel Central Leader
VSS	Vogel Slender Spindle
WIC	Water inoculated control with bactericides
WUC	Water uninoculated control with bactericides
WI2	Water inoculated two week control
WU2	Water uninoculated two week control
ZVA	Zahn Vertical Axe

CHAPTER 1: RECENT DEVELOPMENTS IN SWEET CHERRY ROOTSTOCKS AND TRAINING SYSTEMS

INTRODUCTION

Sweet cherry (*Prunus avium* L.) production has increased significantly in the past 20 years. However, reliable production can be difficult because cropping can be decimated by rain-induced cracking (Sekse, 1995), spring frost, birds, hail, and a myriad of insect and microbial pests. Sweet cherry orchards have traditionally featured large, vigorous trees that take many years to fill their orchard space and bear fruit. Developments in rootstock breeding over the past 35 years have provided precocious fruiting and dwarfism (Lang, 2000), and allowed high density training systems to be developed (Lang, 2005; Robinson, 2005). Smaller trees in high density orchards enable better protection of fruit by nets or covers, and better coverage of applied pesticides. High density training systems also help facilitate mechanization of harvest and pruning. By understanding the characteristics of new rootstocks and their interactions with high density training systems, combinations can be selected to maximize early yields of high quality fruit.

ROOTSTOCKS

Many new rootstocks have been developed that provide the traits necessary for high density sweet cherry production. Rootstock can influence traits such as vigor control, precocious fruiting, scion productivity, disease resistance, tolerance to adverse climatic or soil conditions, and scion compatibility. Each of these traits contributes to the overall success or failure of a rootstock in an orchard site.

Vigor control is essential for high density sweet cherry orchards because it can reduce pruning, reduce tree size for easier harvest, allow better spray and light distribution, and facilitate the use of covering structures to protect from birds, rain, or hail. Amount of vigor reduction can vary widely for a particular rootstock depending on soil characteristics, environmental conditions, scion, and orchard management (Long and Kaiser, 2010).

Precocious fruiting is promoted by some rootstocks and causes scions to come into production earlier than on traditional seedling rootstocks. Some trees on such rootstocks begin flowering in the third leaf (Lang, 2000), and full production is possible within 5-6, while trees on 'Mazzard' (*Prunus avium* L. seedlings or clones) can take up to 12 years for full production (Long and Kaiser, 2010). Earlier fruiting can increase early returns on the investment of establishing a new orchard. An economic study comparing a high density orchard on 'Gisela 6' (*Prunus cerasus* L. x *Prunus canescens* L., a precocious, semi-vigorous rootstock) with a standard density orchard planted on 'Mazzard' found that the high density orchard would break even in year 8, while the standard density orchard would not break even until year 15 (Seavert and Long, 2007).

Scion productivity also can be influenced by rootstock. Some interspecific hybrid Gisela rootstocks increase productivity 25-50% compared to 'Mazzard' (Lang, 2000). When highly productive rootstocks are paired with highly productive scions, trees can produce too many fruit, causing a reduction in fruit size. However, if crop load is properly managed in proportion to the canopy leaf area, high quality fruit can still be achieved (Lang, 2000).

Some rootstocks also confer resistance to diseases such as bacterial canker (caused by *Pseudomonas syringae* pv. *syringae*) (Krzesinska and Azarenko, 1992), phytophthora and

armillaria root rots (Lang, 2000), and the ilarviruses prune dwarf virus (PDV), and prunus necrotic rings-spot virus (PNRSV) (Lang et al., 1998; Lang and Howell, 2001; Long and Kaiser, 2010).

Rootstocks also vary in tolerance to poor soil or climatic conditions. Poor growing conditions often will increase the dwarfing effects of a rootstock, so soil type must be considered during rootstock selection. Rootstocks may be incompatible with certain scion cultivars and result in premature tree decline and death. For example, 'Mazzard' is compatible with all cultivars, but 'Mahaleb' (*Prunus mahaleb* L. seedlings or clones) is incompatible with some cultivars (Long and Kaiser, 2010).

As new cherry rootstocks are developed around the world and become available for testing, field trials are usually established to determine which are best suited to North American climates, and how they influence traits such as yield, yield efficiency (yield/trunk cross sectional area, TCSA), vigor, disease tolerance, suckering, and fruit quality. Rootstocks that have been tested in the NC140 regional trials across North America include Tabel Edabriz (*Prunus cerasus*) (Kappel et al., 2013), various *Prunus mahaleb* clones (St. Lucie series and Hungarian selections) (Perry et al., 1996; Perry et al., 1998), a wide range of Gisela interspecific hybrids (including *P. avium*, *P. cerasus*, *P. canescens*, and *Prunus fruticosa*) (Kappel et al., 2013; Facteau et al., 1996), the Weiroot series (*P. cerasus*) (Kappel et al., 2013), the Gran Manier interspecific hybrids (including *Prunus incisa*, *Prunus dawyckensis*, *Prunus serulata*, and *P. canescens*) (Perry et al., 1996; Perry et al., 1998; Facteau et al., 1996), and Mazzard x Mahaleb hybrids (Facteau et al., 1996; Perry et al., 1996; Perry et al., 1998) selections. Additional rootstocks derived from a wide range of *Prunus* species and not yet extensively tested in North America

include the PiKu series from Germany; Krymsk rootstocks from Russia; CAB 6P and CAB 11E from Italy; P-HL A, P-HL B, and P-HL C from the Czech Republic; and Cass, Clinton, Crawford, Clare, and Lake from Michigan State University (NC-140, 2015).

Testing is essential for selecting rootstocks that perform well in a particular climates and soil types, and also to assess the benefits and limitations of each rootstock. Many rootstocks are unsatisfactory candidates for high density cherry production because of traits such as: insufficient vigor control (or unsatisfactory dwarfing effects) (Facteau et al., 1996; Lang, 2000; Kappel et al., 2013; Perry et al., 1996), sensitivity to pollen-borne viruses such PDV and/or PNRSV (Lang et al., 1998; Lang and Howell, 2001; Long and Kaiser, 2010), excessive sucker production (Facteau et al., 1996; Kappel et al., 2013; Kemp and Wertheim, 1996; Perry et al., 1996; De Salvador et al., 2005), lack of precocity (Facteau et al., 1996; Kemp and Wertheim, 1996; Lang, 2000; Perry et al., 1996), scion incompatibility (Long and Kaiser, 2010), susceptibility to disease (Lang, 2000), insufficient winter hardiness (Lang, 2000), or low yield efficiency (Facteau et al., 1996; Kappel et al., 2013; Perry et al., 1996). Krymsk 5 (VSL 2) and Krymsk 6 (LC 52) are both hypersensitive to PDV and PNRSV (Long and Kaiser, 2010), and P-HL A, P-HL B, and P-HL C are thought to be drought sensitive (Lang, 2000). Gisela 5 and 6 have performed poorly in hot climates like California and Spain (Lang, 2000). CAB 6P and CAB 11 E both produce a number of suckers (De Salvador et al., 2005) that makes management difficult. This review will only include specifics on the most promising rootstocks for high density training systems in North America.

The current commercially-available Gisela rootstocks are hybrids of *P. cerasus* (sour cherry) and *P. canescens* (grey leaf cherry) and tend to perform well in temperate zone areas of

North America. Four rootstocks ('Gisela 3', 'Gisela 5', 'Gisela 6' and 'Gisela 12') exhibit superior traits. All are precocious with high productivity (Franken-Bembenek, 2004; Long and Kaiser, 2010; Kemp and Wertheim, 1996; Perry et al., 1998) but can have trouble with over-cropping if poorly managed (Lang, 2001; Long, 2001). These rootstocks also have low levels of suckering (Facteau et al., 1996; Franken-Bembenek, 2004; Kappel et al., 2013; Long and Kaiser, 2010), are winter hardy (Franken-Bembenek, 2004; Lang 2000; Long and Kaiser, 2010), have good scion compatibility (Franken-Bembenek, 2004; Long and Kaiser, 2010), and are tolerant to PDV and PNRSV (Lang et al., 1998; Lang and Howell, 2001).

'Gisela 3' (tested as Gi 209/1) is the most dwarfing of the recommended Gisela rootstocks. It reduces vigor by 20% more than 'Gisela 5' (Balmer and Blanke, 2005; Franken-Bembenek, 2004). Averaged across sites in the 1998 NC-140 trial, 'Gisela 3' reduced trunk cross-sectional area (TCSA) by 45% of 'Mazzard' (Kappel et al., 2013). In addition to its dwarfing nature, 'Gisela 3' is highly productive with cumulative yield efficiency (cumulative yield/TCSA) five times as high as 'Mazzard' and twice as high as 'Mahaleb' (Kappel et al., 2013). It is recommended for testing in high density orchards in good soils, provided it has irrigation, support, and good cultural management (Franken-Bembenek, 2004). However, in the NC-140 trial, 'Gisela 3' also produced the smallest fruit (Kappel et al., 2013), indicating that tree crop loads and leaf area must be managed to prevent over-cropping. In Germany, 'Gisela 5' planted at 2.5 m in-row spacing had a higher yield per hectare than 'Gisela 3' planted at 1.5 m, 2 m, or 2.5 m in-row spacing (Stehr, 2014). That study also found higher tree mortality after seven years on 'Gisela 3' than 'Gisela 5'.

‘Gisela 5’ (tested as 148/2) reduces vigor by 35–62% of ‘Mazzard’, ‘MxM.2’ and ‘F12/1’ (Facteau et al., 1996; Kappel et al., 2013; Kemp and Wertheim, 1996; Lang, 2000; Long and Kaiser, 2010; Perry et al., 1996; Robinson and Hoying, 2008; Whiting et al., 2005). It has high cumulative yield efficiency (twice that of ‘Mahaleb’ and five times that of ‘Mazzard’) (Kappel et al., 2013); however, the high fruit production and medium to low vigor can result in small fruit when leaf area and crop load are not in balance (Long and Kaiser, 2010). In the 1998 NC-140 trial, fruit size for ‘Gisela 5’ was between that of ‘Gisela 3’ and ‘Gisela 6’ (Kappel et al., 2013). Gislea 5 advances flowering and fruiting by 2-4 days (Long and Kaiser, 2010). This can extend the season with early cultivars, but also increases the risk of frost damage. ‘Gisela 5’ is recommended for high density orchards if planted in deep, fertile soils, irrigated, and pruned properly to manage crop load. Trees may need support to prevent leaning with prevailing winds (Long and Kaiser, 2010). ‘Gisela 5’ is drought sensitive (Lang, 2000) and irrigation should be considered. It also requires good drainage and does not do well in heavy soils (Long and Kaiser, 2010).

‘Gisela 6’ (tested as 148/1) has medium to high vigor, producing trees 38-120% the size of ‘Mazzard,’ but usually trees are 80-100% of ‘Mazzard’ or ‘MxM 2’ (Facteau et al., 1996; Kappel et al., 2013; Lang, 2000; Perry et al., 1996; Robinson and Hoying., 2008; Whiting et al., 2005). The high vigor more readily provides new shoot growth essential for balancing leaf area to fruit ratios for high quality fruit, compared to Gisela 3 or 5 (Long and Kaiser, 2010). In the 1998 NC-140 trial, ‘Gisela 6’ had larger fruit than ‘Gisela 5’ or ‘Gisela 3’, and had cumulative yield efficiency four times that of ‘Mazzard’ (Kappel et al., 2013). Like other Gisela rootstocks, pruning is required for high quality fruit. ‘Gisela 6’ tolerates both light and heavy soils, provided it has good drainage (Long and Kaiser, 2010), and is considered tolerant to anoxia (Lang, 2000).

It may need support to prevent leaning with prevailing winds (Long and Kaiser, 2010). 'Gisela 6' also may be susceptible to phytophthora root rot (Lang, 2000). Resistance of 'Gisela 6' to bacterial canker was similar to 'F12/1'in a lab assay (Krzesinska and Azarenko, 1992); however, in field testing and observations it appears to be more sensitive than Mazzard (Long and Kaiser, 2010; Spotts et al., 2010).

'Gisela 12' (Tested as 195/2) has vigor ranging 68-100% size of 'Mazzard' depending on scion (Facteau et al., 1996; Long and Kaiser, 2010; Perry et al., 1996; Robinson and Hoying, 2008). Early yield efficiency was 9 times that of 'Mazzard' (Facteau et al., 1996). Although it is productive, pruning is needed for good fruit size (Long and Kaiser, 2010). 'Gisela 12' is well anchored and can grow in a wide range of soils (Long and Kaiser, 2010). Similar to 'Gisela 6,' it may be susceptible to phytophthora root rot (Lang, 2000).

'MxM 60' (or 'Brooks 60') is a *P. avium* x *P. mahaleb* cross from Oregon. It tends to produce a large tree that is 63-218% of 'Mazzard' (Facteau et al., 1996; Perry et al., 1996). It had double the yield efficiency of 'Mazzard' 2 years after planting, but is less precocious than 'Gisela 6' or 'Gisela 12' (Facteau et al., 1996). For years 7-9 after planting, cumulative yield efficiency was similar to 'Mazzard' (Perry et al., 1996). It is drought tolerant (Lang, 2000), resistant to PDV and PNRSV (Lang et al., 1998), and similar to 'F12/1' in resistance to bacterial canker (Krzesinska and Azarenko, 1992).

'Maxma 14' is another *P. mahaleb* x *P. avium* cross from Oregon. It is considered semi-dwarfing (Long and Kaiser, 2010), but has not been widely tested in North America. In Germany, it produced smaller fruit and had lower yield efficiency than 'Gisela 5' or 'Gisela 6' with 'Regina' (Balmer, 2008). It is precocious, has low suckering, and good scion compatibility

(Long and Kaiser, 2010). It is resistant to iron-induced chlorosis caused by calcareous soils, and does well in different environments and a wide range of soil types (Long and Kaiser, 2010). Anecdotal observations suggest it may exhibit less incidence of bacterial canker than Gisela 6 (G.A.Lang, personal communication).

Consequently, a rootstock genotype can have unique trait combinations that make it better suited to different environments or training systems. Rootstock effects on tree vigor can vary widely depending on soil type, environmental conditions, and orchard management (e.g., irrigation). When selecting rootstocks, it is essential to reference the results from trials that had similar climatic (e.g., temperature, evapotranspiration, and rainfall) and soil (e.g. texture, drainage, pH, and fertility) conditions to gauge how a rootstock will perform in that particular site. Rootstocks should also be partnered with appropriate scion cultivars to balance rootstock productivity with scion productivity to reduce over-cropping. Similarly, the desired training system will influence rootstock selection, because vigor appropriate for the system is required for its optimal performance. Selecting the best rootstock for each orchard situation can increase orchard efficiency while optimizing early yield.

Prior to considering the details of particular training systems, it behooves us to go over some general concepts and techniques that are useful in high density training systems. The developmental morphology of a precocious fruiting branch is key to the foundational concepts of some high density training systems. The first year, a shoot grows and produces a single leaf at each node. The next year, that shoot usually has a one-time, small amount of non-spur fruit at its base, and each node is replaced by a non-fruiting multi-leafed spur. For the third (and subsequent) years, those nodes can become reproductive (the fruiting spurs) (Lang, 2005; Long

et al., 2015) and have the potential to bear fruit until they become damaged, shaded, or diseased. Therefore, trees on Gisela rootstocks can achieve significant flowering in the 3rd or 4th year in the orchard. Since each node grown in the first year can become a fruiting spur in year three, maximizing shoot growth in the first year can be an important step to increase yield potential in year 3 and beyond.

TRAINING CONCEPTS FOR HIGH QUALITY FRUIT

To produce high quality fruit, a tree must provide adequate carbohydrates for optimal fruit growth. Maximizing carbohydrates from photosynthesis, balancing crop load with leaf area, and minimizing waste of carbohydrates (such as for extraneous canopy development) are important for maximizing the production of large fruit with high soluble solids.

Adequate light distribution

Good light distribution throughout the tree canopy is essential for maximizing carbohydrate production because light drives photosynthesis. Carbohydrates from photosynthesis provide the building blocks for both new shoot and fruit growth. When light is limiting, techniques to increase light improve leaf sugar biosynthesis. Pruning decisions often are based on ensuring that each leaf receives an optimal amount of light for maximizing its photosynthetic potential, such as in grape (*Vitis vinifera* L.) (Smart and Robinson, 1991). In apple (*Malus* x *domestica* Borkh.), this concept is advanced by selecting adequate in-row and between-row spacing so that all the leaves in the canopy are exposed to sunlight during the day (Heinicke, 1964). A minimum of 30% of full sunlight is considered to be a baseline for leaves within a canopy to serve as photosynthetic sources for fruit development. A greater percentage of leaf

area of dwarf tree canopies reached this level of exposure than did standard or semi-dwarf canopies. By choosing an appropriate canopy shape (with a high proportion of leaf area exposed to sunlight) and keeping trees small (to reduce the volume to surface area ratio), growers can maximize canopy photosynthesis. In one apple study, shading reduced flower bud formation, fruit set, and yield (Jackson and Palmer, 1977). More shade produced a greater reduction in yield, with the most severe shading treatment reducing yield to less than half that of unshaded trees. Large trees have light available on the edges of the canopy but can be shaded in the center. Leaves and branches in the shaded part of the canopy will not produce carbohydrates as efficiently, yet still require carbohydrates for respiration. Some new training systems utilize narrow (or even planar) canopies to reduce shading and facilitate good spray distribution to reduce insect pest and disease problems.

Leaf area to fruit ratios and crop load management

Insufficient leaf area can limit fruit growth. Each leaf can only provide a fixed amount of carbohydrate to support fruit growth. As fruit number increases, carbohydrate supply can become limiting and result in smaller fruit (Whiting and Lang, 2004). Very productive rootstocks or varieties may require intervention to prevent over-cropping and small fruit. Increases in the leaf area to fruit ratio (LA:F) increased fruit size and soluble solids content in sweet cherry (Roper and Loescher, 1987). Chemical thinners have been developed for apple to reduce crop load. However, similar work for cherry has not progressed beyond the developmental stages (Whiting et al., 2006; Whiting and Lelahan, 2006; Schoedl et al. 2009). Currently no thinners are used in commercial sweet cherry production, and crop load is mostly managed through pruning.

Removal of fruiting wood and stimulation of new shoot growth will reduce fruit number and encourage more shoot growth (i.e., more leaf area) to supply the remaining fruit.

This non-fruiting leaf area is important for high fruit quality. When fruit are solely supplied by leaf area from fruiting spurs, fruit are smaller and have lower soluble solids (Ayala and Lang, 2004). In a simplified model, a ratio of 2 shoot segments with leaf spurs (including one segment with fruit) to 1 fruiting spur segment (2:1) is assumed to provide enough carbohydrate to not limit fruit growth on a three-year-old branch (Lang, 2005). Using this model as a guide for reducing crop load, it is recommended to remove 25%, 33%, 38.5%, and 42% of the potential crop load in years 4, 5, 6, and 7, respectively, and then 40-45% in year 8 and onward to maintain the trees at the 2:1 ratio found in year 3 (Lang, 2005).

Renewal Pruning

Fruit on young wood are larger and have higher soluble solids than fruit on older wood (Roper and Loescher, 1987). Furthermore, branches with large amounts of old and dying spurs become a drain on the tree's resources and reduce efficiency. The goal of renewal pruning is to remove these less productive branches and re-grow replacements that will produce higher quality fruit. If branches are thought of as renewable fruiting units, training systems then become a pattern of renewable fruiting units arranged on a structure of permanent wood. This concept of tree structure can simplify pruning decisions compared with traditional orchards that require experience to know which branches to prune and where to make the cuts. Maintenance pruning for some systems is as simple as removing 20% of the largest fruiting units. This not only promotes renewal of older branches, but also reduces crop load and encourages shoot growth to help balance the LA:F ratio. However, renewal pruning might not resolve all fruit quality issues

on highly-spurred cultivars (Lauri, 2005), and other techniques (such as spur extinction described below) may be used.

Minimizing waste of carbohydrate resources

Carbohydrate resources can be limited by the amount of leaf area, sunlight, and storage reserves available to a tree. Each branch requires carbohydrates that could otherwise be allocated to fruit. Some branches provide structural support (such as the trunk and scaffolds) or essential leaf area and are a necessary carbohydrate sink. In contrast, branches that are poorly placed or cause shading utilize carbohydrates that then must be removed through pruning, a loss of carbohydrates that otherwise could have been allocated to structural wood or fruit production. Using new techniques (described below), precision branch placement can reduce the incidence of poorly placed branches and allocate carbohydrate to long-term structural or fruiting branches.

When selecting training systems, important concepts for producing high quality fruit include 1) good light distribution throughout the canopy, 2) balancing the LA:F ratio (Lang, 2005), and 3) having renewable fruiting branches on minimal permanent wood. An ideal high density training system will incorporate these concepts and be easy to prune, train, and harvest.

TRAINING TECHNIQUES

Pruning

Dormant pruning is used in both traditional and high density sweet cherry orchards to remove fruiting wood to balance crop load, remove branches for renewal, or remove poorly placed, dead, or diseased wood. Two main types of pruning cuts are thinning and heading cuts.

Thinning cuts are made at the base of the branch to permanently remove branches that may be diseased or poorly positioned. They help to increase light distribution but do not stimulate as much re-growth as heading, and thereby reduce potential invigoration that may delay fruiting (Long et al., 2015). Heading cuts only remove part of a branch above a bud. When a branch is headed, it causes "reiteration" in which growth tends to replace the removed shoot (Lauri, 2005). Heading cuts can be used to renew or shorten branches, redirect growth, or stimulate new growth. Heading also promotes branching, but the resulting branches may be poorly distributed and/or have acute crotch angles (Hoying et al., 2001). Heading can be used to reduce crop load (sometimes called tipping) by removing potential fruiting spurs (Long et al., 2015).

Pruning in the summer can reduce the risk of bacterial canker infection. However, pruning during the dormant season can be used to reduce the following season's crop, as well as re-direct spring growth. Pruning during the summer may result in re-growth later in the summer that could be more susceptible to winter damage. Renewal pruning is usually done with a heading cut, by removing the branch but leaving a bud for reiteration. When heading with no visible vegetative buds, a 4-6 inch stub should be left (Long et al., 2015).

Short pruning is a new technique that uses heading cuts to moderate LA:F ratios and initiate new fruiting laterals. One-year-old precocious shoots are headed to retain the basal fruiting buds plus 1-3 vegetative buds for new shoot formation. This technique is used extensively in the Super Slender Axe (SSA) system (Long et al., 2015; Musacchi et al., 2015).

Techniques for precision branch placement

In sweet cherry, the terminal shoot meristem suppresses the growth of subtending lateral buds due to apical dominance, a type of paradormancy (Lang et al., 1987). This results in vigorous top growth and minimal lower canopy development. Although not fully understood, it is generally accepted that apical dominance is caused by the downward movement of auxin produced in the terminal meristem which suppresses the growth of the lower buds and branches (Leyser, 2005). Disruption of this flow of auxin may allow buds to elongate to become shoots. In training systems that need to distribute branches evenly, different techniques can be utilized to reduce the effect of apical dominance.

Hormone manipulation

Notching (or scoring), by cutting into the cambium just above a bud (Long et al., 2005), promotes branch placement by disrupting hormone flow and allowing the bud to begin growing (Hoying et al., 2001). A moderately coarse blade should be used (about 3/32 of an inch wide) and the cut should extend through the bark and green cambial layer (Long et al., 2005; Long et al., 2015). Scoring is effective over a range of dates (Long et al., 2005); however, caution should be used to avoid times when wounds could become infected by opportunistic pathogens. Promalin® is a combination of cytokinin (which promotes cell division) and gibberellins (which promote shoot extension). When applied to buds, it can alter the bud's hormone levels and cause a release from paradormancy, but effectiveness can vary by temperature (Lang, 2005). It should be applied to buds at the green tip stage of bud swell, and is most effective when warm temperatures follow application (Long et al., 2015). Promalin has also been shown to increase precocity cropping of 'Lapins' on 'Gisela 11' ('Gisela 11' is more precocious than 'Mazzard')

for the first two years of production (3rd and 4th leaf) (Long et al., 2005); this is logical, since promotion of a greater number of shoots during the first two years would lead to greater numbers of basal and spur flower buds in subsequent years. The technique of double sectorial pruning can promote formation of horizontal laterals, presumably by manipulating auxin concentrations. Branches are pruned to upward-oriented buds instead of a downward-oriented bud. This usually results in the most terminal new shoot growing vertically, but the secondary and tertiary new shoots that emerge will grow at more horizontal angles. Later, the most terminal new shoot is removed, leaving the flatter-angled subtending branches (Brunner et al., 1996).

Bud selection

When a significant portion of the vegetative buds on previous season growth are removed, the remaining vegetative buds are more likely to be released from paradomancy and grow into shoots (Lang, 2005; Long et al. 2015). Selective bud removal (selecting buds for placement of new branches and removing all others) has been more effective than Promalin or notching for producing laterals from remaining buds (or nodes) in the lower portions of the trunk (Hoying et al., 2001). Bud removal should be done during warm, dry weather when risk for bacterial canker infection is low. Furthermore, if some of the remaining buds fail to grow, the removal of buds can leave gaps in the fruiting canopy.

Bending

Bending can be used to help redistribute growth normally directed to terminals without creating wounds that might allow pathogen entry. Severe bending causes a reaction similar to reiteration caused by heading, although not as pronounced (Laurie, 2005). In apple, changing the

orientation of vertical branches released lower buds from apical dominance (Ferree and Schupp, 2003). As deviation from vertical increased, more buds were released. Timing and degree of bending is more important in cherry than in apple. Bending below horizontal reduced subsequent growth of terminal shoots and their subtending shoots, compared to unbent branches. The number of flower buds, and flowers per bud, increased with bending (Lauri et al., 1998). Some early high density cherry plantings tried to maintain trees size on full vigor rootstocks through limb bending, but with questionable success (Long et al., 2005). If bending is used, the increase in flower buds could necessitate additional manipulations to reduce crop load.

Techniques for crop load reduction

When trying to balance LA:F ratios, tipping of the previous season's growth can be used to reduce the future crop load (one year hence). As the growing season progresses, new vegetative node spacing decreases. This creates a potential future high spur density in the terminal portion of branches. By removing the future spur-dense region, less wood and leaf area is removed than if the same crop load reduction was done by removing entire branches. These tipping heading cuts also stimulate vegetative growth that will help increase the LA:F ratio.

"Spur extinction" is another technique used to permanently reduce crop load. In spur-dense situations, a portion of the spurs can be removed to reduce crop load. In the Solaxe training system (a system that utilizes bending), removing 30-50% of fruiting spurs is recommended to achieve a balance between increasing fruit size and decreasing crop yield (Claverie and Lauri, 2005).

HIGH DENSITY TRAINING SYSTEMS

Many different tree architectures have been developed, including spindles, vases, fruiting walls, and v-shaped trellis systems. Some systems are better suited to dwarfing or vigorous rootstocks, and performance of particular rootstock and training system combinations will vary depending on location due to site differences. Some training systems have similar canopy structures, but establish or manage trees differently. Becoming familiar with the benefits and requirements of different training systems will help determine which system will perform best for each orchard.

Spindle and Axe systems

High density training systems have been very successful in apple, which has served as a model for sweet cherry. Spindle systems provide good light distribution, are minimally pruned, and utilize branch bending to promote earlier cropping and help control vigor. Several variations of spindle training have been adapted to sweet cherry. The basic structure is a central leader tree with varying planting densities depending on training system and rootstock. There are many variations of spindle training and although many of the following spindles are similar and have similar names they are presented separately to provide more accurate information. Dwarfing or semi-dwarfing rootstocks work well for Tall Spindle Axe (TSA), Vogel Central Leader (VCL) (sometimes called Vogel Slender Spindle [VSS]) and Super Slender Axe (SSA) (Long et al., 2015). Modified Brunner-Spindle (MBS) works well with semi-dwarfing to vigorous rootstocks (Hrotko et al., 1998b). TSA can also perform well with a semi-vigorous rootstock (Long et al., 2015). Zahn Vertical Axe (ZVA), VCL, TSA, and Slender Spindle (SS) have in-row spacing of about 1.5-2.7 m and between-row spacing of 3.6-4.8 m (Hrotko et al., 1998a; Long et al., 2015;

Robinson et al., 2004). The MBS is less dense, with an in-row spacing of 3-4 m and between -ow spacing of 4.8-5.8 m (Hrotko et al., 1998b)). The SSA has the highest density, with in-row spacing of 0.5-1 m and only 3-3.5 m between rows (Long et al., 2015). Some spindle systems may require trellising, especially when using a dwarfing rootstock (Lauri, 2005; Long et al., 2015).

All spindle systems have a permanent central leader, but have different ways of distributing the fruiting wood that is developed on the leader. Central Leader (CL) trees typically have 2-3 whorls of scaffolds distributed up the trunk approximately 1 m apart (Whiting et al., 2005). TSA, VCL, ZVA, and MBS systems all develop a continuous whorl of wide-angled, lateral branches distributed evenly along the trunk (Hrotko et al., 1998b; Lang et al., 2014; Long et al., 2015; Robinson et al., 2004). The TSA, VCL, and ZVA systems utilize weights or clothespins to develop flat laterals (Long et al., 2015; Robinson et al., 2004). Other training techniques such as double sectorial pruning, heading, and bud removal, are used in various training systems to promote more horizontal or more evenly distributed branches. Hrotkó (2005) provided a comparison of particular training techniques for several of the spindle systems.

Renewal pruning is used in most of the spindle systems. The TSA and VCL systems renew all branches directly off the central leader over about 5 years (Lang et al., 2014; Long et al., 2015). The Spindle (S) and Slender Spindle (SS) both renew fruiting branches off of a few permanent scaffolds (Hrotko et al., 1998a; Musacchi et al., 2015). In contrast, the MBS and Free Spindle (FS) do not include renewal pruning in their training description (Hrotko et al., 1998b; Meland, 1998).

Pruning to maintain good light distribution is important. TSA, VCL, and VA are pruned to resemble a Christmas tree shape with longer branches low in the canopy and shorter branches toward the apex (Long et al., 2015; Meland, 1998). The ZVA differs from VCL by being more densely planted, retaining small feathers at planting, and using bud removal to improve distribution (Long et al., 2015; Robinson et al., 2004). TSA is based off of both ZVA and VCL. It uses bud activation techniques (e.g., bud selection or Promalin®) to stimulate a continuous whorl of branches. Tipping is used to balance crop load and all laterals are renewed over time (Long et al., 2015). TSA works better than VCL for productive varieties because TSA includes crop load management (Long et al., 2015).

The SSA is a unique spindle system because fruiting primarily occurs on the basal buds of precocious one-year-old shoots. This training system is not suitable for varieties that only produce spur fruit (Long et al., 2015; Musacchi et al., 2015). All fruiting laterals are renewed each year with short-pruning (Lang et al., 2014; Long et al., 2015; Musacchi et al., 2015). This training system produces very good quality fruit, but requires very high density because of its low tree yield. It is a simple training system but requires a lot of labor for the extensive annual short-pruning (Long et al., 2015).

Where comparison trials have been performed, ZVA had higher cumulative yields per tree than VCL (in this paper called VSS) (Robinson et al., 2004). The VA system had higher cumulative yields than FS (Meland, 1998). The S had higher cumulative yields per tree than SSA, but the SSA had higher cumulative yield per hectare because of the higher planting density (Musacchi et al., 2015). ZVA has performed better than some of the other spindle systems due to its high density and minimal pruning. The TSA and SSA systems are being evaluated for

performance on precocious dwarfing, semi-dwarfing, and semi-vigorous rootstocks at multiple sites across North America (Lang et al., 2014).

Vase or Bush types

The Vase-shaped (Vase), Goblet, Kym Green Bush (KGB) and Spanish Bush (SB) are self-supporting training systems that do not require trellising. These open centered, multi-leader training systems tend to be planted with semi-vigorous or vigorous rootstocks (Long et al., 2015; Moreno et al., 1998) because of the amount of vigor needed to build the scaffold branches.

KGB, SB, and Goblet are planted at lower densities of 2-3.5 m between trees and 4-5.5 m between rows (Green, 2005; Lauri, 2005; Long et al., 2015) and tested spacing for Vase range from 1.5-6 m in row and 4-6 m between rows (Meland, 1998; Moreno et al., 1998).

All systems are headed at planting to promote branching to develop multiple leaders (Lauri, 2005; Long et al., 2015; Meland, 1998). The Goblet system ties down branches to promote wide angles and develops 5-6 scaffold branches which support fruiting wood (Lauri, 2005). Vase, KGB, and SB also are headed during the first dormant season (Long, 2001; Long et al., 2015; Meland, 1998). The Vase system is left unpruned after scaffold development (Meland, 1998). The KGB and SB systems are headed once more during the following growing season to develop 12-20 upright leaders for SB and 20-30 for KGB (Green, 2005; Long et al., 2015). SB leaders are tipped during the second dormant season to promote branching because they fruit on laterals on the upright scaffold branches (Long et al., 2015). To help manage crop load, the KGB and SB systems utilize tipping to help balance LA:F ratios (Long et al., 2015).

Each year SB and KGB require renewal of 20% of laterals or upright leaders, respectively (Green, 2005; Lang et al., 2014; Long et al., 2015). KGB and SB differ in that the KGB fruits on renewed vertical leaders, and SB fruits on renewed laterals that arise from the permanent upright leaders (Long et al., 2015). If there is insufficient tree vigor, the number of KGB and SB leaders can be reduced to balance vigor with crop load (Long et al., 2015). Renewal also is used for the Goblet to ensure that fruiting wood on the scaffolds is never more than 4 years old (Lauri, 2005). The Goblet is a time consuming, labor intensive training system with delayed production. Furthermore, it allocates a lot of growth to 1-year-old shoots on the oblique leaders and scaffolds that must be removed (Lauri, 2005). In SB and KGB, severe pruning delays production, and modifications using bending and chemical treatment have been used to promote branching in SB without pruning to try to achieve a higher yield in the fourth year (Pérez, 2005). KGB and SB can be harvested without platforms, providing truly pedestrian orchards (Long et al., 2015). The main differences between these systems are: 1) the KGB fruits on spurs on upright leaders that are renewed, 2) SB fruits on lateral shoots that are renewed on permanent upright scaffolds, 3) the Goblet has fewer upright scaffolds (5-6) than the SB or KGB and fruits on laterals on those scaffolds, and 4) Vase scaffolds are developed and then left unpruned. Yields on SB trees have been reported to be lowe than on Palmette, CL, Y-trellis, ZVA, and VSS trees (Robinson et al., 2004; Whiting et al., 2005). The Vase had less light interception and more pruning than Palmette and Marchand, which led to a lower cumulative yield (Moreno et al., 1998). The KGB is currently being evaluated in North America in the NC-140 trial in comparison to several other canopy architectures (Lang et al., 2014). Severe pruning in vase and bush systems appears to reduce yield compared to minimally pruned systems such as

ZVA and Y-trellis. The tradeoff between reduced cumulative yield and reduced input cost for these non-trellised systems should be considered for growers considering this training system.

Unique Architectures

The Steep Leader (SL) was designed to mimic a spindle tree but with the single trunk replaced by 3-4 spindle-like leaders. The SL is usually developed with semi-vigorous or vigorous rootstocks and is spaced 3-4.8 m within rows and 4.25-5.5 m between rows (Long et al., 2015; Robinson, 2005). Trees are headed 75-90 cm at planting and wide angles are developed to provide 3-4 future vertical leaders with a very narrow open center. During the dormant season, laterals are selected for leaders and are headed or bud-selected to help promote branching along each leader. These leaders are treated as a portion of a spindle tree, allowing the vigor of the tree to be dispersed among multiple leaders, but with each leader fruiting and branching like one side of a spindle. Lower lateral scaffolds and upper lateral shoots are pruned to create a pyramid shaped tree, and 20% of the laterals are renewed yearly. Tipping may be required if trees are very productive. The SL is one option for growers who prefer vigorous rootstocks (Long et al., 2015). The Quad Axis is another four-leader system that has been tested recently. It had the lowest yield efficiency compared against SS and SB training systems and is not considered a good candidate for sweet cherry (Robinson and Hoying, 2014).

The single leader Solaxe training system from apple has been adapted for use in cherry. This system is best with low vigor rootstocks with trees spaced 1.5-2 m within rows and 4.5-5 m between rows, and may require light trellising. Branches are bent to reduce vigor, increase precocity, maintain tree size and minimize pruning. Summer pruning may be needed to remove water shoots where growth is bent. Lateral fruiting wood arises directly from the trunk and is not

renewed. This training system improves precocity and maintains tree height with low vigor rootstocks. If trees become over-cropped, spur extinction must be used to improve fruit quality. This system allows for a semi-pedestrian or pedestrian orchard with relatively low labor input, primarily for bending or tying in lieu of pruning (Lauri, 2005).

Palmette has been used for many different fruit trees and performs well with sweet cherry. Palmette canopy architectures work better for vigorous trees (Corelli-Grappadelli, 2000). Typically, trees are spaced 3.5-5 m within rows and 4-7 m between rows. If trees are too close, the production of lower tiers is lost due to shading (Corelli-Grappadelli, 2000; Moreno et al., 1998). Trees require trellising, and are grown to form a fruiting wall. They typically have 4-5 permanent scaffolds trained in a single plane on a trellis, with laterals grown toward the alleyway to bear fruit (Whiting et al., 2005). Well-feathered trees are used at planting, although heading can be used to encourage branching if the trees are not well feathered. The leader and tiers of branches are selected in following years to form a hedgerow (Corelli-Grappadelli, 2000). Not heading the leader can bring the palmette into earlier production. This system provides good light interception, good distribution of vigor, and reduced mature pruning to help compensate for the early expense and labor associated with trellising and training.

The Marchand or Marchant Inclined Tree canopy also forms a fruiting wall. Tree spacing is 2.4-3.5 m within the row and about 4 m between rows (Moreno et al., 1998; Robinson et al., 2004). Trees are planted at 45° and leaders are trained to the trellis at 60°. Side branches and buds under the leader are removed, and remaining buds are thinned to 20 cm apart. Laterals are trained to 45° above horizontal (Robinson et al., 2004). This is similar to the Upright Fruiting Offshoot (UFO) training system (discussed below) except that the leader and laterals maintain a

diagonal orientation instead of horizontal and vertical. Marchant trees had lower yield and yield efficiency than expected for its density, suggesting this system does not work well for sweet cherry (Robinson and Hoying, 2014).

The UFO system creates a narrow fruiting wall somewhat similar to the Marchand. It is recommended for semi-dwarfing to vigorous rootstocks, with trees spaced 1.2-2.1 m within the row and 2.7-3 m between rows depending on rootstock vigor (Long et al., 2015). Unheaded and unbranched trees are planted at an oblique angle. Bud activation techniques (e.g., bud removal or scoring) are imposed on upper-oriented buds every 20 cm. When shoots are 30 cm long, the nursery tree leader is attached to the lowest trellis wire, creating a horizontal cordon. Multiple upright shoots arising from the cordon are attached to upper trellis wires to develop into a fruiting wall. Secondary laterals that form on uprights are either removed (for productive varieties) or short pruned (for less productive varieties) (Long et al., 2015). Each year, 15-20% of the largest uprights should be renewed to help balance vigor and reduce shading (Lang et al., 2014; Long et al., 2015).

The UFO system can be adapted to a Y-shaped canopy with the use of semi-dwarfing to vigorous rootstocks, but with a closer in-row spacing of 0.9-1.8 m and wider between-row spacing of 3.7-4.3 m (Long et al., 2015). Planting is the same as UFO except that upper buds are retained every 10 cm. The UFO-Y uses a y-shaped trellis with each plane 20-30° from vertical. Upright shoots arising from the cordon are attached to the trellis in an alternating pattern to distribute the uprights to form two planar canopies angled into the adjacent tractor alleys. Renewal practices are similar to UFO (Long et al., 2015). UFO-Y has a higher yield potential per orchard area due to greater light interception. Establishing UFO and UFO-Y training systems are

expensive and labor-intensive compared to other training systems. However, they also provide simplified training, pruning, and crop load management during the overall life of the orchard, allowing the use of a less-skilled labor force. The planar architecture also can facilitate at least partial mechanization of orchards for pruning or possibly even harvest.

Other training systems have also utilized a divided canopy to improve light distribution. The untrellised Perpendicular V (PV) and Y-trellis are both developed by heading the nursery tree and growing two laterals toward the row middle (Meland, 1998; Robinson et al., 2004). In the second year, lateral shoots are promoted along the V leaders in the PV system by removing 67% of the buds (Robinson et al., 2004). The two main scaffolds of the Y-trellis are attached to a trellis with 60º between them and the subscaffolds and fruiting laterals arising from them are trained to the trellis (Whiting et al., 2005) and upright shoots in the middle of the V are removed (Meland, 1998). The Tatura Trellis (TT) (originally developed for vigorous rootstocks) creates two scaffolds that grow over the alleyway on a V-trellis (Robinson, 2005). The TT uses minimal pruning, removing only water shoots and pendant shoots. Renewal pruning is used to promote production on spurs less than 3 years old (Lauri, 2005).

V-shaped canopy architectures also have been developed without heading. In Taturaxe (Lauri, 2005), 30º V-spindle, 60º V-shaped trellis hedge ((Balmer, 2001), and V-system (Musacchi et al., 2015), the V-shape is achieved by planting trees at 20º-60º from vertical and leaning them on a V-trellis, alternating the direction of each tree. This increases light interception, but avoids the severe pruning of the headed systems that can delay production. However, remedial pruning is necessary to remove shoots that grow inside the V-canopy planes (Lauri, 2005). Yield for the Y-trellis declined with age, resulting in similar cumulative yields per

hectare to Vase and VA canopies, and slightly higher cumulative yields than FS when all are planted at the same density (Meland, 1998). The PV outyielded the SB, Marchand and CL (Robinson and Hoying, 2014). Cropping of the 60° V-shaped hedge decreased as it aged (Balmer, 2001). The V-system had a lower yield than S trees, but came into cropping earlier and, because of its higher density, had higher yields per hectare (Musacchi et al., 2015).

TRAINING SYSTEM TRENDS

A major goal of high density training systems is to maximize early yield without reducing fruit quality. Some systems do this by increasing density, minimizing pruning, and/or balancing crop load. Understanding the tradeoffs inherent in the different training systems is essential for determining the best training system for an orchard. Increasing tree density to the optimal spacing increases yield per hectare (Meland, 1998), but it also raises orchard establishment costs. Less than optimal density reduces precocious yields, while greater than optimal density can reduce mature yields due to shading or excessive pruning. Minimal pruning usually brings trees into cropping earlier, and spindle systems that use little pruning (such as ZVA and VCL) tend to have high yield efficiency and cumulative yields, although they may not be the most precocious systems (Meland, 1998; Robinson et al., 2004). The SSA system requires intense pruning and high tree density, but it produces high quality fruit with a simplified training program and can achieve high yields per hectare (Musacchi et al., 2015) but yields per hectare can vary widely among cultivars

When evaluating production costs, labor availability and cost will determine if pedestrian orchards will significantly reduce harvest expenses. Most bush-type training systems facilitate pedestrian orchards which simplify harvest and do not require trellising (Long et al., 2015).

However, they have low early yield because of the significant early pruning needed to develop the canopy structure. Spindle systems are semi-pedestrian orchards, requiring moderate use of ladders (Long et al., 2015). Some of the trellised systems facilitate pedestrian orchards, but the trellis needed for training the canopies of such systems as the UFO or divided V canopies, or for support of trees on weaker rootstocks, increase establishment costs.

When comparing training systems, V-shaped canopies can achieve high early yields, but remedial pruning may be needed for branches that grow in the interior of the V-structure, resulting in carbohydrate loss. The most promising current dual-plane systems are the PV, V-system, (Musacchi et al., 2015; Robinson and Hoying, 2014) or the UFO-Y. The UFO-Y should perform well because most of the upright growth (which requires removal pruning in other systems) can be attached to the trellis to prevent shading and then be removed in the renewal process if needed. The UFO system has the potential for mechanized harvest or pruning, which makes it advantageous where labor is scarce or expensive. The ZVA and TSA are high yielding due to their minimal pruning and high density. The KGB shows promises as a low cost, lower yielding option for growers who want to avoid trellising. Continued evaluation is needed for the newest systems such as TSA, KGB, UFO, UFO-Y, and SSA to assess their performance in various sites and with different rootstocks.

Many factors should be considered in training system selection. Different varieties have higher yields depending on training system (Long et al., 2015; Moreno et al., 1998; Musacchi et al., 2015). This could be related to different genotypi propensities for spur or non-spur fruit. Rootstock influences on vigor, growth habit, and reproductive development must be appropriately matched with training system to optimize canopy development, vigor and LA:F

ratios. Balancing the tradeoffs associated with different training systems and rootstocks will require combinations that are tailored to the needs of each orchard situation. Labor availability, availability of quality planting sites, use of nets or coverings, trellising, possible mechanization, and initial capital can impact training system selection. A sound understanding of the benefits and requirements of each system will help growers pick the one that will work best for their orchard.

LITERATURE CITED

LITERATURE CITED

Ayala, M. and G. Lang. 2004. Examining the influence of different leaf populations on sweet cherry fruit quality. Acta Hort. 636:481–488.

Balmer, M. 2001. Sweet cherry tree densities and tree training. Compact Fruit Tree 34:74–77.

Balmer, M. 2008. Evaluation of semi-dwarfing rootstocks for sweet cherry orchards in the Rhine River Valley (Germany). Acta Hort. 795:203–208.

Balmer, M. and M. Blanke. 2005. Developments in high density cherries in Germany. Acta Hort. 667:273–275.

Brunner, T., L. Juhász and E. Páldi. 1996. New data on the training of dwarf sweet cherry trees on a physiological basis. Acta Hort. 410:287–290.

Claverie, J. and P.È. Lauri. 2005. Extinction training of sweet cherries in France – appraisal after six years. Acta Hort. 667:376–372.

Corelli-Grappadelli, L. 2000. The palmette training system. Acta Hort. 513:329–336.

De Salvador, F.R., G. Di Tommaso, C. Piccioni and P. Bonofiglio. 2005. Performance of new and standard cherry rootstocks in different soils and climatic conditions. Acta Hort. 667:191–200.

Facteau, T.J., N.E. Chestnut and K.E. Rowe. 1996. Tree, fruit size and yield of 'Bing' sweet cherry as influenced by rootstock, replant area, and training system. Scientia Hort. 67:13–26.

Ferree, D.C. and J.R. Schupp. 2003. Bending, p. 337–339. In: D.C. Ferree and I.J. Warrington (eds.). Apples: Botany, production and uses. CABI Publishing. Cambridge, MA

Franken-Bembenek, S. 2004. GiSelA 3 (209/1) – A new cherry rootstock clone of the Giessen series. Acta Hort. 658:141–143.

Hoying, S.A., T.L. Robinson and R.L. Andersen. 2001. Improving sweet cherry branching. New York Fruit Qrtly. 9:13–16.

Green, K. 2005. High density cherry systems in Australia. Acta Hort. 667:319–324.

Heinicke, D.R. 1964. The micro-climate of fruit trees. III. The effect of tree size on light penetration and leaf area in red delicious apple trees. Proc. Amer. Soc. Hort. Sci. 85:33–41.

Hrotkó, K. 2005. Developments in high density cherry production in Hungary. Acta Hort. 667:279–283.

Hrotkó, K., G. Simon and L. Magyar. 1998a. Training of slender spindle trees for intensive sweet cherry orchards. Acta Hort. 468:465–470.

Hrotkó, K., G. Simon and L. Magyar. 1998b. Modified brunner-spindle as a training system for semi-intensive sweet cherry orchards. Acta Hort. 468:459–464.

Jackson, J.E. and J.W. Palmer. 1977. Effect of shade on growth and cropping of apple trees. II. Effects on components of yield. J. Hort. Sci. 52:253–266.

Kappel, F., G. Lang, A. Azarenko, T. Facteau, A. Gaus, R. Godin, T. Lindstrom, R. Nunez-Elisea, R. Pokharel, M. Whiting and C. Hampson. 2013. Performance of sweet cherry rootstocks in the 1998 NC-140 regional trial in western North America. J. Amer. Pomol. Soc. 67:186–195.

Kemp H. and S.J. Wertheim. 1996. First results of two international cherry rootstock trials. Acta Hort. 410:167–176.

Krzesinska, E.Z. and A.N.M. Azarenko. 1992. Excised twig assay to evaluate cherry rootstocks for tolerance to *Pseudomonas syringae pv. syringae*. HortScience 27:153–155.

Lang, G.A. 2000. Precocious, dwarfing, and productive-how will new cherry rootstocks impact the sweet cherry industry? HortTechnology 10:719–725.

Lang, G.A. 2001. Intensive sweet cherry orchard systems–rootstocks, vigor, precocity, productivity and management. Compact Fruit Tree 34:23–26.

Lang, G.A. 2005. Underlying principles of high density sweet cherry production. Acta Hort. 667:325–336.

Lang, G.A., J.D. Early, G.C. Martin, and R.L. Darnell. 1987. Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. HortScience 22:371–377.

Lang, G.A. and W. Howell. 2001. Lethal sensitivity of some new cherry rootstocks to pollen borne viruses. Acta Hort. 557:151–154.

Lang, G., W. Howell and D. Ophardt. 1998. Sweet cherry rootstock/virus interactions. Acta Hort. 468:307–314.

Lang, G.A., S. Blatt, C. Embree, J. Grant, S. Hoying, C. Ingels, D. Neilsen, G. Neilsen and T. Robinson. 2014. Developing and evaluation intensive sweet cherry orchard systems: the NC140 regional research trial. Acta Hort. 1058:113–120.

Lauri, P.È. 2005. Developments in high density cherries in France: integration of tree architecture and manipulation. Acta Hort. 667:285–292.

Lauri, P.E., J. Claverie and J.M. Lespinasse. 1998. The effect of bending on the growth and fruit production of INRA Fercer ® sweet cherry. Acta Hort. 468:411–417.

Leyser, O. 2005. The fall and rise of apical dominance. Current Opinion Genet. Dev. 15:468–471.

Long, L. 2001. Sweet cherry training systems. Compact Fruit Tree 34:66–69.

Long, L.E., T. Facteau, R. Nuñez-Elisea and H. Cahn. 2005. Developments in high density cherries in the USA. Acta Hort. 667:303–310.

Long, L.E. and C. Kaiser. 2010. PNW 619 sweet cherry rootstocks. Pacific Northwest Ext. Publ. 619:1–8.

Long, L., G. Lang, S. Musacchi and M. Whiting. 2015. PNW 667 cherry training systems. Pacific Northwest Ext. Publ. 667.

Meland, M. 1998. Yield and fruit quality of 'Van' sweet cherry density production systems over seven years. Acta Hort. 468:425–432.

Moreno, J., F. Toribio and M.A. Manzano. 1998. Evaluation of palmette, marchand and vase training systems in cherry varieties. Acta Hort. 468:485–489.

Musacchi, S.M., F. Gagliardi and S. Serra. 2015. New training systems for high-density planting of sweet cherry. HortScience 50:59–67.

NC-140. 2015. 2014 NC-140 annual report. 9 February 2016. <http://www.nc140.org/2014/2014annualreport.html>.

Pérez, J.N. 2005. Cherry cultivation in Spain. Acta Hort. 667:293–301.

Perry, R., G. Lang, R. Andersen, L. Anderson, A. Azarenko, T. Facteau, D. Ferree, A. Gaus, F. Kappel, F. Morrison, C. Rom, T. Roper, S. Southwick, G. Tehrani and C. Walsh. 1996. Performance of the nc-140 cherry rootstock trials in North America. Compact Fruit Tree 29:37–56.

Perry, R., G. Lang, R. Andersen, L. Anderson, A. Azarenko, T. Facteau, D. Ferree, A. Gaus, F. Kappel, F. Morrison, C. Rom, T. Roper, S. Southwick, G. Tehrani and C. Walsh. 1998. Performance of the nc-140 cherry rootstock trials in North America. Acta Hort. 468:291–296.

Robinson, T.L. 2005. Developments in high density sweet cherry pruning and training systems around the world. Acta Hort. 667:269–272.

Robinson, T.L., R.L. Andersen and S.A. Hoying. 2004. Performance of Gisela cherry rootstocks in the northeastern United States. Acta Hort. 658:231–240.

Robison, T.L. and S.A. Hoying. 2008. Performance of Gisela rootstocks in six high density sweet cherry training systems in the Northeastern United States. Acta Hort. 795:245–254.

Robinson T.L. and S.A. Hoying. 2014, Training system and rootstock affect yield, fruit size, fruit quality and crop value of sweet cherry. Acta Hort. 1020:453–462.

Roper, T.R. and W.H. Loescher. 1987. Relationships between leaf area per fruit and fruit quality in 'Bing' sweet cherry. HortScience 22:1273–1276.

Schoedl, K., A. Denk, S. Hummelbrunner, P. Modl and A. Forneck. 2009. No improvement in fruit quality thorough chemical flower thinning in sweet cherry (*Prunus avium L.*) J. Sci. Food Agri. 89:1236–1240.

Seavert, C. and L.E. Long. 2007. Financial and economic comparison between establishing a standard and high density sweet cherry orchard in Oregon, USA. Acta Hort. 732:501–504.

Sekse, L. 1995. Fruit cracking in sweet cherries (*Prunus avium* L.). Some physiological aspects – a mini review. Scientia Hort. 63:135–141.

Smart, R. and M. Robinson. 1991. Winegrape canopies and their importance, p. 1–15. In: Sunlight into Wine: a handbook for canopy management. Winetitles, Broadview, South Australia.

Spotts, R.A., J. Olsen, L. Long, and J.W. Pscheidt. 2010. EM 9007 Bacterial canker of sweet cherry in Oregon disease symptoms, cycle, and management. Oregon State Univ. Ext. Serv. EM 9007:1–4.

Stehr, R. 2014. Experiences with dwarfing cherry rootstock Gisela 3 compared to Gisela 5 in Northern Germany. Acta Hort. 1020:732:389–394.

Whiting, M.D. and G. Lang. 2004. 'Bing' sweet cherry on the dwarfing rootstock 'Gisela 5': thinning affects fruit quality and vegetative growth but not net co2 exchange. J. Amer. Soc. Hort. Sci. 129:407–415.

Whiting, M., G. Lang and D. Ophardt. 2005. Rootstock and training system affect sweet cherry growth, yield, and fruit quality. HortScience 40:582–586.

Whiting, M.D. and O.M. Lelahan. 2006. Physiological and horticultural effects of sweet cherry chemical blossom thinners. HortScience 41:1547–1551.

Whiting M.D. and D. Ophardt. 2005. Comparing novel sweet cherry crop load management strategies. HortScience 40:1271–1275.

Whiting, M.D., D. Ophardt and J.R. McFerson. 2006. Chemical blossom thinners vary in their effect on sweet cherry fruit set, yield, fruit quality, and crop value. HortTechnology 16:66–70.

CHAPTER 2: PLANTING ANGLE AND MERISTEM MANAGEMENT INFLUENCE SWEET CHERRY CANOPY DEVELOPMENT IN THE "UPRIGHT FRUITING OFFSHOOTS" TRAINING SYSTEM[z]

INTRODUCTION

High density tree training systems are important for overcoming some of the challenges of sweet cherry (*Prunus avium* L.) production. Cherry fruit are susceptible to many pests and diseases, rain-induced cracking, and bird damage, requiring multiple sprays for pests, rain covers, and nets to ensure marketable crops in locations prone to rain during ripening. High density training systems can make sweet cherry production more efficient, by reducing pesticide and herbicide use and facilitating mechanization of orchards and the use of nets and covers for fruit protection. Recent developments in rootstocks have provided precocious fruiting and dwarfism (Lang, 2000), and allowed high density training systems to be developed (Lang, 2005; Lang et al., 2014; Musacchi et al., 2015; Robinson, 2005).

There are several important factors to consider when designing a high density training system to maximize yields, minimize disease, and facilitate easy pruning and harvesting. Training goals for producing high quality fruit include 1) good light interception and distribution by the canopy, 2) a balanced leaf to fruit ratio (Lang, 2005), and 3) renewable fruit-bearing sites on minimal permanent structure. A good high density training system should address these principles and be efficient to prune, train, and harvest. The "Upright Fruiting Offshoots" (UFO)

[z]Reprint of: Law, T.L. and G.A. Lang. 2016. Planting angle and meristem management influence sweet cherry canopy development in the "Upright Fruiting Offshoots" training system. HortScience 51: 1010–1015.

training system develops a trellised, planar multiple leader tree to create a narrow fruiting wall with evenly-distributed vertical fruiting branches (or "uprights") along a cordon-like trunk (Long et al., 2015). This provides a tall, narrow fruiting canopy that is easy to train and prune for renewal of uprights. The UFO system's planar architecture and pedestrian size also help increase harvest efficiency (Ampatzidis and Whiting, 2013). The light interception of UFO orchards has been described (Zhang et al., 2015), however, too few or uneven spacing of fruiting uprights creates gaps in the fruiting wall and reduces orchard efficiency by failing to optimize both interception and distribution of light throughout the canopy. Little work has been published to determine how to achieve the ideal canopy structure and maximize early shoot growth for UFO trees.

Sweet cherry trees exhibit strong apical dominance (the suppression of subtending buds by the shoot terminal) resulting in vigorous top growth and minimal branch development lower in the canopy. It can be difficult to redistribute that vigor during the first year of establishment into balanced secondary shoots along the trunk, whether oriented vertically or horizontally. The mechanism of apical dominance is not fully understood, but it is generally accepted that basipetal transport of auxin produced in the terminal meristem suppresses growth of lower buds and branches (Leyser, 2005). Different training techniques can alter shoot growth patterns. In apple (*Malus* x *domestica* Borkh.), changing the orientation of vertical branches released lower buds from apical dominance (Ferree and Schupp, 2003). As deviation from vertical increased, more buds were released. Bending sweet cherry branches below horizontal reduced subsequent growth of the leader and subtending shoots, compared to unbent branches (Lauri et al., 1998). Bending also increased the number of flower buds and flowers per flower bud. Placing sweet cherry trunks horizontally caused a reduction in shoot growth, relative to upright trees, by reducing

node number and internode spacing (Wareing and Nasr, 1961). The more horizontal orientation of the trunk in the UFO system may partially reduce the effects of apical dominance, but that alone will not ensure well-distributed uprights.

Various techniques have been used to promote precise placement of new branches, enabling efficient use of storage reserves during tree establishment. In sweet cherry, heading cuts can promote branching, but the branches are poorly distributed and have acute crotch angles (Hoying et al., 2001). Other techniques to alter meristem outgrowth include the topical use of Promalin® (containing gibberellic acids 4 and 7 and 6-benzyladenine) to alter the hormone balance at a bud and cause it to elongate into a new shoot, but effectiveness can vary due to temperature (Lang, 2005). Notching (or scoring), by cutting through the bark and phloem just above a bud, facilitates branch placement by disrupting hormone flow and promoting elongation of the bud into a new shoot (Hoying et al., 2001). Another meristem management technique is bud selection and removal. When a portion of buds are removed, the remaining buds are more likely to grow into shoots (Lang, 2005). Selective bud removal (selecting buds to be retained for placement of branches and removing all others) has been more effective than Promalin® or notching for producing laterals from remaining buds (or nodes) in the lower portions of the trunk (Hoying et al., 2001). However, with bud selection or notching, caution should be taken to remove buds during warm, dry weather when risk for bacterial canker infection is low. Furthermore, gaps may be left in the canopy if any of the selected buds fail to grow.

Current recommendations for UFO tree training are to use precocious rootstocks, such as the Gisela series, to bring trees into production quickly (Long et al., 2015). Trees on precocious rootstocks enable earlier yields, but they can be susceptible to poor structural development

and/or overcropping if poorly managed (Lang, 2001; Long, 2001). Understanding how fruiting branches develop can help growers make wise training and pruning decisions to maximize early yields. The year a shoot forms, a single leaf is produced at each node. The next year, that shoot usually forms a small number of non-spur flower buds at its basal nodes which then become blind wood after fruiting (Lang, 2005). The other nodes each form non-fruiting leafy spurs with 5-9 leaves. In the third (and subsequent) years, those nodes will be the fruiting spurs that will bear fruit until they become damaged or diseased. This fruiting progression often brings trees on Gisela rootstocks into significant flowering in the 3rd or 4th year in the orchard. These different populations of fruit-bearing sites (one-time non-spur fruiting nodes and multi-year fruiting spurs) illustrate how shoot growth in the first year becomes minor fruiting area in year 2 and significant fruiting area in year 3. This underscores the importance of maximizing canopy shoot growth in the first year to optimize yield potential from fruiting spurs in year 3 and beyond.

Successful development of the UFO fruiting wall canopy architecture requires several decisions to be made at planting or soon thereafter. First year establishment of UFO sweet cherry trees was investigated to determine the effects of planting angle, height of cordon bending to horizontal, and selective bud removal on number of structural shoots, shoot growth and distribution, and early fruiting potential.

METHODS AND MATERIALS

Unbranched (whip) nursery trees of ‘Rainier’ on ‘Gisela 3’, with a central leader about 1.5 m long, were divided into 12 treatments and planted at a spacing of ≈1.5 m. The first experimental factor was trunk angle, with the trees planted at 30º, 45º, or 60º from horizontal (Fig. 1.1). Imposed on the angle factor in early summer was the height at which the trunk was

attached horizontally to the first trellis wire, 45 cm or 60 cm, and bent to form the horizontal cordon (Fig. 1.1). The last factor was bud selection, either leaving all buds intact or removing nearly all buds except one upward oriented bud every ≈15 cm. If no upward bud was present, a side bud was used instead.

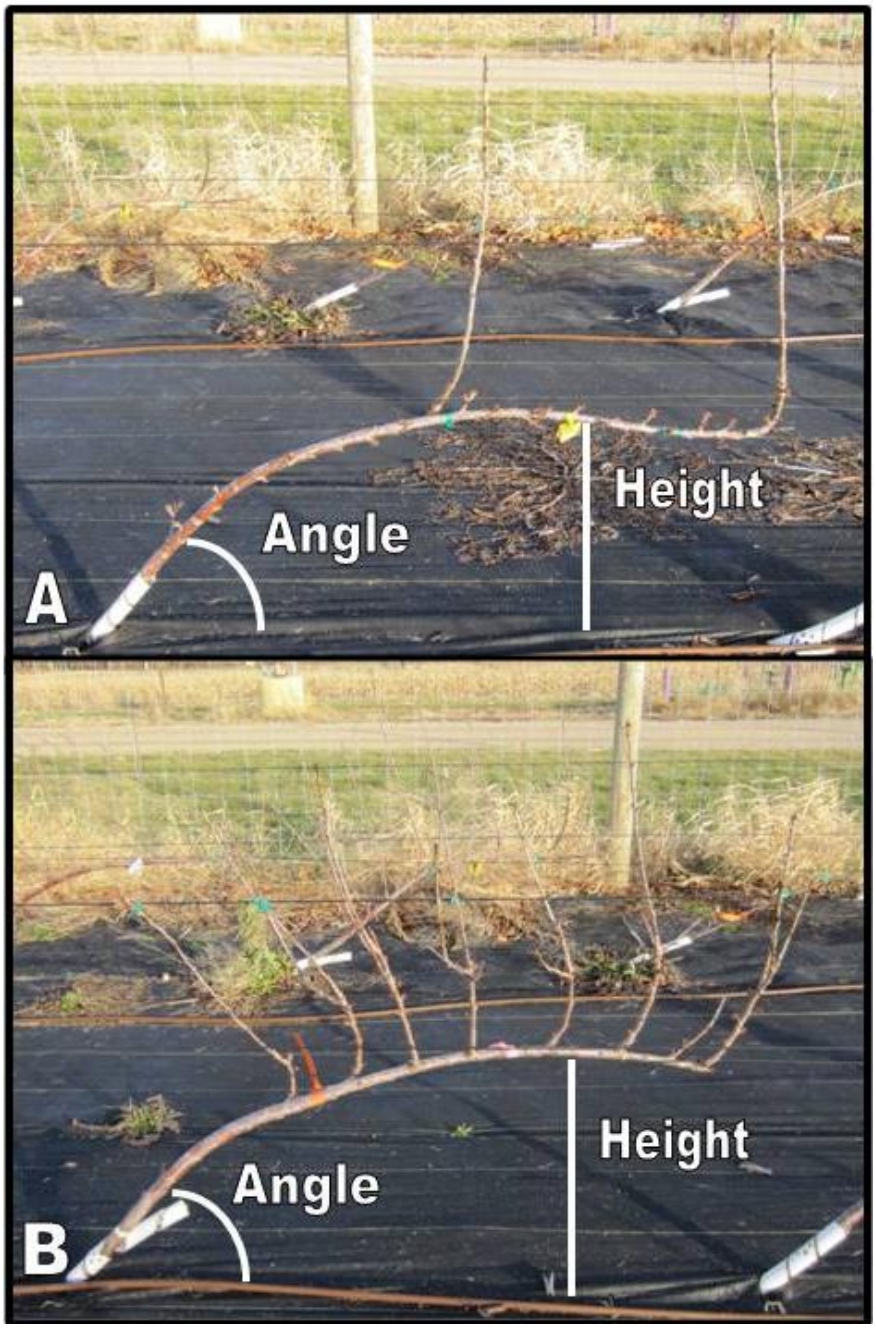

Figure 2.1. Dormant 'Rainier'/'Gisela 3' sweet cherry trees after two growing seasons trained to the Upright Fruiting Offshoot (UFO) canopy architecture illustrating planting angle (30º, 45º, or 60º), cordon height (45 cm or 60 cm), and A) no bud selection at planting vs. B) bud selection imposed at planting.

Six single-tree replications were planted in mid-May of 2010 in a Randomized Complete Block Design at the Clarksville Research Center in Clarksville, MI (lat. 42.8ºN, long. 85.2ºW) in a coarse-loamy, mixed, mesic Typic Hapludalf soil of the Lapeer series. Trees were irrigated and sprayed for pests as needed, and weed barrier fabric (Dewitt Pro 5, Dewitt Co., Sikeston, MO) was used for weed control. Data were taken in fall 2010 for shoot number, length, and spatial distribution, and in spring 2011 for flower bud number (i.e., spur flower buds on the cordon and basal flower buds on the upright shoots).

The length of each upright shoot was measured from its base to its tip. Meristem growth was considered to be a shoot if it was at least 2.5 cm long. Average shoot length was determined by dividing the total shoot length by the number of shoots. Shoot distribution data were quantified by measuring the distance from the base of the tree to each upright. The trunk was then divided into three equal segments and the data for uprights within each segment were segregated for distributional analysis. Segments were designated as basal (closest to ground), middle, and distal (terminal segment).

Yield potential for the first five years of the orchard was determined by counting the number of flower buds in spring 2011 and extrapolating future yield potential based on initial shoot growth, spur and shoot basal flower bud density, and multi-year data for shoot growth from other trees on ‘Gisela 3’ rootstocks trained to UFO. On bud-selected trees (where all spurs were removed), all flower buds were basal. On the trees without bud selection, spur bud number was derived by subtracting the number of basal buds from the total number of flower buds. Spur flower bud density was calculated based on the 150 cm length of the cordon leader minus the cumulative 20 cm portion from which upright shoots arose (i.e., 33 spur flower buds / 130 cm =

0.25 spur flower buds/cm). Shoot growth rates (as a proportion of previous season average shoot length) in Years 2, 3, and 4 were 2.7, 1.3, and 0.5, respectively, based on multi-year annual shoot growth measurements from an adjacent UFO-trained 'Benton'/'Gisela 3' plot. For example, projected mean shoot length in Year 2 for the pooled bud-selected treatments used the actual growth in Year 1 plus 2.7X the growth in Year 1 (17.6 cm + [2.7 x 17.6] = 65.1 cm/shoot). The projected shoot length in Year 2 was used to project total spur flower bud formation in Year 4 (since two years are required for spur formation), such that 8 shoots x (65.1 cm x 0.25 flower buds/cm) = 130 spur flower buds. To this value was added the number of basal flowers buds (assumed to be constant for each year's previous shoot extension) as well as spur flower buds on the cordon for the no bud-selection treatments. Bud density on flowering spurs was assumed to not be affected by differences in total shoot growth. Basal flower buds on upright shoots were assumed to set the same number of fruit per bud as spur flower buds, which was calculated using a value of 2.5 fruit per bud. Potential fruiting on lateral shoots was not considered since any lateral shoots that form are removed annually in the UFO training system. Projected yields per hectare were then estimated from number of fruits per tree, 12 g per fruit, and 2222 trees per hectare (a tree spacing of 1.5 m x 3.0 m). Potential heavy crop load and reduced shoot growth of trees without bud selection could result in fewer flowers or smaller fruit than bud selected treatments. This model was designed to be biased in favor of treatments without bud selection to be more conservative when discussing increases in yield caused by bud selection treatments. It is possible that the yield of trees without bud selection could have even lower yields than projected.

Statistics included analysis of variance (ANOVA) using Proc Mixed in the Statistical Analysis System (SAS) program (SAS Institute Inc., Cary, NC). All pairwise comparisons were done with t-tests using the LSmeans pdiff option and reported as significant if they had a p-value

< 0.05. A natural logarithmic transformation was use for the average shoot length per tree and means were back transformed for the paper.

RESULTS

The angle at planting and bud selection had some independent and some synergistic effects (Tables 1.1 and 1.2) on number of shoots, total and mean shoot length, shoot distribution, and number of flower buds. Number of shoots was only significant for angle and the angle x bud interaction (Table 1.1). Trees developed 7 shoots when planted at a 30º angle, and 8 to 9 shoots when planted at 45ºor 60º (Table 1.3). Bud selection only significantly impacted the number of shoots for trees planted at 30º angles. Without bud selection, trees at 30º only grew 6 shoots, but with bud selection 8 shoots developed (Table 1.3). However, total shoot number for treatments without bud selection included not only the structurally-desirable upright shoots (which predominated in the bud selection treatments), but also undesirable horizontal and downward-growing shoots that developed from the sides and bottom of the cordon.

Table 2.1. P-values from ANOVA testing the effects of Angle, Height, Bud selection (Bud), and all interactions for shoot number, total shoot length, mean shoot length and flower bud number.

Effect	Shoot number	Total Shoot Length	Mean Shoot Length	Flower Bud Number
Angle	0.0043	0.0012	0.0217	0.0189
Height	0.1737	0.0046	0.0369	0.131
Bud	0.7022	<.0001	<.0001	<.0001
Angle x Height	0.6169	0.7388	0.6641	0.1933
Angle x Bud	0.0495	0.0117	0.0788	0.0822
Height x Bud	0.2467	0.2828	0.1227	0.9341
Angle x Height x Bud	0.0782	0.0748	0.3923	0.4155

Table 2.2. P-values from ANOVA testing the effects of Angle, Height, Bud selection (Bud), and all interactions for shoot number and average shoot length in basal, middle, and terminal thirds.

Effect	Basal Third		Middle Third		Terminal Third	
	Shoot No.	Shoot Length (cm)	Shoot No.	Shoot Length (cm)	Shoot No.	Shoot Length (cm)
Angle	0.4047	0.2884	0.0267	0.8731	0.0078	0.0007
Height	0.2583	0.0839	0.6909	0.1015	0.0689	0.0814
Bud	0.001	0.0004	<.0001	<.0001	<.0001	<.0001
Angle x Height	0.1169	0.1789	0.4334	0.5629	0.9886	0.3844
Angle x Bud	0.019	0.0582	0.6187	0.1083	0.0068	0.061
Height x Bud	0.2583	0.3459	0.8423	0.7663	0.1722	0.2458
Angle x Height x Bud	0.0053	0.1419	0.5046	0.2516	0.4344	0.2426

Table 2.3. Establishment season shoot number, total shoot length (cm), mean shoot length (cm), and following season flower bud number for planting angle (30°, 45°, and 60°), meristem management [bud selection (B) and no bud selection (NB)], treatment combinations of angle and bud selection, and cordon height (45 or 60 cm) of 'Rainier' sweet cherry trees on 'Gisela 3' trained to the Upright Fruiting Offshoots (UFO) canopy architecture.

Treatment	Shoot No.		Total Shoot Length (cm)		Mean Shoot Length (cm)		Flower Bud Number	
30°	7.0[z]	b[y]	91.0	b	11.2	b	28	ab
45°	9.2	a	128.8	a	14.0	a	23	b
60°	8.8	a	124.4	a	12.6	ab	32	a
B	8.4	a	148.3	a	17.6	a	12	b
NB	8.3	a	80.9	b	8.9	b	45	a
30°/B	8.1	a	133.1	b	16.2	a	13	c
30°/NB	6.0	b	46.3	d	7.5	c	47	ab
45°/B	8.7	a	143.5	ab	17.7	a	11	c
45°/NB	9.7	a	114.2	b	11.1	b	35	b
60°/B	8.6	a	168.3	a	19.1	a	13	c
60°/NB	9.1	a	80.5	c	8.4	a	53	a
45 cm	8.7	a	127	a	13.7	a	23	a
60 cm	8.0	a	101	b	11.6	b	31	a

[z]Data were pooled to analyze effects of planting angle, meristem management, and cordon height.
[y]Statistical significance of means comparisons were done with t-tests and means in the same column followed by the same letter are not significantly different at $p < 0.05$.

When pooling data to examine trunk angle effects, total shoot length was highest (124 or 129 cm) for trees planted at 45º and 60º, which was 30% higher than for the trees at 30º (91 cm) (Table 1.3). Across treatment combinations, bud selection increased total shoot length by 85% (148 cm vs. 80 cm). The greatest impact of bud selection on total shoot length was for trees planted at 30º and 60º, which increased by 185% and 100%, respectively, compared to the corresponding treatments without bud selection. Trees planted at 45º had the highest total shoot length without bud selection, and growth increased by only 25% when bud selection was applied

(Table 3). Bud selection also increased average shoot length across the entire tree 97% compared with no bud selection. Average shoot length on trees planted at 30º and 60º angles increased by 116% and 127%, respectively, with bud selection. Average shoot length for trees planted at 45º only increased 59% with bud selection (Table 1.3).

The height at which the angled trunk was bent to the wire to create the cordon did not significantly affect shoot number, though it did affect total and average shoot length (Table 1.1). Establishment year shoot growth was 101 cm for trees bent at 60 cm, compared to 127 cm for those bent at 45 cm. Thus, bending at 45 cm caused a 20% increase in total shoot length compared to bending at the higher height. Average shoot length increased from 11.6 cm to 13.7 cm for the 45 cm height compared to 60 cm (Table 1.3).

Flower bud number in Year 2 was significantly affected by both angle and bud selection (Table 1), but the affect of bud selection was more pronounced. Across all treatments with no bud selection, flower bud number per tree was 45, but only 12 for treatments with bud selection (Table 1.3). Without bud selection, flower bud number ranged from 35 (trees at 45º) to 53 (trees at 60º). With bud selection, flower bud number did not differ significantly by tree planting angle, ranging from 11 to 13 (Table 1.3). Height of bending to form the cordon did not affect flower bud number significantly (Table 1.1).

Shoot distribution was impacted significantly byangle, bud selection, and the interactions of angle x bud selection and angle x height x bud selection (this last was only significant for shoot number in the basal third) (Table 1.2). Across treatment combinations, trees without bud selection averaged only half as many shoots in the basal (0.6 vs. 1.2) and middle (1.3 vs. 2.6) sections of the cordon, compared to the bud selection treatments (Table 1.4). Although the trees

without bud selection had higher shoot numbers in the terminal section (6.4 vs. 4.6), these shoots were too close together (6 to 8 cm apart on average) compared to the bud selection trees (8.5 to 11 cm apart), considering that ideal spacing is 15 to 20 cm apart. Trees planted at 30º and 60º had an increase of 0.5 and 1.1 shoots, respectively, in the basal section when bud selection was applied. Trees planted at 45º had an increase of only 0.1 shoot in the basal section with bud selection. The middle section had an increase of 1.7, 1.4, and 1.0 shoots in the 30º, 45º, and 60º treatments, respectively. In the terminal section, the 45º and 60º treatments that were not bud selected had 7.1 or 7.3 shoots, respectively, and all other treatment combinations were not statistically different from each other, ranging from 4.4 to 4.8 shoots (Table 1.4).

Bud selection also increased average shoot length in each section of the cordon (Table 1.2). Across treatments, bud selection increased average shoot length by 330%, 185%, and 93% for the basal, middle, and terminal sections, respectively (Table 1.4).

Table 2.4. Mean number of shoots and shoot length in the basal (closest to ground), middle, and terminal thirds of the trunk (cordon), for meristem management (bud selection [B] and no bud selection [NB]) and planting angle (30°, 45°, and 60°) treatment combinations of 'Rainier' sweet cherry on 'Gisela 3' trained to the Upright Fruiting Offshoots (UFO) canopy architecture.

Treatment	Basal Third				Middle Third				Terminal Third			
	Shoot No.		Shoot Length (cm)		Shoot No.		Shoot Length (cm)		Shoot No.		Shoot Length (cm)	
B	1.2[z]	a[y]	14.2	a	2.6	a	17.4	a	4.6	b	18.4	a
NB	0.6	b	3.3	b	1.3	b	6.1	b	6.4	a	9.5	b
30° B	1.1	ab	10.4	ab	2.3	a	18.9	a	4.7	b	16.0	b
30° NB	0.6	bc	1.7	c	0.6	c	2.9	c	4.6	b	7.9	d
45° B	1.1	ab	9.1	ab	2.8	a	14.8	ab	4.8	b	18.6	ab
45° NB	1.0	ab	6.4	b	1.4	bc	9.5	bc	7.3	a	11.8	c
60° B	1.3	a	23.0	a	2.8	a	18.6	a	4.4	b	20.7	a
60° NB	0.2	c	1.7	c	1.8	ab	5.6	c	7.1	a	8.7	cd
45 cm	1.0	a	11.5	a	1.9	a	13.7	a	5.8	a	14.9	a
60 cm	0.8	a	6.2	a	2.0	a	10.0	a	5.2	a	13.2	a

[z]Data were pooled to analyze effects of meristem management, and cordon height.
[y]Statistical significance of means comparisons were done with t-tests and means in the same column followed by the same letter are not significantly different at $p < 0.05$.

DISCUSSION

When establishing high density orchards on precocious rootstocks, new structural shoot growth and distribution is important for early canopy development. Since first year tree growth on 'Gisela 3' creates sites for fruiting spurs in Year 3, structural shoot growth in the first year impacts early yield potential. The goal in establishing a UFO tree structure is to develop well-distributed upright shoots and maximize vertical shoot growth in the trellis plane. This optimizes yield potential, facilitates good light interception and distribution, good spray penetration, and reduces losses of storage resources from remedial pruning of poorly-placed shoots.

Recommended spacing for UFO upright shoots is ≈20 cm (Long et al., 2015). With a 150 cm nursery tree and an in-row spacing of 120 (for trees planted at a 60º angle) to 140 cm (30º planting angle), 6 to 7 vertical shoots arising from the horizontal cordon structure are needed to fill the canopy. In this study, the target shoot number was achieved in all treatment combinations except the 30º angle without bud selection (which would require 7 shoots), though not all of the resulting shoots were oriented vertically in the treatments without bud selection. Unfortunately, upright vs. non-upright shoot orientations were not quantified. Among the treatments without bud selection, a 45º planting angle gave the best total shoot length, number, and distribution (Fig.1.2A-C). However, all of the treatments without bud selection had poor shoot distribution in the basal and middle sections of the cordon, and an excessive number of shoots in the terminal section that would ultimately result in removal of perhaps 50% due to crowding.

Bud selection improved shoot distribution, orientation (since top-selected buds always grew vertically), and growth uniformity (Fig.1. 2D-F). The number of shoots increased in the basal and middle sections of the cordon and decreased in the terminal section. For shoot

distribution, bud selection was more important than angle, overcoming most of the disadvantages of the 30º angle. Bud selection made planting angle insignificant for vertical shoot number, distribution, and average length. Bud selection has been reported to promote lateral branching in central and multiple leader sweet cherry training systems (Hoying et al., 2001) and, in this study, increased desirable shoot number and improved distribution of future fruiting structure in the cordon leader-based UFO training system.

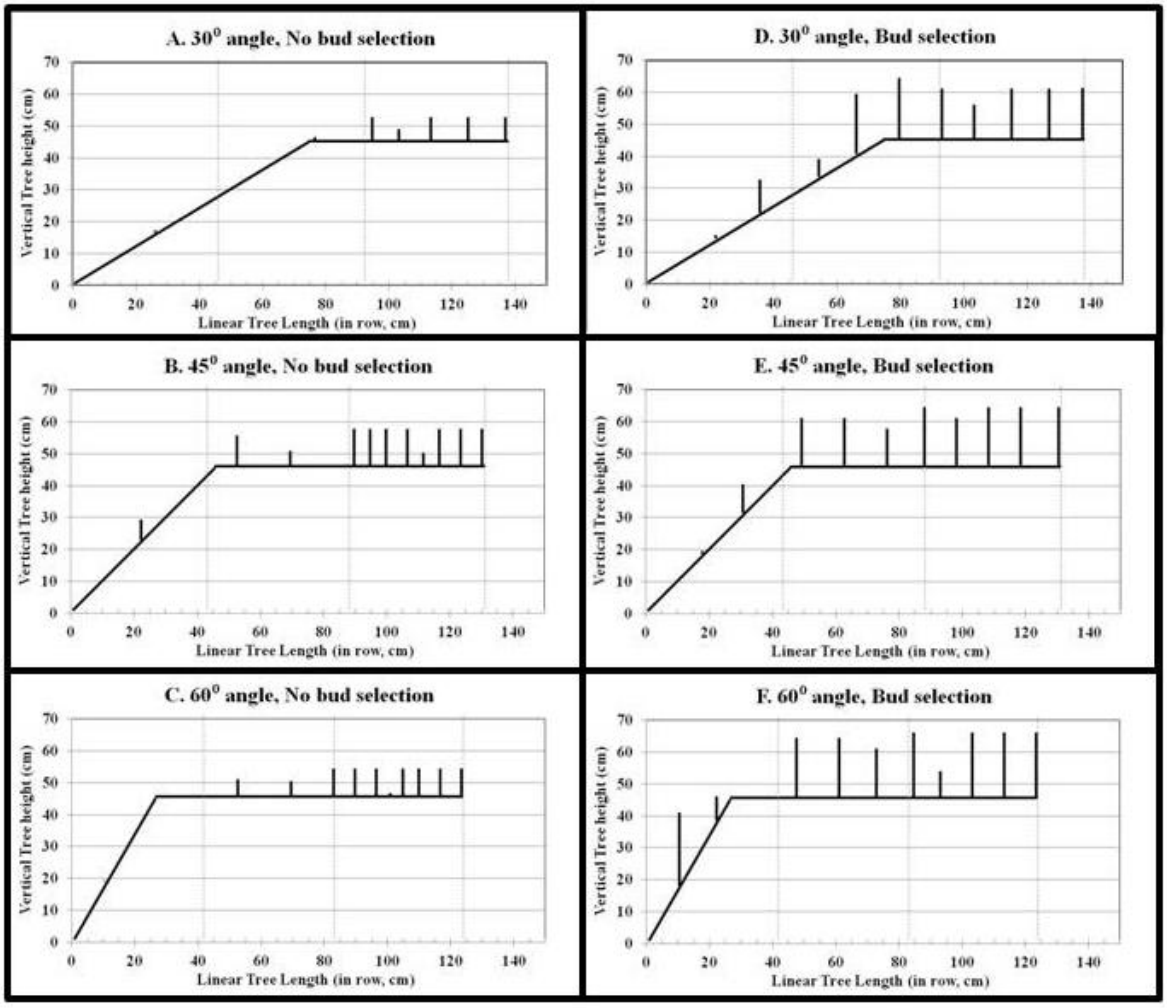

Figure 2.2 Schematic diagram of 'Rainier'/'Gisela 3' sweet cherry shoot formation and growth during the year of planting for Upright Fruiting Offshoots (UFO) tree canopies planted at 30º, 45º, or 60º. Diagrams depict positioning of the "cordon" portion of the leader at a height of 45 cm for simplicity, though the data are the combined means of results at both 45 cm and 60 cm. Dotted background lines depict partitioning of canopies into three equal sections to quantify locational shoot distribution. A. 30º with no bud selection, B. 45º with no bud selection, C. 60º with no bud selection, D. 30º with bud selection, E. 45º with bud selection, and F. 60º with bud selection. Note that diagrams A-C (no bud selection) indicate shoot positions and lengths where growth occurred, but not all shoots were oriented upright as is depicted.

In training UFO trees, once optimal upright shoot number and distribution are attained, the next canopy development goal is to maximize shoot growth. Trees bent at a 45 cm trellis wire height to create the UFO cordon had 20% more total shoot length compared with trees bent at 60

cm (Table 1.3). Height of the bottom trellis wire affects the length of the cordon in the UFO canopy architecture. For a 150 cm nursery tree, the approximate length of the horizontal portion of the cordon leader at a 45 cm wire height is 60, 86, or 98 cm for planting angles of 30º, 45º and 60º, respectively (Fig. 1.2). At the 60 cm wire height, these lengths are reduced to 30, 65, and 80 cm, respectively. This could explain the effect of bending height on total length of shoots arising from the cordon. Apple branches that were horizontal produced more water sprouts, which formed earlier and grew longer, than branches at less horizontal angles (Hamzakheyl et al., 1976). At the 45 cm wire height, a greater proportion of the cordon length is horizontal compared with that at 60 cm. However, although the increased horizontal length was related to increased total shoot length in bud-selected treatments, this was not the case in treatments without bud selection. Bud selection independently increased total shoot length, regardless of planting angle. Maximum new shoot growth was achieved with a 60º planting angle and bud selection. This could due to a greater length of horizontal cordon than the other angles. However, without bud selection, 60º had significantly less growth than 45º. Sweet cherry branches that were bent below horizontal form more flower buds (Lauri et al., 1998) and in our study, non-bud selected 60º had more flower buds (largely spur flower buds) than non-bud selected 45º. These spurs and increased flower buds appear to compete with shoot growth for resources. Removal of these spurs by bud selection eliminated the competition and may have allowed for increased shoot growth. Without bud selection the 45º angle had the most shoot growth. This could be because it had less of the trunk horizontal than 60º. The 30º angle also had less shoot growth than the 45º, possibly because the angle was not very different for horizontal which may have increased the number of flower buds. This 30º angle may have physiology more similar to a fruiting lateral, than a terminal shoot. The 45º angle may strike the right balance when bud selection is not used.

It may have enough of the cordon diagonal to increase growth, but not enough horizontal cordon length to increase flower bud number therefore competition for resources.

Although the UFO trees developed by bud selection generally created canopies closest to the target upright shoot number as well as having the best vertical shoot distribution and total length, the trees without bud selection were significantly more precocious, with 3.75 times the number of flower buds in Year 2 (Table 1.3). This was because bud selection removed meristems on the cordon that would have developed into fruiting spurs. To evaluate the economic trade-off of early canopy structural development vs. precocious cropping, projected future yields through Year 5 were calculated from the pooled data for trees with and without bud selection. Both sets of data average about 8 upright shoots, which was considered to be sufficient for projecting a well-structured future UFO canopy. Therefore, the measured total flower bud count on the bud-selected trees represents the number of basal flower buds that would be expected to form at the base of each year's extension growth on the 8 upright shoots annually (Table 1.3). The 33 spur flower buds present on the cordon of the trees without bud selection are assumed to persist through Year 5. The number of spur flower buds that begin appearing in Year 3 is directly proportional to the shoot growth from Year 1, and the increase in these spur flower buds in Years 4 and 5 are proportional to the projected shoot growth in Years 2 and 3. While some of the shoots that form without bud selection tend to be poorly located or downward growing, this possibility was not taken into account in the potential yield projections; all 8 shoots on each group of trees were assumed to grow vertically and at equal growth rates (therefore, any effect of differential crop loads also was not taken into account).

The estimated potential yield on a per orchard basis was 3.0 t/ha in Year 2 for the trees without bud selection, an impressive level of precocity, and far higher than that for the bud-selected trees at 0.8 t/ha (Table 1.5). Projected yields in Year 3 continued to be higher, 4.2 vs. 3.1 t/ha, for the trees without bud selection. However, projected annual yield for the bud-selected trees surpassed that of the non-bud-selected trees in Year 4, 9.5 t/ha vs. 7.4 t/ha, as did cumulative yield in Year 5, 34.2 t/ha vs. 27.7 t/ha. The average length (225 cm) of the 8 vertical shoots of the bud-selected trees is projected to have essentially filled a 2.5 m trellis by the end of Year 4 and reached full productivity by Year 5, while the trees without bud selection are projected to have only filled half their allotted canopy space. At the end of Year 5, the projected yield differential would be 6.5 t/ha; at a crop value of $6,000 per ton, the projected economic differential would be $39,000 per ha higher for the bud-selected trees.

Table 2.5. Projected year-by-year shoot growth, flower bud formation, and yield potential during establishment, comparing bud selection [B] and no bud selection [NB] for 'Rainier' sweet cherry on 'Gisela 3' trained to the Upright Fruiting Offshoots (UFO) canopy architecture.

Year	Mean length per upright shoot (cm)	Total spur flower buds on the cordon (no.)	Total basal flower buds on upright shoots (no.)	Total spur flower buds on upright shoots (no.)	Total flower buds / tree (no.)	Total yield[z] (t/ha)	Cumula-tive yield (t/ha)
			Bud Selection (B)				
1	17.6	0	0	0	0	0	0
2	65.1	0	12	0	12	0.8	0.8
3	149.7	0	12	35	47	3.1	3.9
4	224.6	0	12	130	142	9.5	13.4
5		0	12	300	312	20.8	34.2
			No Bud Selection (NB)				
1	8.9	0	0	0	0	0	0
2	32.9	33	12	0	45	3.0	3.0
3	75.7	33	12	18	63	4.2	7.2
4	113.6	33	12	66	111	7.4	14.6
5		33	12	152	197	13.1	27.7

[z]2222 trees/ha and 2.5 fruits/flower bud

Heavy crop loads can stunt trees on dwarfing rootstocks, with fruit production significantly reducing shoot growth (Kappel, 1991; Whiting and Lang, 2004). Quickly filling canopy fruiting volume is essential to attain full production early and help recoup orchard establishment costs. In this study, although the trees without bud selection were projected to attain impressive early yields (3-4 t/ha each in Years 2-3), they did not attain projected full yields as quickly as the bud-selected trees (Table 1.5). A balanced crop load is essential for high quality fruit, and a slight delay in precocious cropping can be beneficial for establishing enough leaf area to support subsequent cropping as spurs become reproductive. In the Solaxe training system (a central leader canopy with lateral branches bent below horizontal), removal of 30 to 50% of the fruiting spurs is recommended to promote larger fruit size balanced against the subsequent

reduction in fruit number (Claverie and Lauri, 2005). Others have recommended removal of 25-45% of the potential crop load (depending on tree age) to balance fruit quality with yield (Lang, 2005). Although bud selection in Year 1 reduced crop load potential in Year 2 by 73%, it increased canopy development by nearly 50%, resulting in more rapid attainment of full fruiting capacity and adequate leaf area to support quality fruit production and delay the potential need for crop reduction strategies.

Growers interested in planting a UFO orchard must match rootstock vigor and planting density to the orchard site. These factors affect the length of the cordon leader and the number of vertical shoots to be developed per tree. In this study, 'Gisela 3' did not have enough vigor to quickly fill the orchard space allotted. 'Gisela 3' is a dwarfing rootstock, only imparting 35-50% of the vigor of 'Mazzard', but requires good soil, irrigation, tree support and intensive cultural management (Balmer and Blanke, 2005; Franken-Bembenek, 2004). Other rootstocks to consider would be 'Gisela 6' which is semi-vigorous, imparting 80-95% of the vigor of 'Mazzard', or 'Gisela 5' which is semi-dwarfing, and imparts 50-65% of 'Mazzard' (Facteau et al., 1996; Kemp and Wertheim, 1996; Lang, 2000; Perry et al., 1998; Robinson et al., 2008; Whiting et al., 2005). Trees on dwarfing rootstocks like 'Gisela 3' should be planted at higher densities and developed with fewer upright shoots. Conversely, trees on semi-dwarfing (e.g., 'Gisela 5') to semi-vigorous (e.g., 'Gisela 6,' 'Gisela 12,' 'Krymsk 6') rootstocks should be planted at more moderate densities and developed with more upright shoots. Tree angle at planting can further modulate canopy development. For a vigorous rootstock-site combination, a 30º angle could help reduce excessive vigor, while a 60º angle would increase shoot growth for a less vigorous combination. A lower trellis wire height results in a greater proportion of the cordon being horizontal, and increases upright shoot length. Bud selection can optimize the distribution and

growth of well-distributed uprights, albeit at a temporary cost of precocious cropping. Although bud selection requires more labor during planting, the improved upright canopy formation reduces the labor needed later for corrective pruning. The improved shoot distribution and orientation, increased shoot growth, and moderated early crop reduction of bud-selected trees improves precision canopy development and full yield potential for the UFO sweet cherry production system.

CONCLUSIONS

Optimizing shoot growth on well distributed uprights is important to fill the fruiting canopy quickly and also provide good light and spray distribution into the canopy. A 60º planting angle combined with bud selection provided the most shoot growth. The 45 cm wire height also increased total shoot length 20% compared to the 60 cm height. Bud selection was a valuable technique to both improve shoot distribution and increase projected yield after 5 years. Bud selected sites could become entry points for *Pseudomonas syringae* pv. *syringae* (PSS) which causes bacterial canker in sweet cherry. Care should be taken to avoid bud selection when PSS pressure is high. Utilizing these planting practices can help growers develop optimal canopies for the UFO training system.

LITERATURE CITED

LITERATURE CITED

Ampatzidis, Y.G. and M.D. Whiting. 2013. Training system affects sweet cherry harvest efficiency. HortScience 48:547–555.

Balmer, M. and M. Blanke. 2005. Developments in high density cherries in Germany. Acta Hort. 667:273–277.

Claverie, J. and P.È. Lauri. 2005. Extinction training of sweet cherries in France – appraisal after six years. Acta Hort. 667:376–372.

Facteau, T.J., N.E. Chestnut, and K.E. Rowe. 1996. Tree, fruit size and yield of 'Bing' sweet cherry as influenced by rootstock, replant area, and training system. Scientia Hort. 67:13–26.

Ferree, D.C. and Schupp, J.R. 2003. Bending, p. 337–339. In: D.C. Ferree and I.J. Warrington (eds.). Apples: Botany, production and uses. CABI Publishing. Cambridge, MA

Franken-Bembenek, S. 2004. GiSelA 3 (209/1) – A new cherry rootstock clone of the Giessen series. Acta Hort. 658:141–143.

Hamzakheyl, N., D.C. Ferree, and F.O. Hartman. 1976. Effect of lateral shoot orientation on growth and flowering of young apple trees. HortScience 11:393–395.

Hoying, S.A., T.L. Robinson, and R.L. Andersen. 2001. Improving sweet cherry branching. New York Fruit Qrtly. 9:13–16.

Kappel, F. 1991. Partitioning of above-ground dry matter in 'Lambert' sweet cherry trees with or without fruit. J. Amer. Soc. Hort. Sci. 116:201–205.

Kemp H. and S.J. Wertheim. 1996. First results of two international cherry rootstock trials. Acta Hort. 410:167–176.

Lang, G.A. 2000. Precocious, dwarfing, and productive-how will new cherry rootstocks impact the sweet cherry industry? HortTechnology 10: 719–725.

Lang, G.A. 2001. Intensive sweet cherry orchard systems–rootstocks, vigor, precocity, productivity and management. Compact Fruit Tree 34:23–26.

Lang, G.A. 2005. Underlying principles of high density sweet cherry production. Acta Hort. 667:325–336.

Lang, G.A., S. Blatt, C. Embree, J. Grant, S. Hoying, C. Ingels, D. Neilsen, G. Neilsen, and T. Robinson. 2014. Developing and evaluation intensive sweet cherry orchard systems: the NC140 regional research trial. Acta Hort. 1058:113–120.

Lauri, P.E., J. Claverie, and J.M. Lespinasse. 1998. The effect of bending on the growth and fruit production of INRA Fercer® sweet cherry. Acta Hort. 468:411–417.

Leyser, O. 2005. The fall and rise of apical dominance. Current Opinion in Genetics and Development 15:468–471.

Long, L. 2001. Sweet cherry training systems. Compact Fruit Tree 34:66–69.

Long, L., G. Lang, S. Musacchi, and M. Whiting. 2015. PNW 667 cherry training systems. Pacific Northwest Ext. Publ. 667:50–56.

Musacchi, S.M., F. Gagliardi, and S. Serra. 2015. New training systems for high-density planting of sweet cherry. HortScience 50:59–67.

Perry, R., G. Lang, R. Andersen, L. Anderson, A. Azarenko, T. Facteau, D. Ferree, A. Gaus, F. Kappel, F. Morrison, C. Rom, T. Roper, S. Southwick, G. Tehrani, and C. Walsh. 1998. Performance of the nc-140 cherry rootstock trials in North America. Acta Hort. 468:291–296.

Robinson, T.L. 2005. Developments in high density sweet cherry pruning and training systems around the world. Acta Hort. 667:269–272.

Robinson, T.L., R.L. Andersen, and S.A. Hoying. 2008. Performance of Gisela® rootstocks in six high density sweet cherry training systems in the northeastern United States. Acta Hort. 759:245–253.

Wareing, P.F. and T.A.A. Nasr. 1961. Gravimorphism in trees 1. Effects of gravity on growth and apical dominance in fruit trees. Annals of Botany 25: 321–340.

Whiting, M.D. and G.A. Lang. 2004. ‘Bing’ sweet cherry on the dwarfing rootstock ‘Gisela 5’: thinning affects fruit quality and vegetative growth but not Net CO2 exchange. J. Amer. Soc. Sci. 129:407–415.

Whiting, M.D., G. Lang, and D. Ophardt. 2005. Rootstock and training system affect sweet cherry growth, yield, and fruit quality. HortScience 40:582–586.

Zhang, J., M.D. Whiting, and Q. Zhang. 2015. Diurnal pattern in canopy light interception for tree fruit orchard trained to an upright fruiting offshoots (UFO) architecture. Biosystems Engineering 129:1–10.

CHAPTER 3: LITERATURE REVIEW OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* AND SWEET CHERRY (*PRUNUS AVIUM* L.)

INTRODUCTION

Bacterial canker of sweet cherry (*Prunus avium* L.) is a complex disease that can cause reduced yields, girdling and loss of tree limbs, and even tree death. With the early warming and subsequent multiple frost events of spring 2012, Michigan lost most of its sweet cherry crop and many trees lost a significant proportion of their fruiting spurs in association with subsequent bacterial canker infections. The impact of these widespread infections reduced yields for several years due to the time required to replace lost spurs and for the new spurs to come into production. Finding control options for bacterial canker is important for the long-term health of cherry industries in climates that experience cool wet weather. Copper resistance and the lack of effective control sprays create a difficult situation, leaving horticultural and orchard sanitation techniques as the main control options. Understanding the interactions between the bacteria, their host, and environment, will help in developing new management strategies.

ORGANISM BACKGROUND

Cherry bacterial canker is caused by *Pseudomonas syringae* pv. *syringae* (PSS) and *Pseudomonas syringae* pv. *morsprunorum* (PSM). Sweet cherry can be infected by both pathovars PSS and PSM, while tart cherry (*Prunus cerasus* L.) tends to be infected by PSM (Latorre and Jones, 1979a; Sundin et al., 1988; Wimalajeewa and Flett, 1985). *Pseudomonas syringae* van Hall originally was isolated from lilac (*Syringa vulgaris* L.) (Hirano and Upper, 1990) but is found on many species including tomato, bean, and broadleaved weeds and grasses

(Latorre and Jones, 1979b). The bacteria can also be distributed throughout the water cycle (Morris et al., 2008). Many *Pseudomonas syringae* (PS) pathovars originally were described as separate species, but have since been consolidated into the specie PS which is divided into at least 40 different pathovars. The pathovars are based on host range and represent widespread diversity within PS (Hirano and Upper, 1990). More details on the description of PS pathovars that invade stone fruits and their taxonomic history are available from Ogawa and English (1991) and Crosse (1966).

Morphology/identification

PSS is a gram-negative rod with one or more polar flagella (Ogawa and English, 1991). A useful trait to help in identifying PSS is the ability to produce the yellow-green fluorescent siderophore, pyoverdin, when grown on iron-limiting media (Cody and Gross, 1987). When grown on selective media such as King's medium B (King et al., 1954; Ogawa and English, 1991), the bacteria will fluoresce under UV light after 72 h incubation at 26^0C (The American Phytopathological Society, 1995). On Kings B, they form circular colonies that are smooth and glistening and appear vitreous against light. PSS is an aerobic, oxidase-negative bacterium capable of utilizing a large number of compounds as energy sources (Ogawa and English, 1991). The GATTa scheme (for gelatin liquefaction, aesculin hydrolysis, tyrosinase activity, and tartrate utilization) or Ice Nucleation Activity (INA) test have been used to distinguish between PSS and PSM (The American Phytopathological Society, 1995).

TRAITS THAT MAY CONTRIBUTE TO PATHOGENICITY

Toxins

PS bacteria produce several toxins that have been identified. PSM (but not PSS) produces coronatine which may be involved in the chlorosis of cherry leaves (Bultreys and Kaluzna, 2010; Liang et al., 1994). PSS produces two lipopeptide toxins, syringomycin and syringopeptin. Either of these two toxins can form transmembrane pores that allow free flow of ions across plant cell membranes (Bultreys and Kaluzna, 2010; Scholz-Schroeder et al., 2001). This disrupts the electrical potential across the cell and results in cell death. Syringomycin does not correlate well with pathogenicity of cherry fruit (Latorre and Jones, 1979a; Ogawa and English, 1991). A study using mutants looked at the contributions of syringomycin and syringopeptin to virulence of sweet cherry fruit. Virulence was reduced 26% for a syringomycin mutant, 59% for a syringopeptin mutant, and 76% for the syringomyin syringopeptin double mutant compared to the parent strain (Scholz-Schroeder et al., 2001). While these two toxins both significantly contribute to virulence, some necrosis occurred without either toxin, suggesting that there are other components that are fundamental to pathogenicity (Scholz-Schroeder et al., 2001).

T3SS and Effectors

PS can also impact it's host using the type III secretion system (T3SS) to introduce effectors into the host plant that suppress host defense or promote disease (Lee et al., 2012). One study with PSM found genes very similar to hypersensitive response and pathogenicity (hrp) genes (genes that are associated with the T3SS) found in PSS61 (a PSS isolate from wheat) that was needed for pathogenicity in cherry and the hypersensitive response in tobacco (Liang and

Jones, 1995). Although work with the T3SS effectors has been limited in cherry, some PSS effectors have been shown to enhance epiphytic survival on tomato (*Solanum lycopersicum*) (Lee et al., 2012). There is a significant epiphytic phase for PSS on sweet cherry and epiphytic survival could be linked to T3SS effectors. Effector repertoire can vary between strains of the same pathovar (Bultreys and Kaluzna, 2010), so to truly understand sweet cherry and its interactions with PSS effectors, cherry strains need to be studied.

Ice nucleation activity

Freezing plays an important role in PSS pathogenesis because it can predispose tissues to infection (Ogawa and English, 1991). Some plants are able to supercool (cool below the freezing point of water without forming ice) to a temperature at which uniform ice formation occurs. PSS bacteria can exhibit ice nucleation activity. If an ice nucleus is present, ice formation will be initiated at a higher temperature and reduce the supercooling affect. On some plants, this increases the amount of freeze damage. Excised sweet cherry buds were able to supercool to -5°C before ice nucleation occurred (Gross et al., 1984). When the buds were attached to a branch or inoculated with ice nucleation-active (INA) PSS, the mean nucleation temperature was raised to -3°C (Gross et al., 1984). Further work discovered the presence of intrinsic ice nuclei present in *Prunus* wood (Gross et al., 1988).

Ice nucleation activity of either *Prunus* wood or bacterial ice nuclei increases bud survival during the frost tolerant phase (before bloom) (Gross et al., 1984). This could be caused by the promotion of extracellular ice formation that could redistribute water and reduce freeze damage of more sensitive tissues (Gross et al. 1988). However, after bloom, *Prunus* floral tissues are frost sensitive and ice nucleation activity then makes blossoms more susceptible to frost

(Gross et al., 1984). Since either wood or bacterial ice nuclei raise the freezing temperature to approximately -2°C to -3°C, reducing the population of INA PSS is unlikely to cause a significant reduction in freeze damage unless high populations of INA PSS are coincident with a time when the wood is warmer than the flowers or fruit (Gross et al., 1984; Gross et al., 1988).

PSS AND ENVIRONMENTAL INTERACTIONS

Disease Triangle

For successful endophytic bacterial colonization of plants, there must be a susceptible host, sufficient bacteria present, and appropriate environmental conditions (Ichinose et al., 2013). Disease can be avoided by eliminating any of these factors. Environment is important because it impacts both the host (e.g., by controlling phenology) and the pathogen (e.g., by influencing population size). Some environmental conditions that can influence bacterial canker disease include water availability and temperature. Water availability is important for PSS population growth (Latorre et al., 1985; Wimalajeewa and Flett, 1985) and distribution (Hirano and Upper, 1990; Hirano et al., 1987). Free moisture allows the local movement of bacteria which can swim using flagella. Water also can increase nutrient availability to bacteria. Temperature influences growth rates of bacteria (Young et al., 1977), and freezing can cause wounding of host tissues, allowing entry points for the pathogen. Temperature could also affect the healing rate of host tissues wounded by environmental damage or pruning. Each of these variables (environment, bacterial presence, and susceptible host) is intricately connected and influences the infection success.

Environmental Conditions

In Chile, levels of bacteria isolated from cankers were low or undetectable during spring and summer, and high during winter and early spring (Latorre et al., 1985). Epiphytic populations on sweet cherry in Australia also were highest in spring and fall with maximum temperatures ranging from 19°C-24°C and minima between 7°C-12°C, and low levels were detected in the summer and winter (Wimalajeewa and Flett, 1985). In Michigan, populations of fluorescent pseudomonads increased after a period of cool wet weather (Sundin et al., 1988).

Temperature

The optimum temperature range for PSS bacterial growth is 25°-30°C (Ogawa and English, 1991). *In vitro* study of *Pseudomonas syringae* showed optimum growth at 28°C with a doubling time of 1.27 h. When grown at 0°C, the doubling time was 22 h (Young et al., 1977). A Chilean study reported a generation time of 40.96 h at 5°C and 4.31 h at 20°C (Latorre et al., 2002). Given the warm optimal range for growth, the authors suggest some limiting factor other than temperature for lower levels of PS in the field in summer. The growth at 0°C can be important for colonization of buds or blossoms that may be damaged by frost events. *Pseudomonas syringae* pv. *aesculi* (which causes bleeding canker of European horse chestnut [*Aesculus hippocastanum*]) can survive temperatures down to -80°C for 1 year even if subjected to freeze-thaw cycles (Laue et al., 2014).

Temperature also can affect infection success. Infection usually is associated with cool wet weather (Crosse, 1966). However, cherry fruit had higher infection incidence at higher temperatures. The experiment examined effects of temperature and inoculum, and it appears that

higher temperatures allow bacteria to infect at lower inoculum levels (Latorre et al., 2002). Infection of excised twigs did not occur at 5°C and infection success increased with increasing temperature up to 20°C (Latorre et al., 2002). Unfortunately, the twig experiment did not test lower inoculum levels at higher temperatures, because the slope seems to be the same across temperatures, suggesting that the twig infections might follow the same pattern as cherry fruit inoculations. Furthermore, temperature does not explain the activation and cessation of growth in existing cankers (Wilson, 1939). Although higher temperature may increase infection at lower inoculum levels, the lack of inoculum in the orchard and increased cambial activity probably restrict infection to the shoulder periods of summer when inoculum levels are able to increase with more moisture availability.

Freezing

Freezing is very important for infection success because without an entry site, only epiphytic colonization can occur. Epiphytic bacteria are still important because they provide opportunistic inoculum for potential infection events. Both freeze damage of blossoms and cracking from freezing and thawing of trunks in the winter can be potential entry points for PSS.

Needle inoculation of thawing stems (after being frozen) with PSS caused larger lesions than inoculation before freezing or after thawing in almond (*Prunus dulcis)* (Cao et al., 1999). Inoculation during thawing of frozen peach (*Prunus persica* (L.) Batsch.) stems increased lesion length more than 550% compared to unfrozen stems (Cao et al., 2011). This supports the hypothesis that water relocated to the apoplast during freezing helps distribute bacteria during the thawing process by facilitating the passive movement of bacteria as water is reabsorbed by cells after freezing (Cao et al., 1999).

In sweet cherry studies, freezing after inoculation with PSS or PSM increased necrosis, but PSS caused up to three times more necrosis than PSM (Sobiczewski and Jones, 1992). The extent of xylem necrosis for sweet cherry was temperature dependent, and phloem necrosis was less severe than xylem necrosis (Sobiczewski and Jones, 1992). Shoots collected in winter required temperatures of -14.5 to -25.5°C to injure cambium and xylem in 50% of 'Napoleon' shoots (Sobiczewski and Jones, 1992).

Water

Rain events can enhance infection in two ways: 1) increasing population size facilitated by increased water availability, and 2) allowing mobility to, and penetration of bacteria into, infection sites (Hirano and Upper, 1990; Hirano et al., 1987; Crosse, 1966). Higher PSS populations were detected on autumn buds of sweet cherry after rain events than before the rain event (Latorre et al., 1985). In Australia, rainfall of 40-60 mm in spring and fall were associated with high PSS populations (Wimalajeewa and Flett, 1985). Greenhouse studies confirm the relationship of wetness and dryness with increases and decreases in epiphytic bacterial populations, respectively (Latorre et al., 1985). Rain events on snap bean (*Phaseolus vulgaris* L.) were shown to wash the bacteria off, but also stimulated bacterial replication due to mechanical stimulation (Hirano and Upper, 1990; Hirano et al., 1987). Rainfall also appears to affect endophytic bacteria, with an increase in both distribution and bacterial population during periods of symptom development (Cameron, 1970). With cherry fruit and twigs, free moisture only moderately increased infection, most effectively at 10°C (Latorre et al., 2002). There seems to be a small window where free moisture can impact the actual infection process. However, the

authors suggested that free moisture could facilitate nutrient distribution and increase bacterial populations on healthy tissue.

Other factors

Studies of nutrient effects on bacterial canker do not show strong trends. Ogawa and English (1991) review some of the nutrient studies that have been done. Some results showed that increasing nitrogen in peach reduced bacterial canker infection, especially in conjunction with additions of potassium and phosphorus. Phosphorus deficiency reduced lesions from pinprick inoculations, and nitrogen deficiency slightly increased infection through leaf scars in peach (Cao et al., 2011). Inconclusive nutrition results could be because of the close connection between nutrient availability and soil pH. Soil pH can control the solubility of nutrients and unsuitable pH can make nutrients either unavailable or present at levels that are toxic to the plant. Some research has shown that low pH is conducive to bacterial canker in peach (Ogawa and English, 1991).

Soil pH also may interact with nematodes. In a study of the decline of sweet cherry in Michigan in the 1990s, there was no clear cause but it appeared to be the combination of low soil pH, high populations of the nematode *Pratylenchus penetrans*, and the presence of *Pseudomonas syrinae* (Melakeberhan et al., 1993). In the study, nematode populations did not vary between healthy and declining cherry trees. This could be because older trees may be more resistant to nematodes, or because there were also fewer nematodes than other studies reported (Melakeberhan et al., 1993). In peach, nematodes have been associated with bacterial canker, but nematode numbers did not correlate with bacterial canker disease when the pH was raised (Weaver and Wehunt, 1975). It appears there may be intricate interactions between soil pH,

nematode populations, and nutrition. Field observations indicated that stressed trees are more susceptible to bacterial canker infection than trees with moderate vigor (Spotts et al., 2010b) and it may be that combinations of improper nutrition, incorrect pH, or nematode infestation could stress trees and increase infection rate.

PSS AND ITS INTERACTIONS WITH SWEET CHERRY

PSS is commonly found as an epiphyte on plant tissues (Crosse, 1966; Latorre and Jones, 1979a; Ogawa and English, 1991) and forms aggregates which increase survival under adverse conditions such as UV light, fluctuating moisture availability and temperature (Lee et al., 2012; Monier and Lindow, 2003). PSS also are capable of producing cellulose that can be used in formation of biofilms and adhesion to plant surfaces in mango (*Mangifera indica* L.) (Arrebola et al., 2015). Biofilms have been reported in *Pseudomonas syringae* pv. *actinidiae* (PSA) infections (Renzi et al., 2012). Biofilms can be important for epiphytic survival by protecting bacteria from water stress. Epiphytic colonization appears to be harmless to trees, but under the right environmental conditions, PSS can invade cherry leaves, blossoms, fruit, and woody tissues and become endophytic (Kennelly et al., 2007; Ogawa and English, 1991). Endophytic infection was observed in apparently healthy sweet cherry trees (Cameron, 1970). Bacterial canker was first identified as a problem in Michigan in 1968, but growers had observed the symptoms earlier (Jones, 1971). Young trees can be very susceptible (Spotts et al, 2010a) and older trees can lose limbs to the disease. Inconsistent infection results in trials stresses the need for more controlled study systems in which to test potential controls. Some excised dormant shoot and twig infection assays have been developed (Krzesinska and Azarenko, 1992; Thomidis et al., 2005) and

preliminary investigation of sweet cherry callus has been considered as a potential approach to study plant pathogen interactions with PSM (Smith and Hodson, 2011).

Inoculum source and infection sites

The main source of inoculum varies throughout the year. PSS can overwinter in cankers, dead buds, or healthy-looking buds. These bacteria are sources for epiphytic colonization when environmental conditions are conducive for growth and survival. The epiphytic bacteria can then be transferred by rain splash to new sites and provides inoculum for new infections (Kennelly et al., 2007).

Since PSS is an opportunistic pathogen that cannot force entry into host plants, there must be a breach in plant defenses where it can infect. These can be naturally occurring and uncontrollable such as leaf scars in autumn, hydathodes, or stomata on leaves and fruit. Other entry points may be preventable such as frost-damaged tissue, bark inclusions, wounding from pruning, herbicide damage, tractor blight, scale insect infestations, or winter injury. The notorious canker symptom that develops in the trunk and scaffold branches only develops after a successful infection of such an entry point. Not all PSS infections lead directly to cankers, and some endophytic colonization can occur without any obvious symptoms. The most common entry points, their infection process and symptoms will be discussed to illustrate how they fit into the disease cycle.

Blossom infections

Blossoms become epiphytically colonized by bacteria that have overwintered in buds or cankers. Blossoms can be predisposed to infection by frost events that damage the blossoms. The

ice nucleation activity of PSS is unlikely to increase frost damage of blossoms due to the intrinsic ice nuclei present in the wood (Gross et al., 1984; Gross et al., 1988). The blossoms may wither, turn dark brown, and in some cases the infection may spread into the supporting wood, creating a small canker that may produce gum (Kennelly et al., 2007; Ogawa and English, 1991). These small cankers do not appear to reactivate during the dormant season (Ogawa and English, 1991). Although blossom infections may not lead to tree death, the loss of fruiting spurs can cause significant yield reduction for years to come until new spurs become productive.

Leaf and fruit infections

Leaves and fruit become infected through stomata or frost damage (Ogawa and English, 1991). This is similar to PSA which also can infect leaves through stomata (Renzi et la., 2012). The leaves are only susceptible when young, and no infection occurs once leaves are mature (Crosse, 1966). Leaf infections are 2-4 mm in diameter and form chlorotic halos around the dry, dark brown, necrotic center which falls out to form a "shot hole" (Jones, 1971; Kennelly et al., 2007; Ogawa and English, 1991). Fruit infections manifest as small, dark brown, sunken, water-soaked lesions (Ogawa and English, 1991). Fruit infections can cause misshapen fruit and increase susceptibility to other fruit diseases. Leaf and fruit symptoms on tart cherry include: necrotic spots on leaves, yellowing of leaves and defoliation, and soft brown pedicels on fruit (Latorre and Jones, 1979a). Since fruit and leaf infections rarely lead to devastating cankers, growers do not spray for them.

Leaf scars

Epiphytic bacteria can be mobilized by rain and pulled into broken ends of leaf trace vessels by negative tension in the tree's vascular system. In severe cases, the infection can kill the fruiting spur and spread into the branch and cause a canker (Crosse, 1966). Experimentally, the rate of infection of both PSS and PSM depended on bacterial concentration and pathovar, with PSM being more successful at infecting leaf scars than PSS (Crosse, 1966; Sundin et al., 1988). Leaf scars can be infected starting in early September but successful infection decreases rapidly after mid-October, probably due to decreased vascular tension due to reduced transpiration and increased soil moisture (Crosse, 1966). In bleeding canker of European horse chestnut (*Aesculus hippocastanum* L.) caused by *Pseudomonas syringae* pv. *aesculi*, leaf scars are most susceptible to infection May through October, but they are less susceptible after that, similar to what has been observed with PSS (Laue et al., 2014).

Woody tissue infection and wounding

Natural entry points such as stomata and leaf scars are not the only entry points for PS. Bacteria also will infect directly into woody tissue if a wound is created. Damage to the bark caused by scale insect infestations or herbicide damage can be infected by PSS. Acute crotch angles for branches also can get infected as the bacteria can get incorporated into tissues as the branch grows. Winter injury caused by freezing and thawing of trunk tissues can cause cracking that may become infected.

Some horticultural practices also can create sites for bacterial canker infection. Wounds caused by trellis wires, pruning, scoring (or notching), and bud removal all create breaks in the

periderm that could become infected. In one study, 47-100% of inoculated scored wounds made in March in Oregon became infected depending on year and variety (Spotts et al., 2010a). A main source of wounding is through annual pruning. Pruning in sweet cherry is used to balance crop load, maintain tree size and architecture, and renew old fruiting wood. Pruning wounds extend through entire branches creating entrance points directly into susceptible tissue.

Once a wound is infected, it may form a canker. Canker infections that occur in late autumn and winter appear to progress during dormancy but stop progressing as the tree becomes resistant during the active growing season (Ogawa and English, 1991). Most cankers are thought to be annual rather than perennial, and the infected tissue is walled off by callus in mid- to late spring (Ogawa and English, 1991). Perennial cankers reactivate in late autumn and become dormant in early summer (Crosse, 1966; Wilson, 1939). Inactive cankers have defined lateral margins delimited by what Wilson (1939) calls a "roll" of new tissues which appear to be partially callus in nature. Reactivation is marked by water soaking along the margins of canker (Wilson, 1939). Infected canker tissues are buried by either the vascular cambium or phellogen to minimize the spread of canker. Cambial activity is not the sole explanation of why cankers extend in late fall to early spring, but it seems to be an important part of the process (Wilson, 1939). Furthermore, the period of time when cankers could be induced coincides with same time that existing cankers are active (Wilson, 1939). In *Prunus*, between late January to mid-February the number of PSS bacteria in cankers increased by 1000% while tissue was undergoing rapid necrosis (Wilson, 1939).

DISEASE CYCLE AND PROGRESSION

To summarize the disease cycle, PSS bacteria overwinter in cankers or healthy-looking buds. In the spring, the bacteria epiphytically colonize the tree but do not cause disease without an entry point such as damage from spring frosts or wounding from pruning. Infection of the blossoms (known as blossom blast) can lead to canker formation similar to pruning wounds. During the spring and early summer, epiphytic bacteria can infect developing leaves and fruit, causing the "shot hole" symptom in leaves and dark water-soaked lesions on fruit. These infections do not generally lead to cankers, but fruit infections can be more susceptible to other pathogen infections. In the fall, symptomless infection can occur through leaf scars and bacteria can migrate into the buds where they may overwinter. Knowledge of the important entry points within the disease cycle helps guide possible control programs.

POTENTIAL CONTROL STRATEGIES

Chemical sprays commonly are used to control pests and diseases of fruit trees, but effective spray materials for bacterial canker are limited. Copper or Bordeaux mixture have been used for control of bacterial canker (Ogawa and English, 1991); however, resistance to copper has been reported (Sundin et al., 1989; Sundin et al., 1994). One study found that copper, and copper with streptomycin, spray programs had similar leaf populations of PS on *Pyrus calleryana* (ornamental pear) in nurseries (Sundin et al., 1994). One nursery that did not spray copper or streptomycin had a distinctly different population compared to a nearby (2 km away) nursery that did spray, but 43% of PS isolates were still resistant to either copper or streptomycin (Sundin et al., 1994).

In Michigan, plasmid-mediated copper resistance has been found in PSS, and copper-resistant isolates maintain the plasmid and resistance in the presence of copper (Sundin et al., 1989). Resistance plasmids could transfer between PSS isolates both *in vitro* and *in planta* (tested in bean) but didn't transfer to PSM (Sundin et al., 1989; Sundin et al., 1994). It was found that copper is no longer an effective way to reduce PSS populations in surveyed Michigan orchards (Sundin et al., 1989) or to reduce bacterial canker infection of pruning wounds (Carroll et al., 2010). Streptomycin, which has been used in apple for control of the bacterial disease fire blight (cause by *Erwinia amylovora*), also is ineffective for bacterial canker control (Ogawa and English, 1991; Sundin et al., 1994). It has been hypothesized that the failure of protective sprays could be due to endophytic colonization (Cameron, 1970; Sundin et al., 1988).

Bactericides

The lack of effective bactericides has led to the search for other possible spray controls. There are few new antibiotics available for agriculture due to competing needs in the medical and animal industries and the fear that resistance of importance to human health might develop from widespread agricultural use. Oxytetracycline and kasugamycin are two antibiotics that are used for control of fireblight. Kasugamycin (Kasumin, Arysta Lifescience, Cary, NC) has been shown to be effective in controlling fireblight and kasugamycin resistance has not yet been found in *Erwinia amylovora* (McGhee and Sundin, 2011). The study also included non-target bacteria, and 3 of the 26 PS isolates recovered from orchards were resistant to 100 µg/mL of kasugamycin. Further testing found two of the PS isolates were resistant on Kings B medium amended with Kasugamycin at 100 µg/mL but susceptible to Kings B with Kasumin having a comparable amount of kasugamycin (McGhee and Sundin, 2011). Kasugamycin was tested on

sweet cherry fruit and had no impact on infection when applied before inoculation, and about 25% control after inoculation. This study also found that oxytetracycline achieved about 44% control when sprayed before inoculation and 54% after inoculation of sweet cherry fruit (Carroll et al., 2010).

When tested against PSS, essential oils from the herbs Oregano (*Origanum compactum* Benth. and *Origanum vulgare* L.) and Thyme (*Thymus vulgaris* L.) were over 50% more effective than streptomycin (Kokoskova et al., 2011). These oils could be used as possible control candidates or as templates for new compounds to fight bacterial canker. Another potential control would be CAMAL, a synthetic peptide with antimicrobial activity, because it reduced populations of both PSS and PSM in assays (Golanowska et al., 2012).

Plant defense inducers

Another option would be to upregulate plant defenses to prevent bacterial invasion. An apple and pear (*Pyrus communis* L.) field trial was conducted to assess the efficacy of several products that induce systemic resistance to apple and pear scab (caused by *Venturia inaequalis* and *Venturia pirina*, respectively) (Percival et al., 2009). Messenger (Harpin protein), Phoenix (potassium phosphate), and Rigel (salicylic acid, SA, derivative) were compared with penconazole (a conventional control) and all provided some protection. At least three sprays were needed to achieve a detectable level of control, and penconazole still provided better protection than any of the resistance inducers. The authors recommended possibly using them in conjunction with other spray programs, but exclusive use would provide insufficient control.

Another possible control which has been studied in several species is acibenzolar-S-methyl (also known as Actigard, Bion, BTH, or ASM). Lee et al. (2012) used BTH which is a synthetic agonist of SA to try and induce resistance to PSS in *Nicotiana benthamiana* leaves. They found lower bacterial populations and lower disease ratings in BTH-sprayed treatments.

In apple, Actigard upregulated PR gene expression (PR genes are associated with Systemic Acquired Resistance) in seedlings beginning 2-5 days after treatment (Maxson-Stein et al., 2002). When tested in the field, weekly sprays were required for the best control and it was not any more effective than streptomycin in reducing fireblight infection. It is recommended as a supplement rather a replacement for the bactericides currently in use and to be used in combination to help reduce the development of resistance to streptomycin.

In Japanese pear (*Pyrus pyrifolia*), ASM gave some control over Japanese pear scab (caused by *Venturia nashicola*) (Faize et al., 2004). ASM appeared to prime the plant's defense response, because neither ASM or pathogen challenge alone had as pronounced a response as plants that had both ASM and subsequent inoculation.

Several phosphorus acid (possible defense inducer) sprays and Bion were tested on Arctic Bramble (*Rubus arcticus*) for protection against downy mildew (caused by *Peronospora sparsa*) (Hukkanen et al., 2008). Bion at 0.2 g/L provided 80-90% control and some of the phosphorus acid products had about 50% control. However, phosphite did not reduce bacterial canker infection in pruning wounds in sweet cherry (Carroll et al., 2010).

Biocontrols

Several biocontrol products are available to combat fireblight of apple. However, some of these are specifically selected to compete with *Erwinia amylovora* on the flower stigma. BloomTime (a *Pantea agglomerans* strain) is one such product that is a good stigma colonizer but also produces antibiotics highly specific to *Erwinia amylovora* (Pusey et al., 2008). Stigma surfaces are not an important entry point for PSS or PSM, and the strain of PS tested was resistant to the antibiotic. However, some *Pantoea agglomerans* strains tested to evaluate suppression of basal kernel blight in barley, which is caused by *Pseudomonas syringae* pv. *syringae*, reduced disease 45-74% in field trials (Braun-Kiewnick et al., 2000).

Other biocontrol products have been developed to fight fireblight and might be worth testing as possible bacterial canker controls. These include: BlightBan A506 (*Pseudomonas fluorescens* strain A506), Blossom Protect (*Aureobasidium pullulans* strains 14940 and 14941) and Serenade (*Bacillus subtilis* strain QST 713). BlightBan A506 and Blossom Protect have both reduced *Erwinia amylovora* symptoms in control environments (Mikiciński et al., 2016).

Testing of bacteria is still ongoing to find new potential biocontrols. Putative strains of *Pseudomonas putida and Pseudomonas fluorescens* were able to create inhibition zones on PSS and PSM on media plates, but have not been field tested (Golanowska et al., 2012). There is also a strain of *Pseudomonas graminis* (49M) that shows promise against fireblight (Mikiciński et al., 2016) and possibly could be tested against PSS or PSM.

Phage therapy has been considered for biocontrol of some organisms that have developed resistance or have few other control options such as PSA, the cause of bacterial canker of

kiwifruit. One phage candidate was found for possible control of PSA, and two PSM strains tested also were susceptible (Di Lallo et al., 2014). Phage therapy has been tested in peach for control of *Xanthamonas campestris* pv. *pruni* and showed some disease reduction, but not sufficient control with the phage alone (Saccardi et al., 1993). Some bacteriophage isolates of PSS specifically were investigated for host range (Nordeen et al., 1983) and in the United States, there is one phage biocontrol product available (AgriPhage from Omnilytics, Sandy, UT) for control of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria*. However, due to the specific host range of some phages, this product may not be effective against PSS.

Other potential controls

Without reliable sprays to control bacterial canker, it is critical to utilize any other control options available. Selecting resistant varieties, reducing inoculum in the orchard, and reducing the amount of susceptible entry points can reduce the risk of infection.

Variety selection

Ideally, cultivars should be selected that are more resistant to bacterial canker. Both rootstocks and scion cultivars can vary in their susceptibility to bacterial canker. It is important to recognize that a cultivar's susceptibility could be caused by either increased susceptibility to initial infection or increased susceptibility to canker progression once infected (Wilson, 1939). Unfortunately, most comparisons of resistance are usually between only 2 or 3 cultivars, as large variety trials to evaluate bacterial canker susceptibility have not been done.

Rootstocks with more observed resistance include: *P. avium* clone F 12/1 (a vegetatively-propagated selection of Mazzard) (Long and Kaiser, 2012), Colt (more tolerant than Mazzard), and Krymsk 5 (similar tolerance to Mazzard) (Long and Kaiser, 2010; Spotts et al., 2010b). Although in lab assays Gisela 6 appeared nearly as tolerant as F 12/1 (Krzesinska and Azarenko, 1992), in field tests and observations, it appears to be more sensitive than Mazzard (Long and Kaiser, 2010; Spotts et al., 2010b).

Resistant rootstocks can be high-budded with scion cultivars to slow down or stop a branch infection before it extends into the trunk. The stock is grown to the height of the desired lower branches and then scion wood is budded onto the rootstock (Long and Kaiser, 2010; Ogawa and English, 1991).

There is little agreement on cultivar susceptibility (Ogawa and English, 1991). 'Corum' has been reported to be more resistant in Oregon (Ogawa and English, 1991), and 'Rainier' and 'Regina' to be more resistant, while 'Bing' and 'Sweetheart' were more susceptible (Spotts et al., 2010b). 'Gold' and 'Roundel' were more resistant than 'Napoleon' (Crosse, 1966; Melakeberhan et al., 1993) and 'Nelson' had less cankers than 'Emperor Francis' (Melakeberan et al., 1993). Bacterial canker also was worse on 'Schmidt' and 'Hardy Giant' in Michigan (Jones, 1971). 'Early Robin' appears to be very susceptible to bacterial canker (personal observation). Susceptibility during dormancy also can be variety dependent. In twigs given a freezing treatment, 'Hedelfingen' was more resistant as dormancy increased; however, the susceptibility of 'Gold' increased as dormancy increased (Sobiczewski and Jones. 1992). Active cambium is important for suppression of bacterial canker infection, which could contribute to the effect of dormancy stage on infection.

Cultural

There are horticultural practices that can help reduce bacterial canker infections. Field observations show that trees with moderate vigor have lower levels of infection than trees that are stressed. Planting in well-drained soil, and maintaining proper nutrition, pH, and irrigation can promote tree vigor. Minimizing tree wetness reduces free moisture available for bacterial population growth and for movement of bacteria via water splashing. This can be done by planting in areas that will dry quickly and keeping irrigation off aboveground tree parts for the first few years when trees are most susceptible. Planting in frost-free areas is recommended to reduce infections associated with spring frost. In high infection areas, trees may be planted later in the spring to avoid cool wet conditions (Spotts et al., 2010b).

High bacterial populations are key to successful infection. Minimizing inoculum sources in the orchard can help reduce high bacterial populations. This can be done by removing and destroying all branches and trees killed by PSS. Weeds (especially grasses) harbor PS populations and growers should consider using clean cultivation with grass-less alleyways for the first three years (Spotts et al., 2010b).

Reduce entry points

Since PS is an opportunistic pathogen, it is important to eliminate as many plant entry points as possible. Avoidable entry points can occur due to frost, pest, and mechanical injury. Frost in spring can be mitigated by planting in frost-free areas and using frost fans or heated high tunnels. Training for wide crotch angles can reduce bark inclusions. Southwest injury caused by freezing and thawing of trunks in winter can be reduced by painting trunks white to minimize

thawing during the day. Pests such as scale or nematodes should be managed to reduce infections.

Careless management can also create plant injuries due to damage from tractors or herbicides. However, other mechanical injury may be unavoidable, like that from trellis wires or pruning cuts. Some high density training systems or dwarfing rootstocks require trellising for support. Rubbing caused by trellis wires can create wounds that can become infected by PSS. Growers should consider either using non-traditional trellising materials, or consider planting orchards that don't require a trellis. Pruning, however, is utilized in all modern cherry orchards. Researchers have compared stub versus flush cuts to see if canker expansion could be contained in the stub and prevent cankers from reaching the trunk or scaffolds. Unfortunately, stub cuts did not have less canker expansion than flush cuts, and a few stub infections still progressed to the trunk or scaffolds (Carroll et al., 2010). Heading cuts in Oregon during May and June caused the most tree death when inoculated; all inoculated wounds, and 93-100% of uninoculated wounds, became infected (Spotts et al, 2010a).

Wounds to the periderm of peach and sweet cherry achieve suberin continuity in 14-24 days (Biggs, 1985, 1986). Suberization stops moisture loss and also impedes microbial invasion (Biggs, 1985). In peach, infection of bark wounds by *Cytospora leucostoma* dropped to 10% at 14 days after wounding, which coincided with an average thickness of 3 cells of necrophylactic periderm; at 24 days, the necrophylactic periderm was 6 cells thick and no infection occurred (Biggs, 1986). These wounds were only to the periderm. Pruning wounds cut through the whole branch, thereby exposing more area to pathogen attack. Given the amount of time that wounds take to heal, it is clearly important to prevent wounds when bacterial populations are high.

Traditionally, pruning is done during the dormant season. However, heading cuts made during the summer were susceptible to infection for about 1 week, whereas the cuts made in winter were susceptible for up to 3 weeks (Spotts et al., 2010a). Current recommendations are to prune during dry weather in summer, or if that is not an option, when it is dry in winter and temperatures are around or slightly above freezing (Spotts et al., 2010b). Summer pruning in August resulted in less, though still significant amounts of, infection, with 50-81% of inoculated wounds and 50-97% of uninoculated wounds becoming infected (Spotts et al, 2010a). Another study showed pruning during July reduced canker expansion (measured in October) by at least 50% compared to pruning in March, April, or May. However, fewer lateral shoots grew from stubs that were pruned in May and July than at the earlier pruning dates (Carroll et al., 2010). If the pruning goal is renewal of fruiting limbs, later pruning would probably not produce new shoots until the following spring.

OUTLOOK

Bacterial canker is a difficult pathogen to control because of its wide host range, multiple periods of tree susceptibility to infection, and the lack of effective spray materials for control. With the wide host range of PSS, it is difficult to eliminate it from the orchard. However, sprays to temporarily reduce bacterial populations during highly infectious periods could be beneficial. PSS has developed resistance to streptomycin even though it is not labeled for use in cherry, likely due to its use in nearby apple orchards. Consequently, resistance to other antibiotics used for fireblight or other diseases could carry over into PSS populations as well. Allowing the use of new agricultural antibiotics in sweet cherry could provide some control of bacterial canker before resistance develops in other plant pathogen populations and carries over into PSS.

Conversely, since resistance that develops in PSS may be transferable to other plant pathogens, it may be advisable to use only bactericides that are already widely applied to prevent the possibility of rapid development of resistance in other pathogens.

New products that show promise as potential bacterial canker controls still need to undergo field testing. Testing products in the field is essential because plate assays or greenhouse testing has very controlled environmental conditions. Constantly changing field conditions can impact tree responses, survival of epiphytic biocontrols, and PSS populations. These factors can impact the effectiveness of possible controls. One of the difficulties with field testing is achieving consistent comparative levels of infection. Artificial inoculations are not always successful. Achieving consistent infection of leaf scars can be difficult even with supplemental inoculation.

Until new spray materials to control bacterial canker become available, control strategies must focus on horticultural methods. Infection entry points that have potentially severe consequences deserve more attention. These include blossoms, leaf scars, and pruning wounds. The potential for freeze damage during bloom can be reduced by using frost fans, heating tunnels, or planting varieties that bloom later. Leaf scars are more difficult to control since leaf abscission is inevitable each fall. Horticultural techniques to avoid leaf scar infection are limited, e.g., defoliation of trees using products such as zinc sulfate before high bacterial populations are present. However, premature defoliation can reduce the amount of photosynthates acquired late in the growing season, with possibly negative effects on fall nutrient remobilization and subsequent spring bloom.

Pruning may be the most problematic entry point because it provides direct access into woody tissue where cankers can form. Adjusting pruning timing to summer would be a relatively simple change, especially if it could be timed to avoid high bacterial populations and limit re-growth of shoots. Re-growth in summer may not cold acclimate sufficiently to prevent winter damage. The goal should be to allow pruning cuts to heal before infection conditions increase in the fall and not initiate re-growth until spring.

With the development of copper resistance in PSS, options to control bacterial canker are limited. Advances are being made for biocontrols of other bacterial diseases, which may eventually extend to bacterial canker. A rapid repeatable infection system to enable prompt testing of new potential controls could speed the development of new products and techniques. Strategies that show promise in greenhouse or growth chamber environments could then be tested in the field.

LITERATURE CITED

LITERATURE CITED

Arrebola, E., V.J. Carrión, J.A. Gutiérrez-Barranquero, A. Pérez-García. P. Rodríguez-Palenquela, F.M. Corzola and A. de Vicente. 2015. Cellulose production in *Pseudomonas syringae pv. syringae*: a compromise between epiphytic and pathogenic lifestyles. FEMS Microbiology Ecol. 91:1–12.

Biggs, A. 1985. Suberized boundary zones and the chronology of wound response in tree bark. Phytopathology 75:1191–1195.

Biggs, A. 1986. Wound age and infection of peach bark by cytospora-leucostoma. Can. J. Bot.-Rev. Can. Bot. 64:2319–2321.

Braun-Kiewnick, A., B.J. Jacobsen, and D.C. Sands. 2000. Biological control of *Pseudomonas syringae* pv. *syringae*, the causal agent of basal kernel blight of barley, by antagonistic *Pantoea agglomerans*. Phytopathology 90:368–375.

Bultreys, A., and M. Kaluzna. 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. J. Plant Pathol. 92:S21–S33.

Cameron, H. 1970. Pseudomonas content of cherry trees. Phytopathology 60:1343–1346.

Cao, T., B.C. Kirkpatrick, K.A. Shackel and T.M. DeJong. 2011. Influence of mineral nutrients and freezing-thawing on peach susceptibility to bacterial canker caused by Pseudomonas syringae pv. syringae. Fruits 66:441–452.

Cao, T., R.J. Sayler, T.M. DeJong, B.C. Kirkpatrick, R.M. Bostock, and K.A. Shackel. 1999. Influence of stem diameter, water content, and freezing-thawing on bacterial canker development of dormant stone fruit. Phytopathology 89:962–966.

Carroll, J., T. Robinson, T. Burr, S. Hoying, and K. Cox. 2010. Evaluation of pruning techniques and bactericides to manage bacterial canker of sweet cherry. New York Fruit Qrtly 18:9–15.

Cody, V.S. and D.C. Gross. 1987. Characterization of pyoverdinpss, the fluorescent siderophore produced by pseudomonas syringae pv. syringae. Appl. Environmental Microbiology 53: 928–934.

Crosse, J.E. 1966. Epidemiological relations of the pseudomonad pathogens of deciduous fruit trees. Annu. Rev. Phytopathol. 4:291–310.

Di Lallo, G., M. Evangelisti, F. Mancuso, P. Ferrante, S. Marcelletti, A. Tinari, F. Superti, L. Migliore, P. D'Abbaddo, D. Frezza, M. Scortichini, and M.C. Thaller. 2014. Isolation and partial

characterization of bacteriophages infecting *Pseudomonas syringae* pv. *actinidiae*, causal agent of kiwifruit bacterial canker. J. Basic Microbiology 54:1210–1221.

Faize, M., L. Faize, N. Koike, M. Ishizaka, and H. Ishii. 2004. Acibenzolar-S-methyl-induced resistance to Japanese pear scab is associated with potentiation of multiple defense responses. Phytopathology 94:604–612.

Golanowska, M., H. Ankiewicz, A. Taraszkiewicz, W. Kamysz, R. Czajkowski, A. Krolicka, and S. Jafra. 2012. Combined effect of the antagonistic potential of selected pseudomonas spp. Strains and the synthetic peptide "CAMEL" on pseudomonas syringae pv. syringae and P. syringae pv. Morsprunorum. J. Plant Pathol. 94:S1.69–S1.73.

Gross, D.C., E.L. Proebsting, Jr. and P.K. Andrews. 1984. The effects of ice nucleation-active bacteria on temperatures of ice nucleation and freeze injury of prunus flower buds at various stages of development. J. Amer. Soc. Hort. Sci. 109:375–380.

Gross, D.C., E.L. Proebsting, Jr., H. Maccrindle-Zimmerman. 1988. Development, distribution, and characteristics of intrinsic, nonbacterial ice nuclei in prunus wood. Plant Physiol. 88:915–922.

Hirano, S., and C. Upper. 1990. Population biology and epidemiology of pseudomonas-syringae. Annu. Rev. Phytopathol. 28:155–177.

Hirano, S.S., C.B. Tanner, and C.D. Upper. 1987. Rain-triggered multiplication of pseudomonas syringae on snap bean leaflets. Phytopathology 77:1694.

Hukkanen, A., K. Kostamo, S. Kärenlampi, and H. Kokko. 2008. Impact of agrochemicals on Peronospora sparsa and phenolic profiles in three Rubus arcticus cultivars. J. Agr. Food Chem. 56:1008–1016.

Ichinose, Y., F. Taguchi, and T. Mukaihara. 2013. Pathogenicity and virulence factors of pseudomonas syringae. J. Gen. Plant Pathol. 79:285–296.

Jones, A. 1971. Bacterial canker of sweet cherry in Michigan. Plant Dis. Rptr. 55:961–965.

Kennelly, M.M., F.M. Cazorla, A. de Vicente, C. Ramose, and G.W. Sundin. 2007. Pseudomonas syringae diseases of fruit trees - Progress toward understanding and control. Plant Dis. 91:4–17.

King, E., M. Ward, and D. Raney. 1954. 2 simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clinical Medicine 44:301–307.

Kokoskova, B., D. Pouvova, R. Pavela. 2011. Effectiveness of plant essential oils against Erwinia amylovora, Pseudomonas syringae pv. syringae and associated saprophytic bacteria on/in host plants. J. Plant Pathol. 93:133–139.

Krzesinska, E.Z., and A.N.M. Azarenko. 1992. Excised twig assay to evaluate cherry rootstocks for tolerance to *Pseudomonas syringae pv. syringae*. HortScience 27:153–155.

Latorre, B., and A. Jones. 1979a. Pseudomonas-morsprunorum, the cause of bacterial canker of sour cherry in Michigan, and its epiphytic association with pseudomonas-syringae. Phytopathology 69:335–339.

Latorre, B., and A. Jones. 1979b. Evaluation of weeds and plant refuse as potential sources of inoculum of pseudomonas-syringae in bacterial canker of cherry. Phytopathology 69:1122–1125.

Latorre, B., J. Gonzalez, J. Cox, and F. Vial. 1985. Isolation of pseudomonas-syringae pv syringae from cankers and effect of free moisture on its epiphytic populations on sweet cherry trees. Plant Dis. 69:409–412.

Lattore, B., C. Lillo, and M. Rioja. 2002. Effect of temperature, free moisture duration and inoculum concentration on infection of sweet cherry by Pseudomonas syringae pv. syringae. Phytoparasitica 30:410–419.

Laue, B.E., H. Steele and S. Green. 2014. Survival, cold tolerance and seasonality of infection of European horse chestnut (*Aesculus hippocastanum*) *by Pseudomonas syringae* pv. *aesculi*. Plant Pathol. 63:1417–1425.

Lee, J., G.M. Teitzel, K. Munkvold, O. del Pozo, G.B. Martin, R.W. Michelmore and J.T. Greenberg. 2012. Type III secretion and effectors shape survival and growth pattern of Pseudomonas syringae on leaf surfaces. Plant Physiol. 158:1803–1818.

Liang L.Z., A.L. Jones. 1995. Organization of the *hrp* gene cluster and nucleotide sequence of the *hrpL* gene from *Pseudomonas syringae* pv. *morsprunorum*. Phytopathology 85:118–123.

Liang, L.Z., P. Sobiczewski, J.M. Paterson, and A.L. Jones. 1994. Variation in virulence, plasmid content, and genes for coronatine synthesis between pseudomonas syringae pv. morsprunorum and P. s. syringae from prunus. Plant Dis. 78:389–392.

Long, L.E., and C. Kaiser. 2010. PNW 619 sweet cherry rootstocks. Pacific Northwest Ext. Publ. 619:1–8.

Maxson-Stein, K., S. He, R. Hammerschmidt, and A. Jones. 2002. Effect of treating apple trees with acibenzolar-S-methyl on fire blight and expression of pathogenesis-related protein genes. Plant Dis. 86:785–790.

McGhee, G.C. and G.W. Sundin. 2011. Evaluation of kasugamycin for fire blight management, effect on nontarget bacteria, and assessment of kasugamycin resistance potential in erwinia amylovora. Phytopathology 101:192–204.

Melakeberhan, H., A.L. Jones, P. Sobiczewski and G.W. Bird. 1993. Factors associated with the decline of sweet cherry trees in Michigan: nematodes, bacterial canker, nutrition, soil pH, and winter injury. Plant Dis. 77:266–271.

Mikiciński A., P. Sobiczewski, J. Pulawska, R. Maciorowski. 2016. Control of fire blight (Erwinia amylovora) by a novel strain 49M of Pseudomonas graminis from the phyllosphere of apple (malus spp.). European J. Plant Pathol. 145:269–276.

Monier, J.-M. and S.E. Lindow. 2003. Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. Proc. Natl. Acad. Sci. 100:15977–15982.

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

Nordeen, R.O., M.K. Morgan, T.C. Currier. 1983. Isolation and partial characterization of bacteriophages of the phytopathogen *Pseudomonas syringae*. Appl. Environmental Microbiology 45:1890–1898.

Ogawa, J.M., and H. English. 1991. Chapter 4: Diseases attacking several genera of fruit and nut trees Sub chapter; Bacterial disease of fruit and nut trees, p. 246–257. In: Diseases of temperate zone tree fruits and nut crops. Univ. CA, Div. Agr. Natural Resources, Oakland, CA. Publ. 3345.

Percival, G.C., K. Noviss, and I. Haynes. 2009. Field evaluation of systemic inducing resistance chemicals at different growth stages for the control of apple (*Venturia inaequalis*) and pear (*Venturia pirina*) scab. Crop Protection 28:629–633.

Pusey, P.L., V.O. Stockwell, D.R. Rudell. 2008. Antibiosis and acidification by *Pantoea agglomerans* strain E325 may contribute to suppression of *Erwinia amylovora*. Phytopathology 98:1136–1143.

Renzi, M., P. Copini, A.R. Taddei, A. Rossetti, L. Gallipoli, A. Mazzaglia and G.M. Balestra. 2012. Bacterial canker on kiwifruit in Italy: Anatomical changes in the wood and in the primary infection sites. Phytopathology 102:827–840.

Saccardi A., E. Gambin, M. Zaccardelli, G. Barone, and U. Mazzucchi. 1993. Xanthamonas campestris pv. pruni control trials with phage treatments on peaches in the orchard. Phytopathologia Mediterranea 32:206–210.

Scholz-Schroeder, B.K., M.L. Hutchinson, I. Grgurina and D.C. Gross. 2001. The contribution of syringopeptin and syringomycin to virulence of pseudomonas syringae pv. syringae strain B301D on the basis of sypA and syrB1 biosynthesis mutant analysis. Mol. Plant-Microbe Interactions 14:336–348.

Smith, A.R.W., and A. Hodson. 2011. Effects of *Pseudomonas syringae* pv. *morsprunorum* strain C28, outer membrane and LPS preparations on cherry callus. J. of Phytopathol. 159:358–367.

Sobiczewski, P. and A.L. Jones. 1992. Effect of exposure to freezing temperatures on necrosis in sweet cherry shoots inoculated with *Pseudomonas syringae* pv. *syringae* or *P. s. morsprunorum.* Plant Dis. 76:447–451.

Spotts, R.A., J. Olsen, L. Long, and J.W. Pscheidt. 2010b. EM 9007 Bacterial canker of sweet cherry in Oregon disease symptoms, cycle, and management. Oregon State Univ. Ext. Serv. EM 9007:1–4.

Spotts, R.A., K.M. Wallis, M. Serdani, and A.N. Azarenko. 2010a. Bacterial canker of sweet cherry in Oregon-Infection of horticultural and natural wounds, and resistance of ccultivar and rootstock combinations. Plant Dis. 94:345–350.

Sundin, G.W., D.H. Demezas, and C.L. Bender. 1994. Genetic and plasmid diversity within natural populations of pseudomonas syringae with various exposures to copper and streptomycin bactericides. Appl. Environmental Microbiology 60:4421–4431.

Sundin, G., A. Jones, and D. Fulbright. 1989. Copper resistance in pseudomonas-syringae pv. syringae form cherry orchards and its associated transfer in vitro and in planta with a plasmid. Phytopathology 79:861–865.

Sundin G., A. Jones, and B. Olson, 1988. Overwintering and population-dynamics ofpseudomonas-syringae pv syringae and PS-pv morsprunorum on sweet and sour cherry trees. Can. J. Plant Pathol.-Rev. Can. Phytopathol. 10:281–288.

The American Phytopathological Society. 1995. Bacterial Canker p. 48–50. In: J.M. Ogawa, E.I. Zehr, G.W. Bird, D.F. Ritchie, K. Uriu, and J.K. Uyemoto (eds.) Compendium of stone fruit diseases.. APS Press. Amer. Phytopathol. Soc. St. Paul, Minnesota.

Thomidis, T., C. Tsipouridis, E. Exadaktylou, and P. Drogoudi. 2005. Comparison of three laboratory methods to evaluate the pathogenicity and virulence of ten *Pseudomonas syringae* pv. *syringae* strains on apple, pear, cherry and peach trees. Phytoparasitica 33:137–140.

Weaver, D.J. and E.J. Wehunt. 1975. Effect of soil pH on susceptibility of peach to Pseudomonas syringae. Phytopathology 65: 984–989.

Wimalajeewa D., and J. Flett. 1985. A study of populations of pseudomonas-syringae pv syringae on stonefruits in Victoria. Plant Pathol. 34:248–254.

Wilson, E.E. 1939. Factors affecting development of the bacterial canker of stone fruits. Hilgardia 12:259–298.

Young J., R. Luketina, and A. Marshall. 1977. Effects on temperature on growth in vitro of pseudomonas-syringae and xanthamonas-pruni. J. Appl. Bacteriology 42:345–354.

CHAPTER 4: PRELIMINARY TRIALS OF BACTERIAL CANKER CONTROL STRATEGIES IN THE LABORATORY AND ORCHARD

INTRODUCTION

Bacterial canker of sweet cherry (caused by *Pseudomonas syringae* pv. *syringae* [PSS]) is a complex and opportunistic disease, with multiple infection points and few control options for growers. With PSS resistance to copper (Sundin et al., 1989; Sundin et al., 1994) and its lack of effectiveness in the field (Carroll et al., 2010), the search for new control spray materials is a top priority. Several antibiotics, plant resistance inducers, and biocontrols were tested to assess their control of PSS in the orchard. Tested products included kasugamycin and oxytetracycline, which are antibiotics used for control of fireblight (caused by the bacteria *Erwinia amylovora*). Acibenzolar-S-methyl is a plant resistance inducer that has shown some activity against fireblight (Maxson-Stein et al., 2002). Phosphorus acid has been used as a fungicide, but is thought to possibly induce plant resistance. The biocontrol organisms (some of which are recommended for fireblight) tested include *Aureobasidium pullulans*, *Bacillus subtilis*, and *Pantoea agglomerans*. Field testing is needed at both bloom and leaf drop to determine the ability of these products to reduce bacterial canker infection.

Results from bacterial canker field trials can be highly variable, likely due to environmental influences such as temperature and water availability that can impact PSS populations. Bacterial canker infections often are associated with cool wet weather (Crosse, 1966). Studies consistently have shown high bacterial populations in early spring, and some high populations in winter or fall. Often low or undetectable populations were found in summer (Latorre et al., 1985; Wimalajeewa and Flett, 1985). The optimal temperature range for PSS

growth is 25° to 30°C (Ogawa and English, 1991; Young et al., 1977), which suggests that low bacterial populations in summer are more a result of limited water availability than temperature.

Water is important for increasing inoculum levels for infection. Higher PSS populations have been detected after rain events, and greenhouse studies confirmed that wetness increased epiphytic bacterial populations (Latorre et al., 1985). Furthermore, the mechanical impact of rain on snap beans stimulated bacterial replication (Hirano and Upper, 1990; Hirano et al., 1987). Latorre et al. (2002) found that free moisture only moderately increased infection of cherry fruit and twigs, but they suggest that it could play a role in nutrient distribution and build-up of bacterial populations.

With the many environmental variables that can influence infection, results of product testing in the orchard can be inconsistent. Development of a repeatable infection system for greenhouse or growth chamber studies would help provide more consistent comparisons between products before investing in large scale field testing. Infection assays for PSS have been developed with excised dormant shoots and twigs (Krzesinska and Azarenko, 1992; Thomidis et al., 2005) and some product testing has used fruit assays (Carroll et al., 2010), but there is a need for a whole tree assay that will better reflect intact system factors such hormones produced in roots. The goal of this work was to test some potential control products in the field and to determine important environmental and host susceptibility factors that impact infection to develop a rapid repeatable infection assay that gives consistent results to better reflect likely success in orchard testing.

MATERIALS AND METHODS

Products tested, bacterial strains used, and inoculum preparation

Products and rates tested for control of leaf scar and blossom infections are presented in Table 4.1. The PSS strains used in all experiments are presented in Table 4.2. PSS strains 13-7, 26-3, 19-6, and rifampicin-resistant 6-9 were obtained from Dr. George Sundin at Michigan State University, as was the inoculum for the 2012 leaf scar trial. Rifampicin-resistant (Rif) strains were generated by growing the parent strain in Kings B (KB) broth (King et al., 1954) and then spreading it on KB agar plates containing rifampicin at 75 µg/mL. Although strain 6-9 was already rifampicin-resistant when isolated from the field, a resistant isolate was still generated in the lab to assure that it retained its resistance. Inoculum was prepared in the Lang lab by culturing strains separately in KB broth, then mixing equal parts of each and measuring the absorbance of the mixture with a UV300 double beam spectrophotometer (Thermo Electron Corporation, Madison, WI) set at 600 nm. Dilutions with an absorbance of ~0.155 were considered to be ~2 x10^7 CFU/mL. Unless otherwise noted, this absorbance was used to determine population size and further dilutions were made for lower inoculum loads.

Table 4.1 Products tested, including name, manufacturer, designation as a bactericide, resistance inducer, or biocontrol, rate used, and leaf scar (LS) or blossom (B) experiment in which they were tested.

Product	Available from	Designation	Rate	LS 2012	LS 2013	B 2013	B 2014
Kocide (Copper)	Certis USA LLC, Columbia, MD	Bactericide	2.00 g/L	x			
Cuprofix (Copper)	United Phosphorus Inc., King of Prussia, PA	Bactericide	2.00 g/L		x	x	x
Fireline	Agrosource Inc., Mountainside, NJ	Bactericide	0.60 g/L	x	x	x	x
Kasumin (8L) or (2L)	Arysta Lifescience, Cary, NC	Bactericide	1.25 or 4.99mL/L, respectively	x	x	x	x
Actigard	Syngenta Crop Protection LLC, Greensboro, NC	Resistance Inducer	0.05 g/L	x	x	x	x
Phostrol	Nufarm Americas Inc., Alsip, IL	Resistance Inducer	1.66 mL/ L	x	x	x	x
Bloomtime	Northwest Agricultural Products Inc., Pasco, WA	Biocontrol	4.42 mL/ L [z]		x	x	x
Blossom Protect and Buffer Protect	Westbridge Agricultural Products, Vista, CA	Biocontrol	0.50 and 3.49 g/ L, respectively		x	x	x
Botector	Westbridge Agricultural Products, Vista, CA	Biocontrol	0.25 g/ L			x	x
Optiva	AgraQuest Inc ., Davis, CA	Biocontrol or bactericide[y]	0.60 g/ L	x		x	x

[z]Rate using Bloomtime reconstituted at 0.1 g in 500 mL of water.
[y]Treated as Biocontrol except in 2012 leaf scar testing.

Table 4.2 *Pseudomonas syringae* pv. *syringae* strains used, including those resistant to rifampicin (Rif), for experiments that included leaf scar (LS) and blossom (B) trials, and infection parameters that included tissue stage, temperature (Temp), inoculum load (Inoc), and delayed inoculation (Day).

PSS strains	LS 2012	LS 2013	B 2013	B 2014	LS Infection Parameters	Tissue Stage	Temp	Temp and Inoc	Day 0, 2, 5 2013	Day 0, 2, 5 2014	Day and Temp
13-7			x	x					x		
13-7Rif[z]		x			x	x	x	x		x	x
26-3			x						x		
26-3Rif[z]								x		x	x
19-6			x	x					x		
19-6Rif[z]		x			x	x	x	x		x	x
30-1Rif	x										
6-9 Rif	x		x						x		
6-9.3 Rif[z]		x			x	x	x	x		x	x

[z]Rifampicin-resistant mutants generated in the lab.

Blossom spray trials

To test the efficacy of potential control products for sweet cherry blossom infections, field trials were conducted in 2013 and 2014 with 'Rainier' on 'Gisela 3' rootstock planted in 2010 at the Clarksville Research Center (CRC) in Clarksville, MI (lat. 42.8ºN, long. 85.2ºW) in a coarse-loamy, mixed, mesic typic hapludalf soil of the Lapeer series.

In 2013, the experimental design was a Randomized Complete Block Design (RCBD) with 6 blocks each containing a single tree replication of each treatment. Due to climatic conditions, bloom progressed rapidly, preventing multiple spray applications. Plant resistance inducers and biocontrols were applied on May 3, two days prior to wounding and inoculation. Bactericides were sprayed on May 4, one day prior to wounding and inoculation. To simulate a frost damage event to blossoms, on May 5, sterile scissors were used to wound the pistils and stamens of all flowers of ~10 blossom clusters per tree, but the number of wounded flowers per cluster was not quantified. All treatments except the uninoculated control were inoculated on May 5, with a cocktail of four PSS strains (Table 4.2) at ~2 $x10^7$ CFU/mL (colony forming units per mL) by spraying to runoff with an atomizer. The branches were then bagged with white opaque plastic bags and the bags were removed after two days (May 7). Infection was assessed 16 days after inoculation (May 21). Blossom clusters were counted as infected if at least one flower or fruitlet was brown and necrosis was advancing along the pedicel (Fig. 4.1). Percent infection was calculated by dividing the number of infected clusters by the total number of treated clusters.

In 2014, the experimental design was a RCBD with 5 blocks each containing a single tree replication of each treatment. Plant resistance inducers were applied 4 and 8 days (May 2 and

May 6) before wounding and inoculation. Biocontrols were sprayed two and four days (May 6 and May 8 of 2014) prior to wounding and inoculation. Bactericides were sprayed on May 8, two days prior to wounding and inoculation, which occurred on May 10. Simulation of frost damage to blossom clusters was done in the same manner as in 2013. Some trees had fewer flowers per cluster, probably due to winter damage from low temperatures, however, flower number was not recorded. Inoculation and bagging protocol were the same as 2013 except that only two PSS strains (Table 4.2) were used. The inoculum had an absorbance of 0.163 at 600 nm, corresponding to a slightly higher bacterial load than in 2013. There was 2.5 cm of rain on May 7, and it also rained 0.3 cm on May 9 (the day before inoculation). The rain may have affected the results by removing some of the control products. Infection assessment was performed 17 days after inoculation on (May 27) and percent infection was calculated as in 2013.

Leaf scar product testing

To test the efficacy of control products on leaf scar infections, field trials were conducted in 2012 and 2013. Treatments for both years were imposed on 'Early Robin' on 'Gisela 5', 'Gisela 6', and 'Gisela 12' rootstocks planted in 2007 at CRC in a coarse-loamy, mixed, semiactive, mesic oxyaquic hapludalf soil of the Dryden series with a small part of the plot changing to a coarse-loamy, mixed, semiactive, mesic typic hapludalf soil of the Lapeer series. Blocking was used to reduce variability by plot location. Sprays tested each year are included in Table 4.1 and PSS strains used are in Table 4.2.

In 2012, the experimental design was a RCBD with four blocks of single tree replications in each block and two subsamples (branches) per tree. Resistance inducers and two water controls (one that was later inoculated [WI2] and one that was uninoculated [WU2]) were

sprayed twice, one and two weeks before inoculation, on September 27 and October 4. Bactericides (Optiva was treated as a bactericide, not a biocontrol) were sprayed the day before and after inoculation along with a two more water controls (one inoculated and one uninoculated), October 10 and 12. Two branches at ~50% leaf drop were inoculated on October 11 with a mixture of two rifampicin-resistant PSS strains (Table 4.2) at 10^8 CFU/mL (inoculum provided by G. Sundin Lab) and each branch was treated as a subsample. The following April, before bud break, branches for subsampling were removed, surface sterilized, and bacteria extracted from a combined sample of 5 buds for each subsample. Bacteria were extracted by surface sterilizing twigs in 10% bleach for 1 min and rinsing twice in 500 mL sterile deionized water. After drying, 5 buds were removed and diced in 2 mL of 0.5 strength phosphate buffered saline (PBS) and allowed to sit for 5 min. Buffer was then removed, serially diluted, and 25 μL drops plated onto KB media amended with either 75 μg/mL rifampicin to select for inoculated strains and 50 μg/mL cycloheximide to inhibit fungal growth, or plates amended with only 50 μg/mL cycloheximide. Resulting colonies were counted to obtain population estimates per gram. Only two samples grew on rifampicin plates, so the reported populations were from plates amended with cycloheximide only and were adjusted for minimal detectable population to the lowest possible number of bacteria isolated in the largest bud sample.

In 2013, the same protocol was followed with the following amendments. The experimental design was a RCBD with 5 blocks using single tree replications and 2 subsamples per tree. Resistance inducers were sprayed on September 19, and re-applied on September 26. Bactericides and biocontrols were sprayed two days before inoculation on October 1. Three strains of rifampicin-resistant PSS (Table 4.2) were prepared at ~2 x 10^7 CFU/mL for inoculation on October 3.

Plant material for growth chamber studies

To assess whole tree physiological responses to PSS infection and potential control materials, the greenhouse and growth chamber studies used potted sweet cherry trees. This allows for any influence of meristematic- or root-produced compounds such as phytohormones that might help combat infection. Unless otherwise noted, trees were planted in ~11 L pots in a commercial potting mix provided by the research greenhouse and then head-pruned at ~30 cm to stimulate branching. 'Bing'/'Gisela 6' and 'Rainier'/'Gisela 6' bare root nursery trees were planted in June 2013 and additional 'Bing'/'Gisela 6' were planted in June 2014. All trees were maintained in the greenhouse and trees planted in 2013 were moved outside in November of 2013 for natural cold treatment to break dormancy.

Infection parameters

Leaf scar chamber study

To develop a repeatable leaf scar infection assay, three types of leaf removal were assessed to test the hypothesis that different leaf removal techniques could simulate natural leaf senescence and leaf scar formation of 'Bing'/'Gisela 6' planted in 2013. The experimental design was a RCBD with 5 blocks and single tree replications within blocks. The experiment was initiated in November 2013. Trees were grouped by inoculated and uninoculated treatments within blocks in the growth chamber to prevent cross contamination. Leaf removal treatments were: 1) manually pulling off healthy green leaves just before inoculation, 2) clipping leaf lamina off at the petiole a week before inoculation and subsequently manually removing the remaining portion of the petiole just before inoculation, and 3) senescence induced by a

thermoperiod of 7.5/4.5°C and 10:14 hr light:dark photoperiod for 5 to 6 weeks. Ultimately, all leaves were removed except for spurs that were left as controls. Following leaf or petiole removal, the trees were inoculated with a mixture of three rifampicin resistant PSS strains (Table 4.2) at ~2 x 10^7 CFU/mL by misting to run-off with an atomizer. Controls were inoculated with deionized water. After inoculation, the trees were kept in the growth chamber at the same settings and after 61 days, bacteria were extracted by surface sterilizing twig samples in 10% bleach for 1 min and rinsing twice in 500 mL of sterile deionized water. After drying the twigs, a combined sample of 5 axillary buds from each tree was removed and chopped in 2 mL of 0.5 strength PBS and allowed to sit for 5 min. Buffer was then removed and 25 µL drops were plated onto KB media amended with 75 µg/mL rifampicin to select for the inoculated strains and 50 µg/mL cycloheximide to inhibit fungal growth. After two days, the plates were evaluated for growth of bacteria and samples were considered infected if bacteria proliferated on the amended media.

Days to inoculation studies

Due to the lack of effective spray materials for PSS control, growers have been advised to prune when there are several days of dry weather to minimize infection from PSS due to rain dissemination of the bacteria. This study evaluated that strategy by examining inoculum load, days to inoculation, and wound size. It was hypothesized that smaller wounds would resist infection better than large wounds by healing more quickly.

In June 2013, unheaded 'Rainier'/'Gisela 6' bare root nursery trees were planted in~3.75 L pots in a commercial potting mix provided by the research greenhouses and maintained in the growth chamber at 20°C with a 12:12 hr photoperiod. The experimental design was a Complete

Randomized Design (CRD) with 5 single tree replications of the 18 treatments. All trees were pruned on the same day and inoculated either 0, 2, or 5 days after pruning. Factors were time of delayed inoculation (0, 2, or 5 days after pruning), inoculum level (0, ~2 x 10^5, or ~2 x 10^7 CFU/mL), and trunk diameter (~1 cm [small, S] or ~1.5 cm [large, L]). Four PSS strains were used for inoculation (Table 4.2). Trees were not surface-sterilized before pruning. All branches were pruned on the same day and the cuts were inoculated on day 0, 2, or 5 after pruning by using a pipette to put inoculum on the cut surface. Re-isolation was done 15 weeks after inoculation on KB media without rifampicin.

The experiment was repeated with 'Bing'/'Gisela 6' planted in 2014 with all trees planted in either ~3.75 or ~11 L pots. To avoid potential problems with native bacteria already present on nursery trees, the trunks were surface sterilized by wiping with 70% alcohol wipes and pruners were surface sterilized between each cut with 10% bleach followed by 75% ethanol. Trunks were pruned on the appropriate treatment day (starting with day 5 before inoculation, pruned on June 20, 2014) and then inoculated with bacteria or deionized water (control) on day 0. Re-isolation was done after 2 weeks on media amended with 75 μg/mL rifampicin to select for inoculation bacteria and 50 μg/mL cycloheximide to inhibit fungal growth.

Pruning and tissue stage study

To test the hypothesis that dormant trees are more susceptible to PSS than actively growing trees, greenhouse-grown 'Rainier'/'Gisela 6' nursery trees (planted in 2013) were moved outdoors in fall 2013 for natural chilling to break endodormancy. The trees were pruned while endodormant (February 2014) or during active growth in summer (June 2014). The tissue stage pruning treatments were inoculated at different times, which prevented using a CRD or

RCBD. Logistic regression was used for statistical analysis. There were 5 replicates of each treatment, and treatments consisted of two factors: Endodormant- or Summer-pruned; and Inoculum levels of 0 or ~2 x 10^7 CFU/mL. Trees were kept in a growth chamber for the duration of the experiment with a 12:12 hr photoperiod at 20°C. Pruning sites were surface sterilized with 70% alcohol wipes and pruners were sterilized between each cut with 10% bleach followed by 75% ethanol. Inoculum contained 3 strains of rifampicin-resistant PSS (Table 4.2). Branches were cut and soon after inoculated with 50 µL of inoculum to the cut surface. Re-isolation was attempted two weeks after inoculation. Branches were surface-sterilized as described for bud re-isolations. Tissue from the wound site was minced in 500 µl 0.5 strength PBS and given 15 min for bacteria to diffuse out of the tissue. The buffer was then extracted and plated on KB media amended with 75 µg/mL rifampicin to select for inoculation bacteria and 50 µg/mL cycloheximide to inhibit fungal growth. Samples were considered infected if bacteria grew on the amended media.

Temperature study

Temperature is hypothesized to impact infection success by affecting plant responses such as healing or plant resistance. To test the impact of spring temperatures on infection, greenhouse-grown 'Rainier'/'Gisela 6' nursery trees planted in 2013 were overwintered outside the greenhouse beginning in November and returned to the greenhouse around June 2014. A split-plot design was used with 3 replications of the experiment (repeated over time for use of the growth chamber) with 2 single tree replications per chamber and with two inoculations per tree. Branches were pruned and inoculated starting in January 2014, and trees were maintained in a growth chamber with a 12:12 hr photoperiod at either 10°C or 20°C. Trees were either

uninoculated or inoculated with 3 strains of rifampicin-resistant PSS (Table 4.2) at ~2 x 10^7 CFU/mL. Pruning sites were surface sterilized, inoculated, and sampled for rifampicin-resistant bacteria as in the tissue stage study.

Temperature and inoculum load study

To test the hypothesis that temperature interacts with inoculum loads to cause infection, 'Bing'/'Gisela 6' trees planted in 2014 were grown in the greenhouse until the experiment began in November 2014. A split-plot design was used with 2 large blocks. Blocking was done over time (to repeat the experiment within the growth chamber) with two single-tree replications per block. Growth chambers were set at 10°C or 20°C with a 12:12 hr photoperiod. Trees were moved to the growth chamber and trees were surface-sterilized and pruned as in the tissue stage experiment. They were then inoculated with a mixture of 4 rifampicin-resistant strains of PSS (Table 4.2) at 0, ~2 x 10, ~2 x 10^3, or ~2 x 10^5 CFU/mL. Re-isolation followed the same procedure as the tissue stage experiment.

Time to inoculation, temperature and dye permeability study

Sweet cherry requires at least 24 days to develop lignified and suberized tissues at wound sites (Biggs, 1985). Therefore, it was hypothesized that even delayed inoculations may successfully infect sweet cherry pruning cuts. A time course of pruning cut permeability to water-soluble dye was used to infer whether infection success could be associated with water permeability of the wound site. It was hypothesized that permeability to water-soluble dyes would be reduced over time as pruning cuts heal. To test these hypotheses, an experiment was developed to assess the effect of time after pruning and temperature on changes in pruning cut

dye permeability and inoculation susceptibility. Greenhouse-grown 'Bing'/'Gisela 6' nursery trees planted in 2014 were used in a split-plot design to block for growth chamber. There were three whole plot replications (for growth chamber) with two single-tree replications per whole plot replication. Factors were pruning 0, 10, 17, and 24 days before inoculation, temperature (10°C or 20°C), and inoculum level (0 or ~2 x 10^5 CFU/mL), plus a dye permeability assessment. Trees were maintained in growth chambers with a 12:12 hr photoperiod for the duration of the experiment. Pruning sites and pruners were surface-sterilized as in the tissue stage experiment. Branches were pruned on the appropriate days before inoculation. All pruned plants were inoculated on the same day (day 0) with a mixture of 4 rifampicin-resistant strains of PSS bacteria (Table 4.2) or a PBS control, or they were evaluated for dye permeability. Dye permeability was assessed by fitting a piece of clear plastic lab tubing over the pruned explants to try to attain a watertight seal, with the open end oriented vertically. An aqueous solution of 0.1% Safranin O dye was added to the tube to cover the cut end with dye and then was left for 2.5 hours. After dye treatment, the branches were cut open longitudinally to determine if dye was able to penetrate beyond the pruning site. Re-isolation of bacteria from the treated pruning cuts was attempted 2 weeks after inoculation as described in the tissue stage experiment. No infection occurred in un-inoculated treatments, so those were removed from logistic regression analysis.

Statistics

Statistical analyses for all experiments were done with the Statistical Analysis System program (SAS Institute Inc., Cary, NC). Statistics for the blossom and leaf scar experiments included analysis of variance (ANOVA) using Proc Mixed and if the ANOVA was significant all pairwise comparisons were done using t tests with the LSmeans pdiff option if. For bacterial

populations extracted from buds near leaf scars a base 10 logarithmic transformation was used. When no bacteria were detected in the bud samples, the population was adjusted to the minimum detectible population. Effects and treatments were reported as significant if the p-value was less than 0.05. Since the infection system experiments had a binomial response variable (infected or not infected), statistics were done with Proc Logistic which performs logistic regression analysis. All pairwise comparisons were done with the LSmeans statement and ilink option, and compared with Wald-tests and the Tukey-Kramer adjustment. Probabilities were reported as significant with a P-value of 0.05. Because Tukey-Kramer minimum significant difference showed no significance, Wald-tests also are reported to avoid being too conservative (which would possibly increase type II errors). Tukey-Kramer accounts for experiment-wise error where Wald-tests do not. With Wald tests, there is a chance of incorrectly concluding that differences are significant, so results should be interpreted with caution.

RESULTS

Blossom spray trials

In 2013, several commercial products showed potential to reduce blossom infection by PSS. Cuprofix (Copper), Fireline (oxytetracycline), and Blossom Protect (which was mixed with Buffer Protect) reduced infection by 44-48%, and Kasumin reduced infection by 89% (Table 4.3). In 2014, none of the spray materials reduced PSS infection significantly and no inoculated treatments were significantly different from the inoculated control.

Table 4.3 Percent blossom cluster infection of 'Rainier'/'Gisela 3' sweet cherry blossoms following inoculation with Pseudomonas syringae pv. syringae in 2013 and 2014. Products tested for control of blossom blast included: Cuprofix (copper), Fireline (oxytetracycline), Kasumin (kasugamycin), Actigard (acibenzolar-S-methyl), Phostrol (phosphorus acid), BloomTime (Pantoea agglomerans), Blossom Protect (Aureobasidium pullulans), Botector (Aureobasidium pullulans), and Optiva (Bacillus subtilis).

Treatment	Percent infected 2013	Significance at 0.05	Percent infected 2014	Significance at 0.05
Uninoculated control	9.73	c[z]	5	c
Inoculated control	72.67	a	38	ab
Cuprofix	36.87	b	46	ab
Fireline	37.59	b	62	ab
Kasumin	7.47	c	34	bc
Actigard	63.29	ab	32.5	bc
Phostrol	51.94	ab	38	ab
BloomTime	68.96	a	36	b
Blossom Protect	40.47	b	34	bc
Botector	55.98	ab	36	b
Optiva	63.64	ab	66	a

[z]ANOVA was statistically significant for both years and years were analyzed separately. Means separation was done with t-tests and means followed by the same letter were not significantly different at a P-value < 0.05.

Figure 4.1. Example of healthy and *Pseudomonas syringae* pv. *syringae* (PSS)-infected sweet cherry blossom clusters from uninoculated and inoculated controls.

Leaf scar product testing

Rifampicin-resistant bacteria were not re-isolated consistently from the leaf scar inoculations, even though additional rifampicin-resistant strains were used in 2013. All data presented were of fluorescent pseudomonads re-isolated on cycloheximide-only medium and therefore could include bacteria that had infected the plant tissues prior to spray treatments. Fluorescent pseudomonads were re-isolated from 60% of the samples in 2012 and only 21% of samples in 2013; all other sample values were adjusted to the minimum detectible level. Data were $\log_{10}$ transformed for statistical analysis (Table 4.4). No bacteria were re-isolated from the copper treatment in 2013, so the standard error is zero since all values were adjusted, resulting in no variation (Table 4.4). ANOVAwas not significant for $\log_{10}$CFU/g (colony forming units per gram) in either 2012 or 2013, demonstrating that none of the treatments significantly affected re-isolation of bacteria.

Table 4.4 Mean and standard error for log10 CFU/g of fluorescent pseudomonads recovered from sweet cherry buds of 'Early Robin' on 'Gisela 5', 'Gisela 6', or 'Gisela 12' rootstocks from fall 2012 and 2013 leaf scar experiments. Products tested included: copper, Fireline (oxytetracycline), Kasumin (kasugamycin), Actigard (acibenzolar-S-methyl), Phostrol (phosphorus acid), BloomTime (Pantoea agglomerans), Blossom Protect (Aureobasidium pullulans) and Optiva (Bacillus subtilis). In addition to spray treatments, controls included a water uninoculated control sprayed with resistance inducers (WU2), a water inoculated control sprayed with resistance inducers (WI2), and Pseudomonas syringae pv. syringae-inoculated (WIC) and uninoculated (WUC) controls sprayed at the same time as the bactericides. Trees were inoculated on October 11 in 2012 and October 3 in 2013. ANOVA was not significant for 2012 or 2013.

Treatment	Mean Log_{10}CFU/g 2012	Standard error 2012	Spray dates 2012	Mean Log_{10}CFU/g 2013	Standard error 2013	Spray dates 2013
WU2	2.34[z]	0.28	Sept 27 & Oct 4	NA	NA	NA
WI2	3.70	0.59	Sept 27 & Oct 4	NA	NA	NA
WUC	3.82	0.35	Oct 10 & Oct 12	2.38	0.33	Oct 1
WIC	2.55	0.29	Oct 10 & Oct 12	2.23	0.25	Oct 1
Copper	2.36	0.34	Oct 10 & Oct 12	1.91	0.00	Oct 1
Fireline	2.87	0.36	Oct 10 & Oct 12	2.29	0.38	Oct 1
Kasumin	3.06	0.39	Oct 10 & Oct 12	2.50	0.40	Oct 1
Actigard	2.95	0.43	Sept 27 & Oct 4	2.14	0.16	Sept 19 & Sept 26
Phostrol	2.64	0.31	Sept 27 & Oct 4	2.23	0.20	Sept 19 & Sept 26
BloomTime	NA[y]	NA	NA	2.47	0.38	Oct 1
Blossom protect	NA	NA	NA	2.69	0.33	Oct 1
Optiva	3.29	0.59	Oct 10 & Oct 12	NA	NA	NA

[z]Adjusted to minimum detected population before log_{10} transformation.
[y]Not all treatments were applied both years; missing treatments (NA) are noted.

Infection parameters

Leaf scar chamber study

Leaf scars resulting from environmentally-induced senescence and mechanical removal of leaves (pulling) yielded a 100% rate of rif-resistant PSS re-isolation, while that from clipped petioles was only 60%. The logistic regression model including inoculation, leaf removal treatment, and their interaction estimated infection probabilities of 0.08 for uninoculated treatments, 0.58 for clipped petiole inoculations, and 0.92 for inoculated trees with environmentally-induced senescence or pulled leaves (Table 4.5). Although the re-isolated populations were not quantified, induced senescence yielded more colonies visually during re-isolation.

Table 4.5 Sweet cherry leaf scar infection protocol testing. Probability of recovering rifampicin-resistant fluorescent pseudomonads from sweet cherry buds with three leaf removal treatments: (environmentally-induced natural senescence [Natural], mechanically-removed green leaves [Pulled], and clipped petioles [Clipped]), with and without inoculation with rifampicin-resistant Pseudomonas syringae pv. syringae.

Treatment	Probability of wound infection	Standard Error of the probability	Significance at 0.05 with Wald tests	Significance at 0.05 with Tukey adjustment
Natural Un-inoculated	0.08[z]	0.12	b[y]	a[x]
Natural Inoculated	0.92	0.12	a	a
Pulled Un-inoculated	0.08	0.12	b	a
Pulled Inoculated	0.92	0.12	a	a
Clipped Un-inoculated	0.08	0.12	b	a
Clipped Inoculated	0.58	0.22	ab	a

[z]Probabilities from logistic regression model which included inoculation and leaf scar treatments. Only inoculation contributed significantly to the model.

[y]Results of Wald tests were reported because there was no significance with Tukey-Kramer adjustment. Results should be interpreted with caution because Wald tests do not control for experiment-wise error and carry a risk of type I error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

[x]Comparisons with Tukey-Kramer adjustment to control for experiment-wise error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

Pruning wounds

Days to inoculation studies

In 2013, some fluorescent pseudomonads were re-isolated even from uninoculated pruning treatments. Since samples may have been infected with native bacteria already present on the trees at the time of inoculation, there were no reliable results for an effect of inoculation time after wounding. The experiment was repeated in 2014 with surface sterilization of pruners and the future wound site prior to pruning to prevent infection by native bacteria present before inoculation. The use of rifampicin-resistant bacteria for inoculation allowed confirmation of infection by re-isolation when using rifampicin in the media. Since external canker length did not give a good indication of infection (data not shown), infection in later experiments was determined by re-isolation of bacteria two weeks after inoculation.

In 2014, three of the 30 uninoculated controls became infected, but logistic regression analysis still showed inoculum load significantly affected the model. However, there was no significant effect of TCSA or inoculation time after pruning on probability of infection. When pooling data for day and inoculum load, uninoculated treatments had 0.15-0.16 probability of becoming infected and inoculated treatments were not significantly different from each other, with probabilities ranging from 0.80 to 0.92 (Table 4.6). Results for TCSA were not reported because it did not significantly impact the model.

Table 4.6 The probability of potted 'Bing'/'Gisela 6' sweet cherry trees becoming infected from inoculation with Pseudomonas syringae pv. syringae at various times after pruning in 2014. Treatment factors include: inoculum load (0, 105, 107 CFU/mL), inoculation time after pruning, and trunk cross-sectional area (TCSA). Data were pooled for TCSA because it did not contribute significantly to the regression model.

Inoculation Load (CFU/mL)	Inoculation Time after Pruning (days)	Probability of wound infection	Standard Error of the probability	Significance at 0.05 with Wald tests	Significance at 0.05 with Tukey adjustment
0	0	0.16[z]	0.13	b[y]	a[x]
10^5	0	0.92	0.09	a	a
10^7	0	0.85	0.12	a	a
0	2	0.15	0.12	b	a
10^5	2	0.85	0.12	a	a
10^7	2	0.92	0.09	a	a
0	5	0.15	0.12	b	a
10^5	5	0.80	0.15	a	a
10^7	5	0.92	0.09	a	a

[z]Probabilities from logistic regression model which included inoculum load, inoculation time (days after pruning), trunk cross sectional area and all their interactions. Only inoculum load contributed significantly to the model.

[y]Results of Wald tests were reported because there was no significance with Tukey-Kramer adjustment. Results should be interpreted with caution because Wald tests do not control for experiment-wise error and carry a risk of type I error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

[x]Comparisons with Tukey-Kramer adjustment to control for experiment-wise error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

Pruning and tissue stage study

At ~$2x10^7$ CFU/mL PSS, all endodormant and summer pruned trees became infected when inoculated.

Temperature study

When sweet cherry trees were pruned and subsequently grown at either 10°C or 20°C, all wounds inoculated at ~$2x10^7$ CFU/mL became infected. Rif-resistant PSS was re-isolated from nearly all inoculated branches and only one uninoculated branch. Logistic regression analysis showed that temperature did not have a significant impact on the probability of infection at this high inoculum load. Inoculated and uninoculated trees had probabilities of 0.96 and 0.04-0.12, respectively, of becoming infected (Table 4.7).

Table 4.7 Probability of potted 'Rainier'/'Gisela 6' sweet cherry branch pruning wound infection following inoculation with ~2x107 CFU/mL Pseudomonas syringae pv. syringae. Treatments include post-pruning wound temperature (10^{o}C or 20^{o}C) and inoculation (Inoculated or Uninoculated).

Treatment	Probability of wound infection	Standard Error of the probability	Significance at 0.05 with Wald tests	Significance at 0.05 with Tukey adjustment
20°C Un-inoculated	0.04[z]	0.06	b[y]	b[x]
20°C Inoculated	0.96	0.06	a	a
10°C Un-inoculated	0.12	0.09	b	b
10°C Inoculated	0.96	0.06	a	a

[z]Probabilities from logistic regression model which included inoculation, temperature, and their interaction. Only inoculation contributed significantly to the model.
[y]Results of Wald tests were reported because there was no significance with Tukey-Kramer adjustment. Results should be interpreted with caution because Wald tests do not control for experiment-wise error and carry a risk of type I error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.
[x]Comparisons with Tukey-Kramer adjustment to control for experiment-wise error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

Temperature and inoculum load study

The previous experiment was repeated at lower PSS inoculum levels. Logistic regression analysis showed no significant effect of temperature, but a significant effect of inoculum load (although one uninoculated control also became infected). According to the model, inoculum levels 0, ~2x10, ~$2x10^3$ and ~$2x10^5$ CFU/mL had infection probabilities of 0.17, 0.50, 0.83, and 0.95, respectively (Table 4.8).

Table 4.8 Probability of potted 'Bing'/'Gisela 6' sweet cherry pruning wound infection at 10°C or 20°C following inoculation with Pseudomonas syringae pv. syringae at 0, ~2x10, ~2x103 and ~2x105 CFU/mL. Data were pooled for temperature because it did not contribute significantly to the regression model.

Inoculum load (CFU/mL)	Probability wound infection	Standard Error of the probability	Significance at 0.05 with Wald tests	Significance at 0.05 with Tukey adjustment
0	0.17	0.13	b	a
~2x10	0.50	0.18	ab	a
~$2x10^3$	0.83	0.13	a	a
~$2x10^5$	0.95	0.08	a	a

[z]Probabilities from logistic regression model which included inoculum load and temperature. Only inoculum load contributed significantly to the model.
[y]Results of Wald tests were reported because there was no significance with Tukey-Kramer adjustment. Results should be interpreted with caution because Wald tests do not control for experiment-wise error and carry a risk of type I error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.
[x]Comparisons with Tukey-Kramer adjustment to control for experiment-wise error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

Time to inoculation, temperature and dye permeability study

When potted sweet cherry trees were pruned, grown at 10°C or 20°C, and pruning wounds were inoculated 0, 10, 17, or 24 days after pruning, temperature showed some affect on susceptibility to infection. Inoculation 0 or 10 days after pruning resulted in all branches becoming infected, regardless of temperature. Inoculation 17 days after pruning resulted in 100% infection at 10°C, but only 67% infection at 20°C. Similarly, inoculation 24 days after pruning resulted in 67% infection at 10°C and 50% infection 20°C. However, neither day nor temperature significantly contributed to the logistic regression model for infection. The probability of infection was not significantly different between days and ranged from 0.58-0.97 (Table 4.9).

Table 4.9. Probability of potted 'Bing'/'Gisela 6' sweet cherry branch pruning wound infection at 10°C or 20°C following inoculation with Pseudomonas syringae pv. syringae at 0, 10, 17, or 24 days after pruning. Data were pooled for temperature because it did not contribute significantly to the regression model.

Treatment	Probability of wound infection	Standard Error of the probability	Significance at 0.05 with Wald tests	Significance at 0.05 with Tukey adjustment
0	0.97[z]	0.05	a[y]	a[x]
10	0.97	0.05	a	a
17	0.82	0.11	a	a
24	0.58	0.15	a	a

[z]Probabilities from logistic regression model which included temperature and day but neither contributed significantly to the model.

[y]Results of Wald tests were reported because there was no significance with Tukey-Kramer adjustment. Results should be interpreted with caution because Wald tests do not control for experiment-wise error and carry a risk of type I error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

[x]Comparisons with Tukey-Kramer adjustment to control for experiment-wise error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

Table 4.10. Probability of potted 'Bing'/'Gisela 6' sweet cherry branch pruning wounds retaining dye permeability at 10°C or 20°C 0, 10, 17, or 24 days after pruning. Data were pooled for temperature because it did not contribute significantly to the regression model.

Treatment	Probability of wound being dye-permeable	Standard Error of the probability	Significance at 0.05 with Wald tests	Significance at 0.05 with Tukey adjustment
0	0.94[z]	0.08	a[y]	a[x]
10	0.88	0.09	a	a
17	0.80	0.12	a	a
24	0.35	0.14	b	a

[z]Probabilities from logistic regression model which included temperature and day. Only day contributed significantly to the model.
[y]Results of Wald tests were reported because there was no significance with Tukey-Kramer adjustment. Results should be interpreted with caution because Wald tests do not control for experiment-wise error and carry a risk of type I error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.
[x]Comparisons with Tukey-Kramer adjustment to control for experiment-wise error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

When wound permeability to dye was examined instead of infection, 100% of wounds were dye permeable at 0 and 10 days regardless of temperature but the permeability decreased dramatically between 17 and 24 days after pruning. At 17 days after pruning, 83% of the wounds were permeable to dye at either 10°C or 20°C. However, 24 days after pruning, only 33% and 20% were permeable at 10°C or 20°C, respectively. Day contributed significantly to the logistic regression model for dye permeability, but temperature did not. Days 0, 10, and 17 were not significantly different from each other with permeability probabilities of 0.94, 0.88, and 0.80, respectively. Day 24 was significantly different (Wald tests) with a probability of 0.35 (Table 4.10).

DISCUSSION

Blossom and leaf scar infection trials

In the 2013 blossom infection trial, spray applications of the bactericides oxytetracycline and kasugamycin most effectively reduced infection, similar to previous research. When applied to sweet cherry fruit after inoculation, kasugamycin reduced lesion diameter by about 25% compared to the inoculated control and oxytetracycline reduced lesion diameter 44-54% compared to inoculated control depending on application before or after inoculation (Carroll et al., 2010). When 26 *Pseudomonas syringae* isolates were tested for resistance to kasugamycin (100 μg/mL), only 3 were resistant. However, 2 of the 3 isolates were susceptible on media amended with Kasumin adjusted to a comparable amount of kasugamycin (McGhee and Sundin, 2011). Although copper sprays reduced infection in the 2013 blossom study, PSS copper resistance has been documented in Michigan and Oklahoma (Sundin et al., 1989; Sundin et al., 1994) and copper sprays are no longer effective at reducing populations in Michigan orchards surveyed (Sundin et al., 1989).

Actigard and Phostrol did not significantly reduce the percent of blossom infections when sprayed two days before bloom. Phosphite did not reduce infection of pruning wounds by PSS in sweet cherry when applied as late as April 25 in New York (Carroll et al., 2010). In apple [*Malus x domestica* (Borkh.)], weekly sprays of Actigard starting one week before bloom reduced the number of fire blight strikes from natural infections and were recommended as a supplement to, rather than a replacement for, streptomycin (Maxson-Stein et al., 2002). Under the testing conditions of the current study, no resistance inducers provided control which could be because there was little leaf area on sweet cherry during bloom. Actigard is used as a foliar spray and it

may be that more leaf surface area is required for it to be effective. In apple more leaves are present before bloom which may explain why it was effective in that study.

The biocontrols had minimal effectiveness against bacterial canker. In field trials, some *Pantoea agglomerans* strains reduced basal kernel blight in barley (caused by *Pseudomonas syringae* pv. *syringae*) 45-74% (Braun-Kiewnick et al., 2000). However, BloomTime did not reduce bacterial canker infection of blossoms or leaf scars. In controlled environments, Blossom Protect reduced *Erwinia amylovora* infection symptoms on apple blossoms (Mikiciński et al., 2016) and in the 2013 study on sweet cherry, it was the most promising biocontrol product tested, reducing blossom symptom inidence 44%. In the 2013 blossom experiment, the biocontrols were applied only once, which may have been insufficient for colonization and competition with PSS. Biocontrol performance may improve with more applications. In 2014, the biocontrols were applied twice; however, there was 2.5 cm of rain after the resistance inducers and the first application of biocontrols were sprayed. There was another 2.5 mm of rain between the last spray date (i.e., the second biocontrol application as well as that of the only antibiotics application) and inoculation. The rain may have washed off some of the products, thereby reducing potential control.

In the orchard leaf scar studies, re-isolation of rifampicin-resistant PSS strains from buds was mostly unsuccessful, although rifampicin-susceptible fluorescent pseudomonads were re-isolated. In a previous study (Sundin et al., 1988), bacteria were re-isolated from 7-24% of buds near leaf scars inoculated in autumn with rifampicin-resistant PSS at 10^6 CFU/mL. The experiment was performed another year and bacteria were re-isolated from buds 55-71% of the

buds. This illustrates how it can be difficult to get consistent re-isolation from leaf scars similar to what was found in the leaf scar product studies.

Since our leaf scar data only indicated fluorescent pseudomonads, we were not able to determine when the buds became infected. Bud infection may have occurred before the control treatments were imposed, thereby limiting their effectiveness. Endophytic colonization of cherry by *Pseudomonas* species has been reported and hypothesized as the reason protective sprays may fail (Cameron, 1970; Sundin et al., 1988). Since several products have shown some control in blossom infection studies, they also may be effective against leaf scar infections. Development of a testing protocol to reliably recover PSS inoculation strains would advance this line of research. Protocols to achieve consistent infections that facilitate endophytic colonization of buds or pruning wounds would improve assessment of the effectiveness of potential control sprays. Further testing of Fireline, Kasumin and Blossom Protect is warranted, based on the 2013 blossom study results.

Leaf scar infection factors

Re-isolation of PSS from leaf scar inoculations following environmentally-induced leaf senescence was similar to that of pulled leaf scars (Table 4.5). In the field, leaf scars can be infected starting in early September, but infection success decreases rapidly after mid-October, probably due to decreased vascular tension (Crosse, 1966). In bleeding canker of European horse chestnut (*Aesculus hippocastanum*) caused by *Pseudomonas syringae* pv. *aesculi*, leaf scars are most susceptible to infection from May through October (Laue et al., 2014). Vascular tension of the tree is hypothesized to pull bacteria into leaf scars, leading to infection (Crosse, 1966). Intact green leaves are likely to transpire and create a negative tension in the water column; this tension

created prior to mechanical leaf removal could pull inoculuminto the resulting scar after leaf removal and scar inoculation. In contrast, de-bladed petioles (leaf removed a week earlier) would have minimal transpiration and less negative water potential, as well as the possible induction of healing processes or defense responses that may have reduced infection potential. However, cherry bark wounds take longer than a week to form a complete boundary zone (Biggs, 1985), and pruning wounds are susceptible for at least one week after cutting (Spotts et al., 2010), so it is unlikely that healing or defense responses reduced infection in the leaf scar experiments.

The difference in PSS recovery from inoculated leaf scars between pulled green leaves and environmental induced senescing leaves could be caused by differential cambial activity. During fall, cambial activity decreases as trees transition from paradormancy to endodormancy, a period that is associated with increased infection potential. Cankers caused by PSS stop progressing during the growing season due to the "walling off" of infected tissue by callus formation (Ogawa and English, 1991). Active cambium is thought to be important in suppressing infection of woody tissue (Wilson, 1939). No follow up was done on this experiment because limited resources stressed the need to narrow the scope of research to pruning wounds.

Pruning wound factors

Pruning wounds were considered to be infected if rif-resistant PSS bacteria could be re-isolated from the wound site. Several factors influenced pruning wound infection success, as follows.

Inoculation time after pruning

After preliminary experiments in 2013 exhibited some incidence of infection in non-inoculated controls, and external canker length was not a consistent indicator of infection (data not shown), plant materials in subsequent experiments were surface-sterilized and rifampicin-resistant PSS was used for inoculations so that treatment effects on potential infection could be determined by re-isolation of bacteria two weeks after inoculation. In 2014, inoculations 2 or 5 days after pruning resulted in infection, which suggests that the time was insufficient for pruning wounds to heal and resist bacteria. This is supported by Biggs (1985) who reported that cherry bark wounds take at least 24 days to create a lignified and suberized boundary zonethat reduces moisture loss and slows microbial invasion. In contrast, peach only took 17 days to form a boundary zone (Biggs, 1985). Peach infection by *Cytospora leucostoma* was 100% when inoculated 10 days after wounding but was only 10% when inoculated after 14 days (Biggs, 1986). These data suggest that more time may be required for a boundary zone adequate for resistance to infection to occur, and this should be considered in a repeatable infection system or for the preventative treatment of wounds.

Regarding potential effects on infection susceptibility related to pruned tissue growth status, in our experiments there was no difference between pruned trees that were dormant and actively growing. However, dormancy has been associated with higher susceptibility to PSS (Crosse, 1966; Shanmuganathan, 1962; Spotts et al. 2010), and cambial or phellogen activity has been proposed as a key factor for tree resistance (Wilson, 1939). In our study, dormant status did not impact initial tree infection, though perhaps the actively growing trees might have overcome the initial infection when evaluated over a longer timeframe.

Temperature and inoculum load

It was hypothesized that temperature could impact plant metabolism and therefore tissue healing and susceptibility to infection. When inoculated at ~$2x10^7$ CFU/mL, all pruned potted trees grown at 10°C or 20°C became infected. Initial pruning wound experiments used this high inoculum load based on previous field wire wound studies. Subsequent experiments showed that lower inoculum loads of ~$2x10^3$ to ~$2x10^5$ CFU/mL were adequate to cause infection under controlled conditions. Latorre et al. (2002) also reported that concentrations above 10^3 CFU/mL were needed for consistent infection of twigs at temperatures between 5°C and 20°C. Therefore, PSS infection studies using potted trees in controlled environments should use inoculum loads in the range of 10^3 to 10^5 CFU/mL.

Surprisingly, the effect of inoculum load did not differ in our experiments conducted at 10°C or 20°C. Latorre et al. (2002) found that increasing temperature (from 5°C to 20°C) increased twig infection symptoms when assessed 7 days after inoculation.The significant effect of temperature in that study could be due to a wider range of temperatures being tested. However, since we inoculated immediately after pruning, infection likely occurred before healing processes could respond to differences in temperature.

Temperature, tissue healing, and inoculation time after pruning

The time between pruning and inoculation was increased to determine at what point eventual healing of the pruned tissue might help resist infection. Water-soluble dye was used to assess changes in the water permeability of wounds following pruning. Through the first 17 days after pruning, the probability of water permeating the cut surface was fairly consistent with the

probability of infection when inoculated. However, when examined or inoculated at 24 days after pruning, the probabilities of water permeating the surface vs. infection was ~0.35 vs. ~0.58. This suggests that entry into the tissues by the bacteria may be due to more than passive diffusion. Possible mechanisms could include boundary zone susceptibility to toxins such as syringomyin or syringpeptin, or effectors produced by PSS. In his investigation of sweet cherry bark wounds, Biggs (1985) found that it took 17 days after wounding for lignification of the cellular boundary zone and suberization increased significantly over the following week (to 24 days). In our study, boundary zone formation (as inferred by dye permeability) was not complete in about one-third of the samples by day 24.

Sweet cherry growers often are advised to reduce PSS infection risk by pruning during hot dry weather in summer. Therefore, it is valuable to note that the probability of infection is only reduced to ~0.82 to ~0.58 even if 17 to 24 days elapse between the pruning event and exposure to significant levels of PSS. Wetting events suitable for PSS population growth and dispersal during that timeframe and even beyond may become infected.

Typically, most pruning occurs during the dormant season or early spring. Higher inoculum loads often are found in early spring (Latorre et al., 1985; Wimalajeewa and Flett, 1985) or after a period of cool wet weather (Sundin et al., 1988). Winter and spring are risky times to prune because inoculum loads are high and rainfall is more frequent. Pruning during the winter may delay wound healing sufficient for imperviousness to bacteria. An orchard study in Oregon measuring canker length showed cherry wounds were susceptible to PSS for about 1 week in summer and 2-3 weeks in winter (Spotts et al., 2010).

CONCLUSIONS

Some products to reduce the risk of PSS infection show promise when applied under dry conditions, which both favor uptake and residue retention as well as hinder PSS population growth. More field testing should be done to validate the effectiveness of Blossom Protect, Kasumin, and Fireline which, if they prove consistently effective, could be potential replacement antibiotics for copper.

A repeatable infection research protocol, using potted trees in controlled environmental conditions, could improve the efficiency of screening for potential control products before they are ultimately tested in the orchard. Given the complexity and opportunistic nature of PSS infection of sweet cherry, there are many factors to standardize for such a protocol in order for accurate assessment of the control measures being tested. Surface sterilization of wound site and pruners is essential to reduce potential infection by native PSS present on potted trees. The use of antibiotic-resistant bacteria for inoculations facilitates confirmation that bacteria re-isolated from infections are the same as that used for inoculation.

In our studies, sweet cherry tissues that were inoculated immediately after wounding were not affected by subsequent temperature or by growth/dormancy status. When testing biocontrols that may compete with PSS, or materials that purport to induce plant resistance, it is essential to time the control applications and follow-up inoculations to allow for adequate microbial or tissue response time before challenging with a suitable inoculum load to accurately assess their influence on the infection process. Testing a range of inoculum levels can help identify spray materials that may be effective at lower inoculum loads and may be useful in conjunction with other control measures.

Until new, effective spray materials are found and become available for reducing PSS populations, summer pruning remains the best control strategy to reduce the risk of pruning wound infection. Infection may still occur, however, if high inoculum levels and subsequent dispersal by rain events occur since pruned tissues remain susceptible to infection for at least 17-24 days. Pruning should be done later in summer to avoid stimulation of re-growth that might be more susceptible to winter freeze injury. Growers need to clearly understand the seasonality of PSS populations and the prolonged duration of wound susceptibility to minimize pruning during periods of high populations and therefore reduce potential infection risks in the orchard.

LITERATURE CITED

LITERATURE CITED

Biggs, A. 1985. Suberized boundary zones and the chronology of wound response in tree bark. Phytopathology 75:1191–1195.

Biggs, A.R. 1986. Wound age and infection of peach bark by Cytospora leucostoma. Can. J. Bot. 64:2319–2321.

Braun-Kiewnick, A., B.J. Jacobsen, and D.C. Sands. 2000. Biological control of Pseudomonas syringae pv. syringae, the causal agent of basal kernel blight of barley, by antagonistic Pantoea agglomerans. Phytopathology 90:368–375.

Cameron, H. 1970. Pseudomonas content of cherry trees. Phytopathology 60:1343–1346.

Carroll, J., T. Robinson, T. Burr, S. Hoying, and K. Cox. 2010. Evaluation of pruning techniques and bactericides to manage bacterial canker of sweet cherry. New York Fruit Qrtly 18:9–15.

Crosse, J.E. 1966. Epidemiological relations of the pseudomonad pathogens of deciduous fruit trees. Annu. Rev. Phytopathol. 4:291–310.

Hirano, S., and C. Upper. 1990. Population biology and epidemiology of pseudomonas-syringae. Annu. Rev. Phytopathol. 28:155–177.

Hirano, S.S., C.B. Tanner, and C.D. Upper. 1987. Rain-triggered multiplication of pseudomonas syringae on snap bean leaflets. Phytopathology 77:1694.

King, E., M. Ward, and D. Raney. 1954. 2 simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clinical Medicine 44:301–307.

Krzesinska, E.Z., and A.N.M. Azarenko. 1992. Excised twig assay to evaluate cherry rootstocks for tolerance to *Pseudomonas syringae pv. syringae*. HortScience 27:153–155.

Latorre, B., J. Gonzalez, J. Cox, and F. Vial. 1985. Isolation of pseudomonas-syringae pv syringae from cankers and effect of free moisture on its epiphytic populations on sweet cherry trees. Plant Dis. 69:409–412.

Latorre, B. A., C. Lillo, and M.E. Rioja. 2002. Effect of temperature, free moisture duration and inoculums concentration on infection of sweet cherry by Pseudomonas syringae pv. syringae. Phytoparasitica 30:410–419.

Laue, B.E., H. Steele and S. Green. 2014. Survival, cold tolerance and seasonality of infection of European horse chestnut (*Aesculus hippocastanum*) *by Pseudomonas syringae* pv. *aesculi*. Plant Pathol. 63:1417–1425.

Maxson-Stein, K., S. He, R. Hammerschmidt, and A. Jones. 2002. Effect of treating apple trees with acibenzolar-S-methyl on fire blight and expression of pathogenesis-related protein genes. Plant Dis. 86:785–790.

McGhee, G.C. and G.W. Sundin. 2011. Evaluation of kasugamycin for fire blight management, effect on nontarget bacteria, and assessment of kasugamycin resistance potential in erwinia amylovora. Phytopathology 101:192–204.

Mikiciński A., P. Sobiczewski, J. Pulawska, R. Maciorowski. 2016. Control of fire blight (Erwinia amylovora) by a novel strain 49M of Pseudomonas graminis from the phyllosphere of apple (malus spp.). European J. Plant Pathol. 145:269–276.

Ogawa, J.M., and H. English. 1991. Chapter 4: Diseases attacking several genera of fruit and nut trees Sub chapter; Bacterial disease of fruit and nut trees, p. 246–257. In: Diseases of temperate zone tree fruits and nut crops. Univ. CA, Div. Agr. Natural Resources, Oakland, CA. Publ. 3345.

Spotts, R.A., K.M. Wallis, M. Serdani, and A.N. Azarenko. 2010. Bacterial canker of sweet cherry in Oregon-Infection of horticultural and natural wounds, and resistance of cultivar and rootstock combinations. Plant Dis. 94:345–350.

Sundin, G.W., D.H. Demezas, and C.L. Bender. 1994. Genetic and plasmid diversity within natural populations of pseudomonas syringae with various exposures to copper and streptomycin bactericides. Appl. Environmental Microbiology 60:4421–4431.

Sundin, G., A. Jones, and D. Fulbright. 1989. Copper resistance in pseudomonas-syringae pv. syringae form cherry orchards and its associated transfer invitro and in planta with a plasmid. Phytopathology 79:861–865.

Sundin G., A. Jones, and B. Olson, 1988. Overwintering and population-dynamics of pseudomonas-syringae pv syringae and PS-pv morsprunorum on sweet and sour cherry trees. Can. J. Plant Pathol.-Rev. Can. Phytopathol. 10:281–288.

Thomidis, T., C. Tsipouridis, E. Exadaktylou, and P. Drogoudi. 2005. Comparison of three laboratory methods to evaluate the pathogenicity and virulence of ten *Pseudomonas syringae* pv. *syringae* strains on apple, pear, cherry and peach trees. Phytoparasitica 33:137–140.

Wilson, E.E. 1939. Factors affecting development of the bacterial canker of stone fruits. Hilgardia 12:259–298.

Wimalajeewa D., and J. Flett. 1985. A study of populations of pseudomonas-syringae pv syringae on stonefruits in Victoria. Plant Pathol. 34:248–254.

Young J., R. Luketina, and A. Marshall. 1977. Effects on temperature on growth in vitro of pseudomonas-syringae and xanthamonas-pruni. J. Appl. Bacteriology 42:345–354.

CHAPTER 5: IMPACT OF TRELLIS WIRES AND WOUND SIZE ON INFECTION OF SWEET CHERRY (*PRUNUS AVIUM* L.) BY *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*

INTRODUCTION

Recent advancements in the sweet cherry (*Prunus avium* L.) industry have led to increased interest in high density training systems because of their high efficiency. Some training systems use dwarfing rootstocks (Franken-Bembenek, 2004; Long et al., 2015; Long and Kaiser, 2010; Robinson 2005; Robinson and Hoying, 2014) and utilize trellises to train and support fruiting canopies (Lauri, 2005; Lang et al., 2014; Long et al., 2015; Musacchi et al., 2015; Whiting et al., 2005). The "Upright Fruiting Offshoots" training system, for example, is planted at an angle, and branches grow upright and are attached to the trellis for support (Long et al., 2015). Other angled training systems (such as the Taturaxe and V-system) plant trees or develop canopies at 20^0-60^0 from the vertical and are supported by a V- or Y-shaped trellis (Balmer, 2001; Lauri, 2005; Musacchi et al., 2015). Traditionally, sweet cherry trees have been free-standing with minimal limb abrasion from other branches. When trees are supported by trellises, additional abrasion can occur as wind blows branches or trunks against the wires, creating wounds (Fig. 5.1) which can be entry points for the bacterial canker pathogen *Pseudomonas syringae* pv. *syringae* (PSS). Bacterial canker is a serious disease and can cause 10-20% mortality in young orchards and up to 75% mortality if conditions are favorable to disease establishment (Spotts et al., 2010a). It also may be one of the factors associated with sweet cherry orchard decline in Michigan (Melakeberhan et al., 1993).

Trees often resist infection by walling off wounded woody tissue to prevent the spread of pathogens. In some woody tissues, wound compartmentalization is initiated in extant wood with responses such as plugging of cells with gum or tyloses to block the spread of pathogens (De Micco et al., 2016; Renzi et al., 2012; Shortle, 1979; Sun et al., 2008; Tippett and Shigo, 1981). It has also been suggested that periderm activity may be associated with the restriction of canker expansion caused by PSS (Wilson, 1939; Cross, 1966).

When wounds do not penetrate to the cambium, tissues can respond by forming a boundary zone and periderm to stop moisture loss and impede microbial invasion (Biggs, 1985). Peach wounds were susceptible to the fungal pathogen *Cytospora leucostoma* until 14 days after wounding, when infection dropped to 10%. At that time, a three cell layer of wound periderm had formed that connected to the old periderm (Biggs, 1986) Sweet potato (*Ipomoea batatas* L.) considered healed when the periderm was 3 to 7 cell layers thick (Walter and Schadel, 1983).

Determining whether wire type affects infection potential, and understanding the process of healing from wounds caused by trellis wires is important for successful adoption of trellising systems for sweet cherry orchards in areas prone to bacterial canker. The goal of this study was to test different trellis wires for their impact on infection, and to document how wire-induced wounds heal in sweet cherry both with and without inoculation with PSS.

MATERIALS AND METHODS

Orchard wire trials

Three wire types (galvanized steel, high-tensile monofilament plastic [Dura-line, Ag-Liner Inc., Mars, Pennsylvania], and polymer-coated galvanized steel [PolyPlus HTP, Centaur HTP Fencing Systems, Oswego, Illinois]) were tested to compare sweet cherry disease incidence resulting from wire abrasion and subsequent inoculation with PSS. Dura-line is a polyamide wire that is UV and weather-stabilized (Dura-line, 2016). The coating used for the PolyPlus wire is a blend of plastic polymers that includes UV stabilizers and anti-fungal agents (Centaur, 2013).

Wounding was simulated by attaching each wire to a disk that was rotated by an electric drill to rapidly simulate long-term rubbing. Disks were 11.43 cm in diameter and wires were attached using epoxy. The steel wire had to be bent to fit the disk; where the cut ends of the wire were joined resulted in a raised bump. To minimize artifactual abrasion that might be caused by the raised bumps, they were filed down to create a smoother junction. Length of branch exposure to the wire wheel was determined time required for the steel wire to rub the trunk until green tissue was exposed. This resulted in exposures of 1 sec for smaller branches (less than 2.5 cm) and 2 sec for larger branches (greater than 2.5 cm). This standard timing was used for all treatments to compare the potential for infection resulting from similar amounts of wire exposure. In one second, the disk rotated an average of 12.8 times. The circumference of the disk and wire totaled 39.2 cm (steel), 39.3 cm (plastic), or 41.3 cm (coated). When multiplied by the rotations/sec, one second of wounding for the wire types equaled ~501 (steel), ~503 (plastic), or ~528 (coated) cm/second of wire exposure. Trees were marked with a small dot of paint above and below wound sites so they could be identified for later assessment of infection.

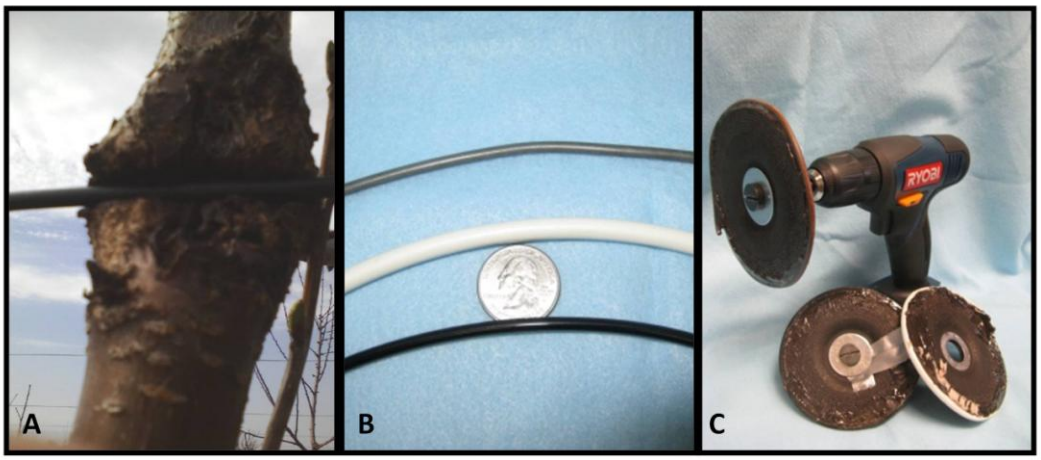

Figure 5.1. A. Wound to sweet cherry tree created by a trellis wire in the orchard. B. The wires tested in field simulations: steel (top), PolyPlus HTP (middle), and Dura-line (bottom). C. Drill with wire-mounted wounding disks.

In 2011, infection of trellis wire abrasions was tested on four-year-old 'Early Robin' (a highly susceptible sweet cherry cultivar) trees on 'Gisela 5', 'Gisela 6', and 'Gisela 12' rootstocks planted in a coarse-loamy, mixed, semiactive, mesic oxyaquic hapludalf soil of the Dryden series with a small part of the plot changing to a coarse-loamy, mixed, semiactive, mesic typic hapludalf soil of the Lapeer series. Blocking was done by orchard location, and the plot was located at Michigan State University's Clarksville Research Center in Clarksville, MI (lat. 42.9ºN, long. 85.2ºW). The experimental design was a RCBD with eight single tree replications and three wounds per tree, with a mixture of branches both smaller and larger than 2.5 cm in diameter. In May 2011, trees were wounded and inoculated with PSS strain 30-1 (provided by G. Sundin Lab, Michigan State University) which is rifampicin (Rif) resistant). The bacteria were grown on agar and suspended in phosphate-buffered saline (PBS) at a concentration of ~10^8 CFU/mL; this suspension was swabbed onto the wounds within a few hours after wounding. This high concentration was used because PSS has a greater chance of survival under desiccation stress at higher populations (Beattie and Lindow, 1999). Infection was determined

symptomatically in January 2012 by observing the development of sunken areas of tissue (sometimes with gummosis).

A second trial in 2012 used fourteen-year-old 'Ulster' (moderately susceptible) trees on 'Gisela 6' planted at the Clarksville Research Center in either a coarse-loamy, mixed, semiactive, mesic typic Hapludalfs of the Lapeer series or a sandy, mixed, mesic lamellic Hapludalfs of the Spinks series. The experimental design was a RCBD with seven single-tree replications of treatments with three wounds per tree that were on branches greater than 2.5 cm in diameter. Wounding and inoculation was done on April 9, 2012. Trees were inoculated using the swab method as in 2011, but a mixture of 4 PSS strains including 13-7, 19-6, 26-3, and 6-9 which is naturally rifampicin resistant (bacterial plates were obtained from G. Sundin Lab, Michigan State University). Infection was determined symptomatically in June 2012 as described above.

Data for field wire trials were analyzed statistically using SAS 9.1.3 (SAS Institute Inc., Cary, NC) Proc Logistic which could not account for the blocking factor. Years were analyzed separately although there was no effect of year. All pairwise comparisons were done with the LSmeans statement and ilink option, and comparisons used Wald-tests and also the Tukey-Kramer adjustment. Probabilities were reported as significant with a P-value of 0.05. Because Tukey-Kramer minimum significant difference showed no significance, Wald-tests also are reported. Wald-tests do not control experiment-wise error (unlike Tukey-Kramer) and there is a chance of incorrect significant difference conclusions, so results should be interpreted with caution.

Microscopic examination of the wire surfaces

To determine the potential for differences in abrasion between treatments, the surface of the wires was examined microscopically. Both unused wires and the disk-mounted wires used for wounding were imaged with a confocal laser scanning microscope (Olympus Fluoview FV1000) which used z-scanning to make the composite images.

Effect of initial wound size on infection and the healing process

Different wires yielded different wound sizes when evaluated in June 2011. This led to the hypothesis that wire-induced wound size may impact infection. An experiment was developed to compare the impact of initial wound size on infection and healing of wire-induced wounds. The experiment was conducted twice, on three-year-old potted 'Bing' trees on 'Gisela 6' (Bing/Gi6) and on one-year-old potted 'Bing' trees on 'Gisela 5' (Bing/Gi5). Bing/Gi6 and Bing/Gi5 were planted in June 2013 and in May 2015, respectively, in ~3 gallon pots in a commercial potting mix provided by the research greenhouse. Both experiments were implemented in January 2016 about one week after removal from cold storage following a ~5 week treatment at 3°C to break dormancy. Wounds were made on branches that were three-years-old on Bing/Gi6 and one-year-old on Bing/Gi5. Small wounds were 1.5 to 2.5 mm wide and large wounds were 4.5 to 5.5 mm. The polymer-coated wire disk was used for all wounds, with time and/or pressure varied until the desired wound size was achieved. The wounds were swab-inoculated with ~2 x 10^5 CFU/mL PSS bacteria in 0.5x phosphate-buffered saline (PBS) or with PBS buffer without bacteria as the control. Swab-inoculations were wet but not to run-off. Inoculum was a mixture of Rif-resistant mutants of PSS strains 13-7, 19-6, 26-3, and 6-9 (original strains were obtained from G. Sundin, Michigan State University and Rif mutants

generated through spontaneous mutation in our lab). Strains were cultured separately in Kings B broth before being mixed in equal parts and the mixture was used for spectrophotometer measurements to determine bacterial concentration at 600 nm; dilutions with an absorbance of ~0.155 were considered to be ~2 x 10^7 CFU/mL. Inoculum was then maintained on ice until inoculation. The boundaries of all wounds or infections were defined by the development of callus tissue after wounding in both inoculated and uninoculated treatments. Wounds were measured externally by measuring the height and width of wounded tissue (when no callus was present) or between the callus margins.

At 45, 75, 90, or 105 days post-wounding, wound length was measured externally, branches were surface sterilized, and the wounded tissue was cut in half. One half was put in formalin-acidic acid-alcohol (FAA) fixative for microscopic analysis, and one half was used for re-isolation of Rif-resistant PSS. Bacteria were re-isolated by removing tissue at the wound site, measuring its width and length, mincing it in 500 µL PBS, and waiting 15 min to allow bacteria to diffuse from the tissue. The PBS was then extracted with a pipette, serially diluted, and 25 µL drops plated on Kings B media (King et al., 1954) amended with 75 µg/mL rifampicin to select for inoculated strains and 50 µg/mL cycloheximide to inhibit fungal growth. Resultant colonies were counted and the average values from three drops were used to calculate population per area of tissue used for re-isolation. Three or four samples were taken at each time except for large inoculated branch wounds on Bing/Gi6. Seven samples were taken at day 45 and 3 samples were taken at day 90. One of the day 90 samples appeared to be dead and no bacteria were re-isolated. Samples for microscopic analysis were fixed for four days in FAA, embedded with paraffin, sectioned at 5-6 microns on a rotary microtome (Biocut 2030, Reichert-Jung, Nusslock, Germany), and stained with Johansen's Safranin O Fast Green (Ruzin, 1999). Images were taken

through a Olympus BX41 microscope. Necrophylactic periderm has been shown to first form below and at the sides of the wound, and lastly near the original periderm (Biggs, 1985). Periderm thickness was quantified at the junction with the original periderm (Fig. 5.4) as done by Biggs (1986) at each end of the wound site, and those numbers were averaged for analysis.

Data were statistically analyzed with SAS 9.1.3 (SAS Institute Inc., Cary, NC) using Analysis of Variance (ANOVA) with Proc Mixed and pairwise comparisons with t-tests, reported as significant with a P-value of 0.05. Bacterial population data were adjusted for minimum detectable population, $\log_{10}$ transformed for analysis, and back-transformed for data presentation.

RESULTS

Orchard trellis wire trials

The probability of infection was highest when wounding was caused by steel wires and PSS inoculations rarely resulted in more infection than uninoculated treatments, which could be due to native PSS strains in the orchard that were equal to or more virulent than those used for inoculation (Fig. 5.2). In 2011, wounds caused by steel wires had a probability of 0.30 and 0.18 of becoming infected for uninoculated and inoculated treatments, respectively. In 2012, these values were 0.52 and 0.39, respectively. Inoculated wounds made by plastic or polymer-coated wires had minimal probabilities of becoming infected either year, though in 2011, uninoculated wounds by those wire types had 0.14 and 0.06 probabilities of infection, respectively. In 2012, inoculated wounds made by the plastic wires had a 0.12 probability of infection. Tukey-Kramer minimum significant difference did not show any statistical significance and pairwise comparisons from Wald-tests should be interpreted with caution because they do not control for experiment-wise error. In June 2011, fewer potential infections and smaller wounds were observed with plastic and polymer-coated wires compared to steel wires (data not shown).

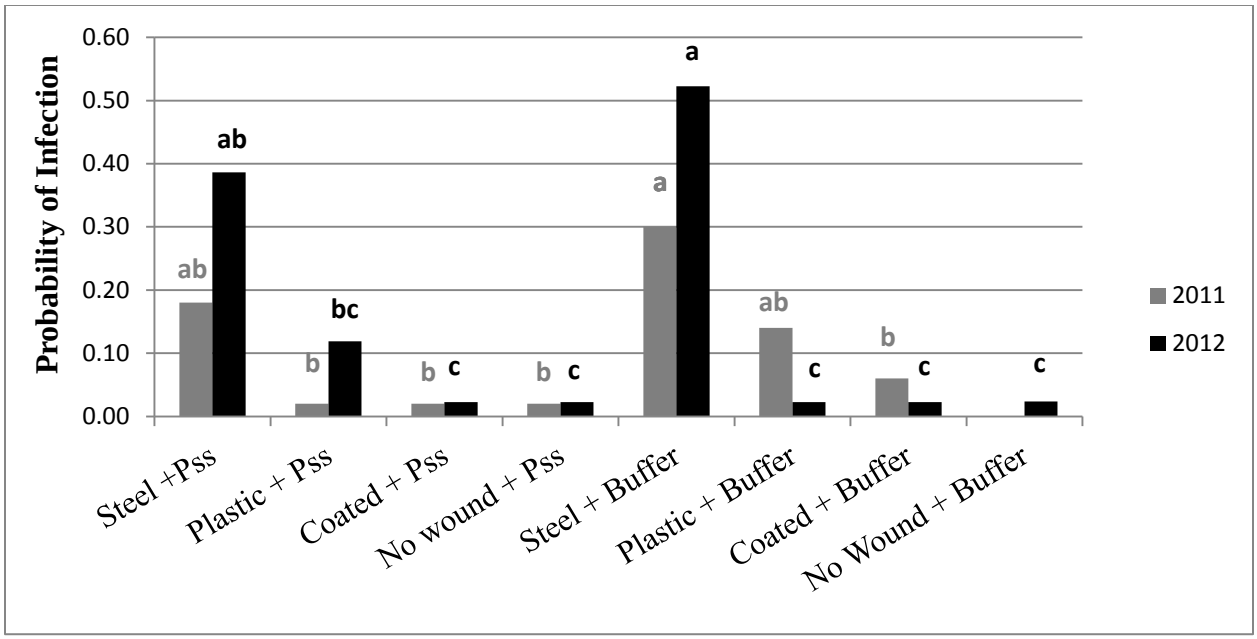

Figure 5.2. Probability of sweet cherry trunks becoming infected after abrasion simulation with three types of trellis wires (high-tensile plastic, polymer-coated steel, and high-tensile steel), following inoculation (PSS) or no inoculation (Buffer) in 2011 (grey) and 2012 (black) orchard experiments. Statistical analysis was done with logistic regression with means separation using Wald-tests and data presented as probabilities. Using Tukey-Kramer minimum significant difference there was no statistical significance between treatments. Years were analyzed separately although there was no year affect. Bars with the same letter were not significantly different with a P-value of 0.05 from other treatments within the same year.

To examine wire characteristics that might explain the differences in wound size observed in the field, each wire type was examined microscopically. Before wounding, the surface of both the steel and plastic wires revealed lengthwise lines that are likely due to the manufacturing process (Fig. 5.3). At higher magnification, horizontal nicks also appear on the plastic wire. The coated wire surface had a bumpy texture with a tactile, rubber-like feel. After wounding, the steel and plastic wires had become coated with a brown residue presumably from the bark of the tree. Under magnification, the striations previously found on those wires are no longer visible due to this residue. The polymer-coated wire, however, did not have as much plant residue and instead developed small balls (or "pills"), presumably of the polymer coating.

Wound Size and Bacterial Population Dynamics after Wounding

Final external wound length and width was measured as an indicator of infection success. The final wound height did not differ statistically between treatments, ranging from 8.3-15.6 mm (Tables 5.1 and 5.2). Final wound width differed significantly between treatments at 45 and 90 days (ranging from 3.5-6.1 mm) in the first experiment, but not at 75 and 105 days (ranging from 4.6-7.7 mm) in the second experiment. To determine the significant factors contributing to wound width at 45 and 90 days, ANOVA with slicing was used to determine significant effects of wound size, inoculation status, and day sampled. Only wound size significantly affected final wound width.

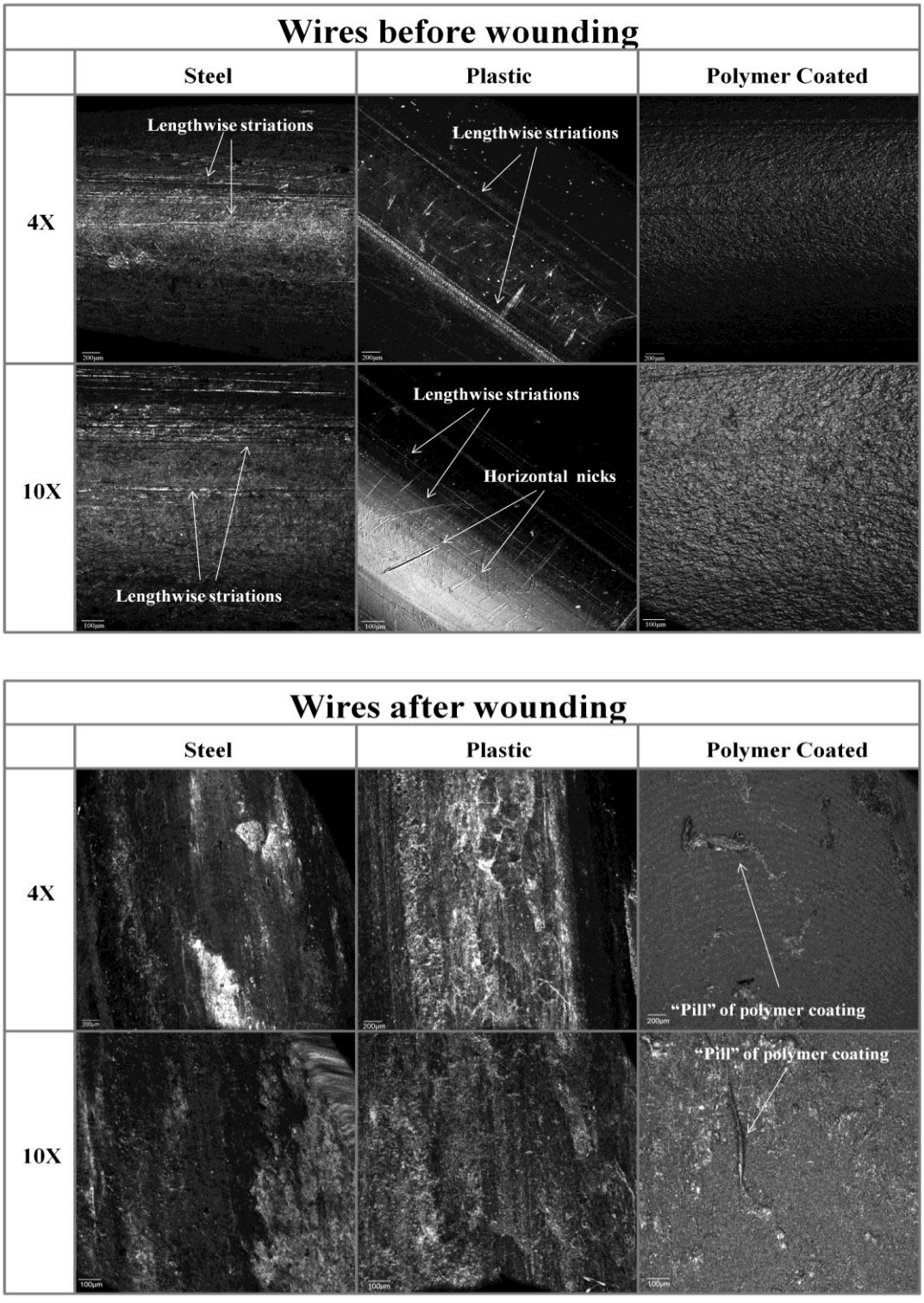

Figure 5.3. Confocal microscopy of wire wheels used in the bacterial canker sweet cherry orchard trial. Steel, plastic, and polymer-coated wires were imaged at 4x and 10x to observe external wire characteristics before and after wounding.

Table 5.1. Final width and length of wounds from branches of three-year-old potted 'Bing'/Gi6 sweet cherry trees. Treatment factors include: Pseudomonas syringae pv. syringae inoculation (Inoculated or Uninoculated), wound size (Large [4.5 to 5.5mm] or Small [1.5 to 2.5mm]), and time of re-isolation (45 or 90 days after wounding).

Treatment	Wound Width (mm)	Standard Error		Wound Height (mm)	Standard Error	
45 Large Inoculated	6.1	0.3	a[z]	11.0	1.3	a
45 Small Inoculated	6.0	1.8	abc	8.3	4.8	a
45 Large Uninoculated	6.1	0.9	ab	15.1	2.7	a
45 Small Uninoculated	3.9	0.4	bcd	11.7	2.3	a
90Large Inoculated	6.1	1.1	ab	14.7	3.7	a
90 Small Inoculated	3.5	0.6	d	15.6	4.8	a
90 Large Uninoculated	5.3	0.6	abcd	13.4	2.9	a
90 Small Uninoculated	3.6	0.8	cd	11.8	3.6	a

[z] ANOVA was only significant for wound width and means separation was done with t-tests. Means followed by the same letter are not statistically significant with a P-value of 0.05.

Table 5.2. Final width and length of simulated trellis wire-induced wounds from branches of one-year-old potted 'Bing'/Gi5 sweet cherry trees. Treatment factors include: Pseudomonas syringae pv. syringae inoculation (Inoculated or Uninoculated), wound size (Large [4.5 to 5.5mm] or Small [1.5 to 2.5mm]), and time of re-isolation (75 or 105 days after wounding).

Treatment	Wound Width (mm)	Standard Error		Wound Height (mm)	Standard Error	
75 Large Inoculated	6.7	0.3	a[z]	13.9	1.6	a
75 Small Inoculated	5.4	1.0	a	11.7	1.8	a
75 Large Uninoculated	7.2	0.3	a	10.6	2.0	a
105 Large Inoculated	7.7	1.1	a	12.5	2.2	a
105 Small Inoculated	4.6	0.9	a	9.5	3.2	a
105 Large Uninoculated	6.4	0.4	a	10.5	1.5	a

[z] ANOVA was not significant for width or length and means were not significantly different when compared with t-tests. Means followed by the same letter are not statistically significant with a P-value of 0.05.

When comparing branch wound sizes and their influence on PSS populations over time, no bacteria were re-isolated from uninoculated or unwounded controls, so those treatments were not included in the population statistical analysis. Sampling was offset in time to sample populations over a longer period. The Bing/Gi6 study had much higher populations than that with Bing/Gi5. During the 30 to 45 days between sampling, all bacterial populations declined, regardless of wound size or initial population size (Tables 5.3 and 5.4). In the Bing/Gi6 study, PSS populations re-isolated from small wounds declined from ~9,200 CFU/mm^2 at day 45 to ~540 CFU/mm^2 at day 90. Re-isolated populations from large wounds decreased from ~58,700 to ~50 CFU/mm^2 over the same period. ANOVA was significant for treatment and ANOVA slicing revealed significant population differences between day 45 and day 90 samples, but initial wound size did not significantly influence population, presumably due to large standard errors. In the Bing/Gi5 study, PSS populations re-isolated from large wounds declined from ~27 CFU/mm^2 to ~11 CFU/mm^2 from day 75 to day 105. Small wounds declined from ~240 CFU/mm^2 to 0.2 CFU/mm^2 over the same time period. ANOVA was again significant for treatment and slicing showed significant differences between day 75 and day 105, but initial wound size did not significantly influence population. Before adjustment for minimum detectible bacteria, no bacteria were re-isolated from small wounds at 105 days.

Table 5.3. Re-isolated Pseudomonas syringae pv. syringae populations from simulated trellis wire-induced wounds on branches of three-year-old potted 'Bing'/Gi6 sweet cherry trees. Data only presented for inoculated treatments because uninoculated treatments were not infected. Treatment factors include: wound size (Large [4.5 to 5.5mm] or Small [1.5 to 2.5mm]), and time of re-isolation (45 or 90 days after wounding).

Treatment	Population CFU/mm^2	Lower Standard Error CFU/mm^2	Upper Standard Error CFU/mm^2	
45 Small	9,208.6[y]	4,840.7	17,518.0	ab[z]
45 Large	58,699.9	37,983.9	90,714.2	a
90 Small	540.8	36.4	8,028.2	b
90 Large	47.4	3.4	668.7	b

[y]Data were adjusted for minimum detectable population, $\log_{10}$ transformed for analysis, and then back-transformed for presentation.
[z]ANOVA was significant for treatment and means separation was done with t-tests. Means followed by the same letter are not statistically significant with a P-value of 0.05.

Table 5.4. Re-isolated *Pseudomonas syringae* pv. *syringae* populations from simulated trellis wire-induced wounds on branches of one-year-old potted 'Bing'/Gi5 sweet cherry trees. Data only presented for inoculated treatments because uninoculated treatments were not infected. Treatment factors include: wound size (Large [4.5 to 5.5mm] or Small [1.5 to 2.5mm]), and time of re-isolation (75 or 105 days after wounding).

Treatment	Population CFU/mm^2	Lower Standard Error CFU/mm^2	Upper Standard Error CFU/mm^2	
75 Small	241.4 [y]	110.9	525.7	a [z]
75 Large	26.7	3.0	241.9	a
105 Small	0.2	0.2	0.2	b
105 Large	10.5	1.7	65.5	ab

[y] Data were adjusted for minimum detectable population, $\log_{10}$ transformed for analysis and then back-transformed for presentation.
[z] ANOVA was significant for treatment and means separation was done with t-tests. Means followed by the same letter are not statistically significant with a P-value of 0.05.

Microscopy of inoculated and uninoculated wounds over time

Sections of young sweet cherry branches taken at 45 and 90 days after wounding were stained with Safranin O and Fast Green to examine periderm development and presence of bacteria. At day 45, all samples had developed a phellogen and begun producing a periderm regardless of wound size or infection status (Table 5.5). By day 90, more periderm cells had developed (5.5) than by day 45 (3.7) (Fig. 5.5, Table 5.6). The periderm was thinner at some points of the samples taken at day 45. Inoculation status and wound size did not significantly affect periderm thickness, but the day and day by size interaction were significant.

Occasionally, the newly-formed wound periderm separated from the underlying parenchyma cells. Outside the wound periderm, at day 45 the beginning of a layer likely composed of older periderm and/or crushed boundary layer was visible, and it became well established by day 90. This layer was not quantified because it did not have distinct cells, but would likely have provided additional protection from infection. When wounds or infections did not reach the xylem, there was a continuous periderm that had joined with the original periderm at the edge of the wound.

Table 5.5. Mean number of periderm cells at the junction with original periderm (Fig. 5.4) from simulated trellis wire-induced wounds on branches of three-year-old 'Bing'/Gi6 sweet cherry trees. Treatment factors included: inoculation (Inoculated or Uninoculated), wound size (Large [4.5 to 5.5mm] or Small [1.5 to 2.5mm]), and time of re-isolation (45 or 90 days after wounding).

Treatment	Mean Number of Periderm Cells	Standard error	
45 Large	3.3[y]	0.4	c[z]
45 Small	4.5	0.5	b
90 Large	6.0	0.2	a
90 Small	5.0	0.4	ab

[y]Data were pooled for day and size combinations.
[z]ANOVA for the interaction was significant and means separation was done with t-tests. Means followed by the same letter are not statistically significant with a P-value of 0.05.

Table 5.6. Mean number of periderm cells at the junction with original periderm (Fig. 5.4) from simulated trellis wire-induced wounds on branches of three-year-old 'Bing'/Gi6 sweet cherry trees. Treatment factors included: inoculation (Inoculated or Uninoculated), wound size (Large [4.5 to 5.5mm] or Small [1.5 to 2.5mm]), and day of re-isolation (45 or 90 days after wounding).

Treatment	Mean Number of Periderm Cells	Standard error	
45	3.7[y]	0.3	b[z]
90	5.5	0.3	a

[y]Data are pooled for day.
[z]ANOVA was significant for day and means separation was done with t-tests. Means followed by the same letter are not statistically significant with a P-value of 0.05.

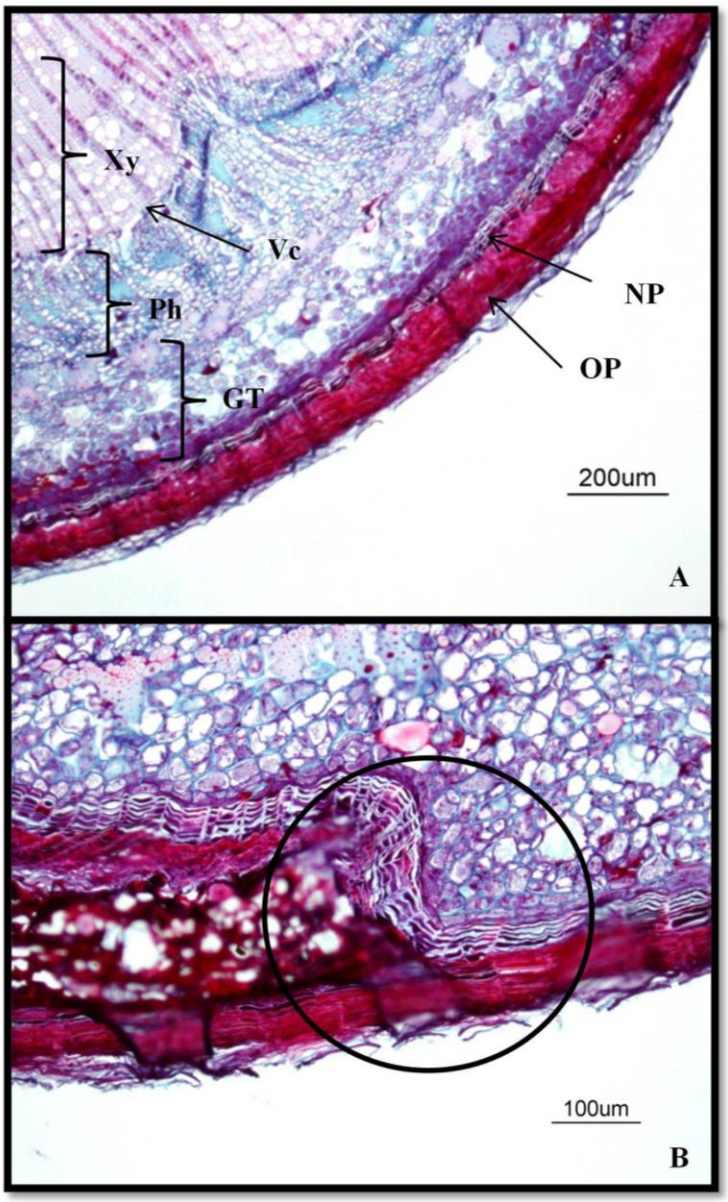

Figure 5.4. A. Unwounded young cherry branch showing xylem (Xy), vascular cambium (Vc), phloem (Ph), ground tissue (GT), new periderm (NP) and accumulated old periderm (OP). B. Simulated trellis wire-induced wounds on sweet cherry branch showing the junction between the old and new periderm where periderm thickness was quantified.

When wounds extended to the xylem, callus tissue formed and periderm developed along its outer edge (Fig. 5.5). The periderm was thinner along the rounded callus edge than at the periderm junction. At days 45 and 90, callus periderm averaged 3.0 and 4.8 cells thick, respectively (Table 5.7). Only sampling day was significant for callus periderm thickness. Inoculation status and wound size did not significantly impact callus periderm thickness. ANOVA by treatment was not significant, likely because of the limited sample size because only a subset of the samples had wounds that extended to the xylem and formed a callus. Means for individual treatments ranged from 2.5-3.3 cells at 45 days and 4-5.3 cells at 90 days (Table 5.8).

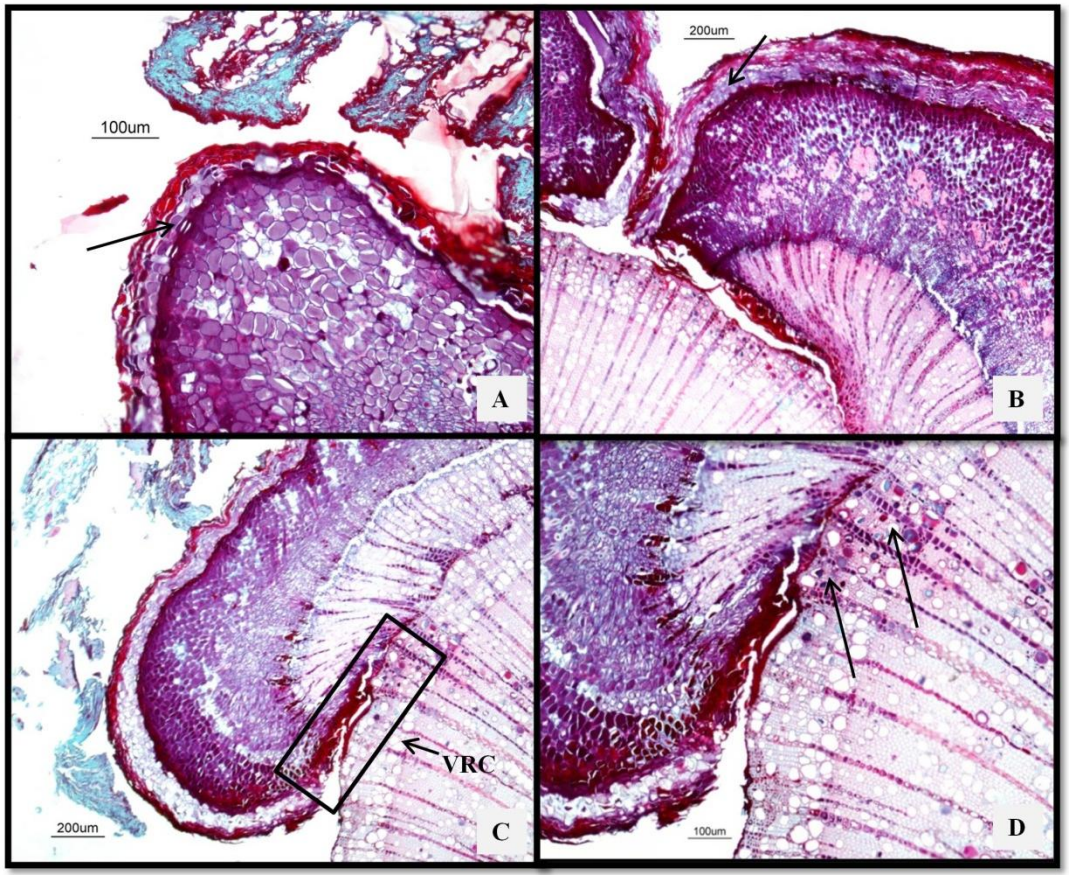

Figure 5.5. Callus and periderm of simulated trellis wire-induced wounds on young sweet cherry branch tissue. A. Wound callus after 45 days with a thinner periderm (arrow) compared with that at 90 days (B, arrow noting wider periderm). C. Wound callus extending over the xylem showing the lack of periderm in the ventral region of the callus (VRC). D. Higher magnification of the interior region of callus extending over exposed xylem showing the plugging of the xylem below the callus (arrows) and lack of periderm in the VRC.

Table 5.7. Mean number of callus edge periderm cells from simulated trellis wire-induced wounds on branches of three-year-old 'Bing'/Gi6 sweet cherry trees. Treatment factors include: inoculation (Inoculated or Uninoculated), wound size (Large [4.5 to 5.5mm] or Small [1.5 to 2.5mm]), and day of re-isolation (45 or 90 days after wounding).

Treatment	Callus Average	Standard Error	
45	3.0[y]	0.2	a[z]
90	4.8	0.3	b

[y]Data are pooled for day.
[z]ANOVA was significant for day and means separation was done with t-tests. Means followed by the same letter are not statistically significant with a P-value of 0.05.

Table 5.8. Mean, standard error, and number of samples for callus edge periderm cell number from simulated trellis wire-induced wounds on branches of three-year-old 'Bing'/Gi6 sweet cherry trees. Treatment factors include: inoculation (Inoculated or Uninoculated), wound size (Large [4.5 to 5.5mm] or Small [1.5 to 2.5mm]), and time of re-isolation (45 or 90 days after wounding).

Treatment	Average Callus Periderm Cells	Standard Error	Number of Samples[z]
45 Large Inoculated	3.3	0.3	4
45 Small Inoculated	2.8	0.3	2
45 Large Uninoculated	3.0	0.8	3
45 Small Uninoculated	2.5	NA	1
90 Large Inoculated	4.0	NA	1
90 Small Inoculated	5.3	0.8	2
90 Large Uninoculated	5.0	0.5	2
90 Small Uninoculated	4.5	NA	1

[z] Number of wounds or infections that reached the xylem and formed a callus.

The callus grew over the exposed xylem tissue, forming new xylem, phloem cortex and periderm tissue. However, a periderm was not developed along the ventral region of the callus that grew over the xylem extant at wounding (Fig. 5.5). Often, plugging of xylem below the ventral portion of callus was observed, but the new xylem in the callus did not always have plugged vessels. Bacteria were observed external to the periderm in 13 of the 15 inoculated wounds (Fig. 5.6). Two of those samples also had bacteria in the ventral area below the callus. In one, the bacteria appear to be penetrating into the cortex tissue that is not protected by a periderm (Fig. 5.6). Other than those two samples, no other samples showed bacteria inside the tissue.

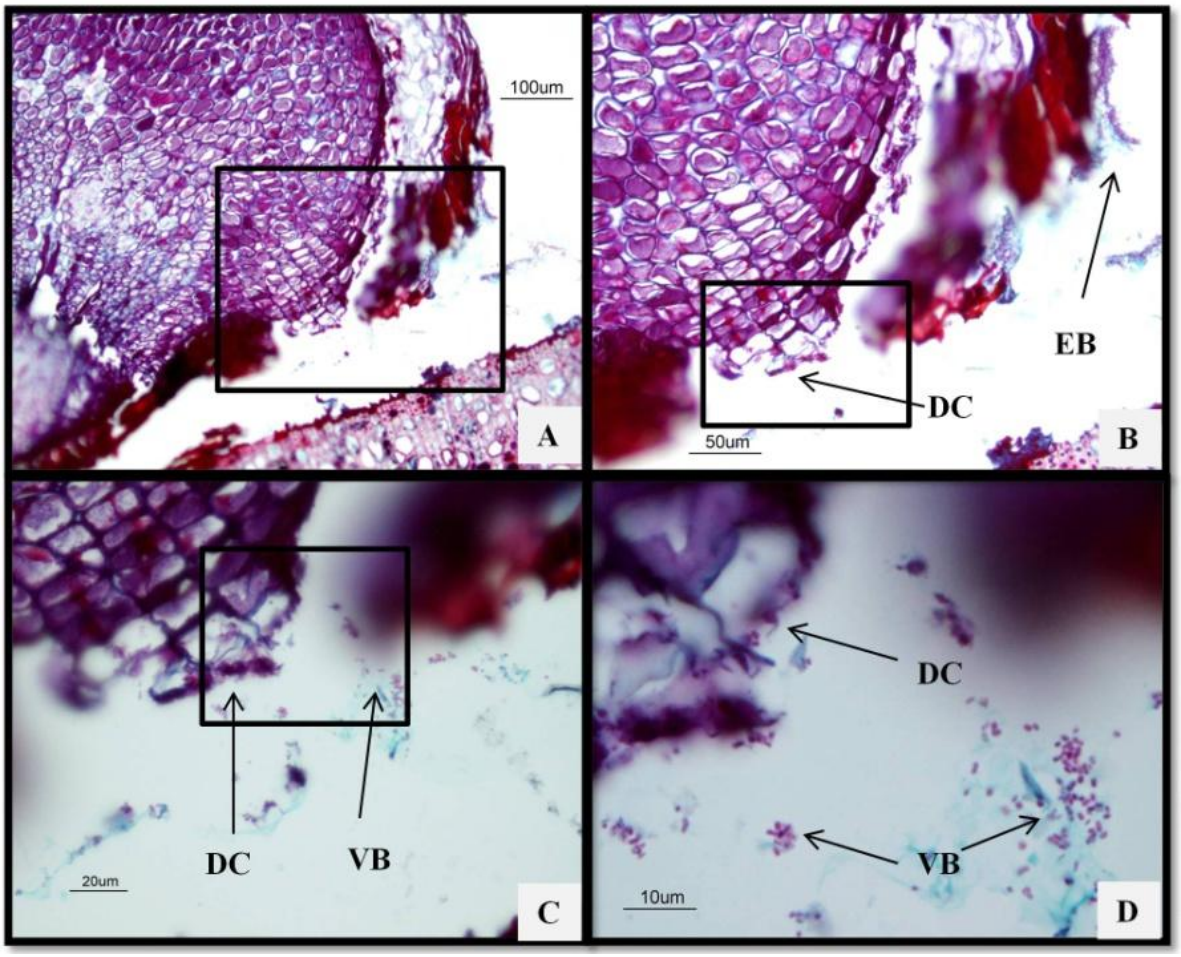

Figure 5.6. Inoculated young sweet cherry branch wound with bacteria present. A. Ventral region of callus showing the absence of periderm underneath the callus. B. Boxed section of image A at higher magnification showing the degraded cells (DC) and external bacteria (EB). C. Boxed section of image B at higher magnification with the bacteria in the ventral region (VB) which are near the degrading cells (DC). D. Boxed section of image C at higher magnification to show location of bacteria.

DISCUSSION

Wire type orchard trials

Infection of ‘Early Robin’ and ‘Ulster’ sweet cherry tissues following simulated trellis wire wounding differed by trellis wire type. Cultivar did not significantly affect infection. Wounds caused by steel wires were at least twice as likely to become infected as those by plastic wires and at least 6X more likely to become infected as those by coated wires across both years tested. Plastic and coated wires reduced the probability of infection by at least 50% to 75%, suggesting their use in trellised sweet cherry orchards, instead of traditional steel wires, may be an important component of an integrated strategy to reduce PSS infection potential. It is possible that filing the steel wire to remove the bump at the wire junction may have increased wounding but that was preferable to leaving a discontinuity that might have added further injury. The polymer coating used on the PolyPlus wire is marketed as having (presumably proprietary) anti-fungal properties that could reduce microbial survival and infection by other pathogens. Since it contains high tensile wire embedded in the polymer, it is less likely to stretch like the solid plastic wire and would be less susceptible to being accidentally cut during pruning. For these reasons, the polymer-coated steel wire is recommended for further testing in trellised sweet cherry orchards, though it is significantly more expensive than the plastic or standard steel trellis wires.

Microscopic examination of the wire surfaces

The greater incidence of infection found with steel trellis wires could be due to more abrasive characteristics that led to more wounding than was created by the plastic and coated

wires. The steel and plastic wires exhibited more microscopic grooves and nicks than the polymer-coated wire that could have facilitated the removal of plant tissue. All wires were found to have bark residue on their surface after wounding, though the polymer-coated wire had less residue and exhibited "pilling" presumably of the polymer coating suggesting that the coating was being removed by the tree. Further testing in the orchard is warranted to observe the long term durability of the coating. An alternative hypothesis is that the coated wire was wider than the plastic or steel wire and therefore would have distributed the abrasive force over a larger area, subsequently reducing the depth of wounding. However, the steel and plastic wires are about the same width and yet there was still a reduction in infection of wounds created by the plastic wire suggesting that innate wire characteristics play a role in the wounding process.

Initial wound size affects canker size

Since different wound sizes were observed in the field, wound width was examined as a potential factor for PSS infection. Final wound size did not differ between inoculated and uninoculated treatments, suggesting that PSS presence does not impact eventual wound sizeduring the growing season. In the orchard, canker expansion often occurs in spring when cambial activity resumes after dormancy. Carroll et al. (2010) reported internal canker lengths of ~8 to 14 cm in the inner bark and cambium resulting from pruning cuts made in April. Internal canker progression may be a better indicator of infection success than external wound size. Internal canker length could not be measured in the present study because a portion of the infected sections was kept intact for microscopic analysis. Final external wound boundaries appeared to be determined by the callus tissue (if present) developed during wound healing, regardless of the presence of PSS bacteria. Wilson (1939) suggested that cambial or phellogen

activity restricted canker expansion in plum (*Prunus domestica* and *Prunus salicina*). Rapidly expanding cankers observed in spring may be from reactivated cankers or infections that were established during the dormant period. The most rapid expansion of preexisting bacterial cankers in *Prunus* occurs in March to late April. When cankers begin to advance in the fall, the margins become watery and poorly defined, which normally becomes noticeable in October or November (Wilson, 1939). Re-isolation of internal bacteria, measurement of internal necrosis or assessing canker reactivation would be better indicators of bacterial canker infection of actively growing trees than external canker length.

Bacterial populations and their decline

Branch wounds of inoculated three-year-old Bing/Gi6 potted trees had much higher bacterial populations than those of one-year-old Bing/Gi5 potted trees. The three-year-old branches of Bing/Gi6 required more pressure and/or time for the wounding simulation device to achieve the same size wound compared to one-year-old branches of Bing/Gi5, possibly due to a thicker periderm that required more abrasion to breach. This increased exposure to abrasion could have crushed more tissue making more cellular nutrients available to bacteria, allowing a higher population to establish.

PSS populations in both studies declined over time. In plum (Shagmuganathan, 1962), bacterial populations resulting from pin prick inoculations with *Pseudomonas syringae* pv. *morsprunorum* (PSM) increased rapidly for about two weeks. Populations from August inoculations declined and were completely undetectable in October or November, whereas inoculations made in October declined similarly but later increased during the dormancy. In our cherry study, inoculations on actively growing trees decreased throughout the experiment, with

re-isolation of bacteria from small wounds becoming undetectable at 105 days. This suggests that PSS colonization of small wounds in the orchard early in the growing season may die out and not become perennial cankers. If wounds become infected late in the growing season, cankers may form that potentially could reactivate in the spring.

Microscopy of sweet cherry wire wounds over time

The other goal of this study was to document the healing process of simulated trellis wire wounds. Microscopic examination of the wounds showed development of periderm with or without PSS inoculation. This demonstrated a plant tissue response to mechanical wounding rather than to bacterial presence. Lignified and suberized boundary zones are generated in response to wounding in multiple species, including cherry, and precede periderm formation (Biggs, 1985). Radial alignment of the new cork cells distinguishes them from the boundary cells, and the cork cells then follow the same progression of lignification and suberization as the boundary-layer (Evert, 2006). In our study, sometimes the new periderm separated from interior parenchyma cells, similar to what has been observed in sweet potato (Walter and Schadel, 1983).

At 45 days after wounding, the periderm averaged 3.3 cells thick but was thinner at some points, and may not have reached the three cell layers that were associated with a reduction in infection in Biggs' (1986) work in peach or that were considered healed in sweet potato (Walter and Schadel, 1983). In contrast, peach is capable of forming callus with a 6-8 cell thick periderm in 28 days (Biggs and Britton, 1988). Sweet cherry takes longer than peach to form a boundary zone, requiring about 17 days after wounding for the boundary zone to lignify, and at least 24 days for maximum suberization (Biggs, 1985). The fact that periderm formation follows the same pattern of lignification and suberization as the boundary layer (Evert, 2006) could explain

why cherry takes so much longer to form a periderm. This suggests that sweet cherry is likely to be susceptible to infection for a longer period of time than peach, and this could help explain why PSS is such a serious problem in pruned or otherwise wounded sweet cherry trees. Outside the periderm, there was a layer of older periderm or boundary layer that built up over time, which could provide more protection. Since sweet cherry takes so long to develop a periderm, it is recommended that any horticultural techniques that create wounds (such as pruning) should be done in the summer when bacterial populations are low and there is ample time for trees to heal. Pruning in summer can help minimize infection (Spotts et al., 2010a) since pruning wounds in winter were susceptible to infection for three weeks, while summer pruning wounds were only susceptible for one week (Spotts, 2010b).

In our study, periderm formation only occured on the outer edge of the wound callus and extended to the original periderm, but the ventral portion of the callus remained unprotected. Peach formed a similar callus when wounded to the cambium and had no ligno-suberized boundary along most of the ventral surface of the callus (Biggs and Britton, 1988). In our study, xylem that had been exposed during the wounding and infection process also showed some plugging of vessels, as has been seen in other plants (De Micco et al., 2016; Renzi et al., 2012; Shortle, 1979; Sun et al., 2008; Tippett and Shigo, 1981).

Bacteria were found under the callus in some samples, but most only had bacteria outside the new callus and periderm. Even though wounds were surface sterilized, live bacteria were still re-isolated from some of the inoculated wounds. Cross-contamination was unlikely since no bacteria were re-isolated from uninoculated wounds. This suggests that PSS bacteria are either

surviving surface sterilization or are internal to the callus and not detectable with the microscopic techniques used.

It is possible that external bacteria in protected sites are surviving surface sterilization. Such sites could be created by biofilms, living within aggregates, or surface tension which could prevent the bleach solution from permeating into crevices created by calluses. Further research could utilize labeled PSS to confirm that the bacteria were only external to living tissue. During infection by *Pseudomonas syringae,* cell degration or disorganization and bacterial proliferation have been observed (Renzi et al., 2012; Shagmuganathan, 1962; Wilson, 1939). When bacteria were only detected outside the periderm, gaps in the callus tissue showed no signs of bacteria that could be causing cell degradation. However, PSM has been detected in sweet cherry leaf veins when no vein deterioration was observed (Roos and Hattingh, 1987), demonstrating bacterial presence without cell degradation. Long-term bacterial proliferation was not observed in this study. Bacterial populations that were re-isolated declined over time, suggesting a nutritional limitation or unfavorable environmental conditions. Nutrient availability would be expected to decline over time as the periderm separates bacteria from living cells. The failure to re-isolate bacteria from small wounds at 105 days after wounding suggests that the bacteria died off, possibly due to a lack of available nutrients. If bacteria were internal and actively infecting tissues, nutrients would be available from dying plant cells and cell degradation would be expected.

Where wounds formed a continuous periderm, internal infection appeared to be prevented. If periderm gaps are present, bacteria may be able to penetrate the exposed tissue. In peach, the unsuberized ventral callus region was the focal point of fungal pathogenesis when

wounds were inoculated with *Botryosphaeria obtusa* or *Botryospaeria dothidea* (Biggs and Britton, 1988). In this study, bacteria also appeared to infect the unprotected portion of the callus. Trees were grown in controlled environments with no exposure to rain. In the orchard, rain could facilitate the movement of bacteria near susceptible portions of wound callus and create more opportunities for infection to take place. Therefore, trellis wire types that create smaller, shallower wounds that will form a continuous periderm would reduce the likelihood of infection.

CONCLUSIONS

Reducing the risk of bacterial canker infection from wire-rub wounds is essential for the adoption of trellised high-density sweet cherry training systems. Polymer-coated wires resulted in the lowest levels of infection in simulated wire-rubbing experiments and are recommended for further testing in cherry orchards. However, PolyPlus wire is more expensive ($0.11 per foot) compared to Dura-Line or steel wires (priced $0.04 and ~$0.045-0.06 per foot, respectively), which may discourage growers from using it. Dura-line also reduced infection significantly compared to steel wire and the lower price may make it a more attractive alternative. Dura-line is easier to cut accidentally while pruning than the other wires, and is more likely to stretch, making it less suitable for maintaining precise tree structure orientation in some training systems like the UFO. This study simulated wire rubbing over the course of a few seconds, whereas less intense but more chronic rubbing may occur in the orchard, which could be physiologically different because of the repeated minor wounding and healing as well as variable bacterial populations. Further wide-scale orchard should be done to evaluate long term canker incidence with wires.

Whichever wire is used, steps should be taken to minimize abrasion of the tree by the wire. Trees should be secured to the trellis soon after installation to reduce wire abrasion caused by wind. New growth during summer should be attached to the trellis before dormancy to reduce abrasion during the dormant season when trees are more vulnerable. Attachment clips or plastic ties that do not put the tree in direct contact with the wire may also reduce abrasion.

When wounds to branches occur, about six weeks may be needed to heal if trees are just coming out of dormancy. This long period of susceptibility highlights the importance of

preventing wounds, especially when trees are not actively growing. Pruning and other horticultural tasks that create entry sites for PSS should be conducted during the summer when bacterial populations are low and trees heal more rapidly. Growers traditionally prune during the dormant season which allows easy visualization of the woody structure of the tree and the direction of spring growth. Pruning during the summer, prior to August, may stimulate re-growth that could be more susceptible to winter damage. The timing for summer pruning should be late enough to not stimulate re-growth but still allow the tree time to heal before bacterial populations rise in the fall and tree resistance decreases.

When sweet cherries are pruned during the growing season, bacteria appear to be excluded from tissue beneath the new periderm that forms after wounding. Wounds that did not extend to the xylem were able to form a continuous periderm that did not have the exposed cortex tissue found in wounds that extended to the xylem. Although the percentage of xylem-depth wounds that appeared to be infected was low in this study, that percentage would be likely to go up in orchard conditions due to rain splash. By reducing wire rubbing and using wires less prone to lead to infection, it is hypothesized that fewer, smaller, shallower wounds will result in fewer significant infections in the orchard. Over time, small wounds appeared to suppress initial infections under good growing conditions. A greater understanding of infection potential and wound healing of trellis wire wounds will help growers better understand the risks associated with trellised training systems and make best management decisions to reduce bacterial canker infection in sweet cherry orchards.

LITERATURE CITED

LITERATURE CITED

Balmer, M. 2001. Sweet cherry tree densities and tree training. Compact Fruit Tree 34:74–77.

Beattie, G.A. and S.E. Lindow. 1999. Bacterial colonization of leaves: a spectrum of strategies. Phytopathology 89:353–359.

Biggs A. 1985. Suberized boundary zones and the chronology of wound response in tree bark. Phytopathology 75:1191–1195.

Biggs, A.R. 1986. Wound age and infection of peach bark by Cytospora leucostoma. Can. J. Bot. 64:2319–2321.

Biggs, A.R. and K.O. Britton. 1988. Presymptom histopathology of peach trees inoculated with Botryosphaeria obtusa and B. dothidea. Phytopathology 78:1109–1118.

Carroll, J., T. Robinson, T. Burr, S. Hoying, and K. Cox. 2010. Evaluation of pruning techniques and bactericides to manage bacterial canker of sweet cherry. New York Fruit Qrtly 18:9–15.

Centaur. 2013. Centaur: the horse friendly fence. 18 January 2016. < http://www.centaurhorsefence.com/PDFs/ComboProductBrochure_%20rev9_2012.pdf >.

Crosse, J.E. 1966. Epidemiological relations of the pseudomonad pathogens of deciduous fruit trees. Annu. Rev. Phytopathol. 4:291–310.

Dura-line. 2016. Dura-line: monofilament agricultural support. 18 January 2016. < http://www.agliner.com/ >

De Micco, V., A. Balzano, E.A. Wheeler and P. Baas. 2016. Tyloses and gums: a review of structure, function and occurrence of vessel occlusions. IAWA J. 37:186–205.

Evert, R.F. 2006. Periderm, p. 427–445. In: Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development. Wiley, Hoboken, NJ.

Franken-Bembenek, S. 2004. GiSelA 3 (209/1) – A new cherry rootstock clone of the Giessen series. Acta Hort. 658:141–143.

Lang, G.A., S. Blatt, C. Embree, J. Grant, S. Hoying, C. Ingels, D. Neilsen, G. Neilsen and T. Robinson. 2014. Developing and evaluation intensive sweet cherry orchard systems: the NC140 regional research trial. Acta Hort. 1058:113–120.

Lauri, P.È. 2005. Developments in high density cherries in France: integration of tree architecture and manipulation. Acta Hort. 667:285–292.

Long, L., G. Lang, S. Musacchi and M. Whiting. 2015. PNW 667 cherry training systems. Pacific Northwest Ext. Publ. 667.

Long, L.E. and C. Kaiser. 2010. PNW 619 sweet cherry rootstocks. Pacific Northwest Ext. Publ. 619:1–8.

Melakeberhan, H., A.L. Jones, P. Sobiczewski and G.W. Bird. 1993. Factors associated with the decline of sweet cherry trees in Michigan: nematodes, bacterial canker, nutrition, soil pH, and winter injury. Plant Dis. 77:266–271.

Musacchi, S.M., F. Gagliardi and S. Serra. 2015. New training sywilsonstems for high-density planting of sweet cherry. HortScience 50:59–67.

King, E., M. Ward and D. Raney. 1954. 2 simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clinical Medicine 44:301–307.

Musacchi, S.M., F. Gagliardi and S. Serra. 2015. New training systems for high-density planting of sweet cherry. HortScience 50:59–67.

Renzi, M., P. Copini, A.R.Taddei, A. Rossetti, L. Gallipoli, A. Mazzaglia and G.M. Balestra. 2012. Bacterial canker on kiwifruit in Italy: Anatomical changes in the wood and in the primary infection sites. Phytopathology 102:827–840.

Robinson, T.L. 2005. Developments in high density sweet cherry pruning and training systems around the world. Acta Hort. 667:269–272.

Robinson T.L. and S.A. Hoying. 2014, Training system and rootstock affect yield, fruit size, fruit quality and crop value of sweet cherry. Acta Hort. 1020:453–462.

Roos, I. and M. Hattingh. 1987. Systemic invasion of cherry leaves and petioles by *Pseudomonas syringae* pv. *morsprunorum*. Phytopathology 77:1246–1252.

Ruzin, S.E. 1999. Johansen's safranin and fast green, p. 93–95. In: Plant microtechnique and microscopy. 1999. Oxford University Press, Oxford.

Shortle, W. 1979. Mechanisms of compartmentalization of decay in living trees. Phytopathology 69:1147–1151.

Spotts, R.A., J. Olsen, L. Long, and J.W. Pscheidt. 2010a. EM 9007 Bacterial canker of sweet cherry in Oregon disease symptoms, cycle, and management. Oregon State Univ. Ext. Serv. EM 9007:1–4.

Spotts, R.A., K.M. Wallis, M. Serdani, and A.N. Azarenko. 2010b. Bacterial canker of sweet cherry in Oregon-Infection of horticultural and natural wounds, and resistance of cultivar and rootstock combinations. Plant Dis. 94:345–350.

Sun, Q., T.L. Rost and M.A. Matthews. 2008. Wound-induced vascular occlusions in *Vitis vinifera* (Vitaceae): tyloses in summer and gels in winter. Amer. J. Bot. 95:1498–1505.

Tippett, J.T., and A.L. Shigo. 1981. Barrier zone formation: a mechanism of tree defense against vascular pathogens. IAWA Bul. 2:163–168.

Walter Jr., W.M. and W.E. Schadel. 1983. Structure and composition of normal skin (periderm) and wound tissue from cured sweet potatoes. J. Amer. Soc. Hort. Sci. 108:909–914.

Whiting, M., G. Lang and D. Ophardt. 2005. Rootstock and training system affect sweet cherry growth, yield, and fruit quality. HortScience 40:582–586.

Wilson, E.E. 1939. Factors affecting development of the bacterial canker of stone fruits. Hilgardia 12:259–298.

CHAPTER 6: THESIS CONCLUSIONS

Planting angle, cordon height, and bud selection all impact growth and distribution of upright fruiting shoots in the "Upright Fruiting Offshoots" (UFO) training system. At planting, a 60^0 angle, 45 cm cordon height, and use of bud selection provided the optimal canopy with good upright shoot formation, distribution and extension growth. Maximizing initial shoot growth in a uniformly balanced canopy increases early fruiting potential. Projected fruiting potential was higher for bud selected trees after 5 years, but these projections should be validated in the field. The potential for bud removal sites to be infected by *Pseudomonas syringae* pv. *syringae* (PSS) needs to be evaluated.

In the controlled infection studies, incidence of infection was not different at 10^0C or 20^0C, even after allowing wounds to heal for 24 days. Inoculation with PSS 24 days after pruning only reduced infection ~50%. Thus, it is critical to prune when bacterial populations are low and subsequent climatic conditions are not conducive to rapid bacterial proliferation and dispersal (as by rain). This is important information since growers typically prune cherry trees during the dormant season when trees are more susceptible. PSS populations are lower in the summer, which is a better time to prune to avoid bacterial canker infection.

The delayed inoculation experiment also demonstrated the utility of the infection system developed thus far. A rapid and repeatable infection research system using potted trees could help speed the process of testing potential products or horticultural techniques to reduce bacterial canker susceptibility of sweet cherry. Some important elements for the infections system include surface sterilization of pruning tools and wound sites and the use of antibiotic-resistant bacteria for inoculation to allow for re-isolation. Re-isolation away from the immediate wound site is also

recommended because it is possible that inoculation bacteria could survive surface sterilization and be present at the wound site and create false positives for infection. Removal of any bark or callus that might have external bacteria present would ensure re-isolation only occurs from inside the tissues. This could however increase the risk of false negatives when bacteria are not re-isolated. It is also important to tailor the experiment to what is being tested, for example, plant resistance inducers require different spray or inoculation timings than bactericides. A range of inoculum loads are also useful to evaluate bacterial spray efficacies. Even though some parameters have been tested in these studies, the repeatable infection system still needs further development.

Reducing the potential for infection of trellis wire-induced wounds is important for the establishment and long-term health of trellised high density sweet cherry orchards. Simulated wire rubbing studies with plastic and polymer-coated wires resulted in lower probabilities of infection than with standard high-tensile steel wires. Long-term orchard testing of polymer-coated wires is warranted to see if it can similarly reduce infection in the field. Wire wounds formed a callus periderm 45-90 days after wounding and healed wire wounds appeared to exclude bacteria outside the new periderm. Follow up work with labeled PSS is recommended to confirm that bacteria were not interior to the periderm. When the depth of wounds extended to the xylem, there appeared to be a region below the new callus that could be susceptible to PSS infection. In contrast, shallow wounds or infections allow development of continuous periderm which avoids the susceptible region below the callus.

In the wound size studies, all bacterial populations declined over time. This suggests that eventual extinction of PSS in wounds may be possible as was observed in the small wounds after

105 days. Assessment of bacterial populations associated with wire wounds in the orchard throughout the growing season would provide valuable insight into the potential for fall or spring canker reactivation. By taking precautions to avoid bacterial canker infections, growers can reduce risk of disease while maximizing productivity in new high density training systems.

APPENDICES

APPENDIX A: STRATEGIES TO MINIMIZE BACTERIAL CANKER IN HIGH DENSITY SWEET CHERRY SYSTEMS[a]

Abstract

Production of fresh market sweet cherries (*Prunus avium* L.) using high density canopy training systems can improve labor efficiencies and early returns on investment. However, some systems, such as the "Upright Fruiting Offshoots" (UFO), require a support trellis that may increase the potential for infection by *Pseudomonas syringae* (the causal agent of bacterial canker) due to plant tissue wounds caused by rubbing against trellis wires. Bacterial canker can cause death of spurs, loss of limbs, decreased yields, and tree mortality. Once the bacteria enter the tree, the infection may become systemic, making treatment difficult. Three types of trellis wires were examined over two years for simulated rubbing and infection potential following inoculation of 'Early Robin' and 'Ulster' sweet cherry trees with lab cultures of *P. syringae* pv. *syringae* (PSS). High tensile plastic or plastic-coated steel wires reduced infection by 50 to 75% compared to traditional high tensile steel wire. Canker bacteria also can gain entry through natural openings, such as leaf scars in the fall, and natural wounds such as spring frost damage to flowers. Research was conducted to examine whether prophylactic application of a range of potential control treatments, including antibiotics (such as oxytetracycline), plant defense inducers (such as Actigard), or microbial biocontrols (such as Optiva), can reduce flower infections. Antibiotics were most effective, reducing infection 48 to 90% compared to the inoculated control. The biocontrols and plant defense inducers were less effective and more

[a] Updated reprint of: Lillrose, T., G.A. Lang, and G.W. Sundin. 2017. Strategies to minimize bacterial canker in high density sweet cherry orchards. Acta Hort. 1161:457-462.

variable, ranging from a 45% reduction (Blossom Protect biocontrol) to little or no apparent effect. Further research on application parameters (e.g., timing) may improve the efficacy of these materials.

Introduction

Sweet cherry (*Prunus avium* L.) infections by bacterial canker, caused by *Pseudomonas syringae* pv. *syringae* (PSS) and *P. syringae* pv. *morsprunorum*, can be severe, causing symptoms that may include death of spurs, loss of limbs, decreased yields, and even tree mortality (Kennelly et al., 2007). Young trees are more susceptible to bacterial canker (Kennelly et al., 2007; Spotts et al., 2010) and must be managed carefully to prevent infection. New canopy training systems can require more pruning and increase the susceptibility of young orchards to bacterial canker, and work is needed to identify ways to reduce infection. There are three factors that must be satisfied for a successful infection to take place: a susceptible host, conducive environmental conditions, and a sufficient population of virulent bacteria. Trees become susceptible through wounding events such as abrasions, pruning, petiole scars from leaf abscission, and freeze damage of blossoms or emerging shoots. Infections can occur throughout the year, but typically are more common during certain climatic conditions. Cool, wet weather can predispose orchards to infection when the tree is susceptible to entry of the pathogen. Infection requires high populations of bacteria, which are promoted by free moisture and favorable temperatures (Young et al., 1977; Hirano and Upper, 1990), and bacteria most often are recovered from the tree during winter and early spring (Latorre et al., 1985). These high populations in the spring increase the potential for blossom infections.

This study tested different types of trellis wires to see if they influence infection potential. We also report a preliminary study to examine prophylactic sprays of antibiotics, plant resistance inducers, and microbial biocontrols as potential strategies for reducing blossom infections.

Materials and Methods

Wire trial

Three different wires (galvanized steel, high-tensile monofilament plastic [Dura-line, Ag-Liner Inc., Mars, Pennsylvania], and polymer-coated galvanized steel [PolyPlus HTP, Centaur HTP Fencing Systems, Oswego, Illinois]) were tested to compare sweet cherry disease incidence resulting from wire abrasion and subsequent inoculation with PSS. Testing was performed on four-year-old 'Early Robin' (a highly susceptible cultivar) on 'Gisela 5', 'Gisela 6' and 'Gisela 12' rootstocks in 2010 and 2011. In 2012, 15-year-old 'Ulster' (moderately susceptible) trees on 'Gisela 6' were used. Both plantings were at the Michigan State University Clarksville Horticulture Research Center in Clarksville, Michigan, USA. Trees were marked so wound sites could later be detected. Wounding was simulated by attaching the wires to a wheel that was rotated by a drill to rapidly simulate long-term rubbing. The drill mechanism was applied for 1 sec to smaller branches (less than 2.5 cm) and 2 sec to larger branches (greater than 2.5 cm). Timing was determined by the length of time it took the steel wire to rub the epidermis down to green tissue, and then using that timing for all treatments to see if the same amount of rubbing resulted in similar infection. In 2010, inoculation was done with an atomizer with PSS bacteria but no infection occurred. In the spring of 2011, the trees were wounded and inoculated again, but this time inoculation was done using PSS colonies grown on agar and suspended in phosphate buffer at a concentration of approximately 10^8 CFU mL^{-1}; this suspension was

swabbed onto the wounds shortly after wounding. In spring 2012, the 'Ulster' trees were wounded and inoculated using the swab method from 2011. This high concentration was used because PSS have a greater chance of survival under desiccation stress when at higher populations (Beattie and Lindow, 1999). Infections were determined symptomatically by observing the development of sunken areas of tissue (sometimes with gummosis) in the fall of 2011 and in the summer of 2012. Data were analyzed statistically using SAS 9.1.3 and using Anova with a Randomized Complete Block Design (RCBD), blocking for location within the orchard with a significance level of 0.05. In 2011, treatments were imposed with eight single tree replications and three wounds per tree. In 2012, there were seven single tree replications with three wounds per tree.

Blossom trial

Resistance inducers (Phostrol [Phosphorous Acid] [Nufarm, Chicago Heights, Illinois] and Actigard [acibenzolar-S-methyl] [Syngenta, Minnetonka, Minnesota]) and microbial biocontrols (Blossom Protect [*Aureobasidium pullulans*] mixed with Buffer Protect [a buffering agent] [bio-ferm, Tulln, Austria], Botector [*Aureobasidium pullulans*] [bio-ferm, Tulln, Austria], Optiva [*Bacillus subtilis*] [Agraquest Inc., Davis, California], and Bloomtime [*Pantoea agglomerans*] [Northwest Agricultural Products Inc., Pasco, Washington]) were sprayed 2 days prior to wounding and inoculation on May 3, 2013. Bactericides (Cuprofix [copper] [United Phosphorus Inc., King of Prussia, Pennsylvania], Fireline (oxytetracycline) [Agrosource Inc., Mountainside, New Jersey], and Kasumin (kasugamycin) [Arysta LifeScience North America, LLC, Cary, North Carolina]) were applied the day before inoculation on May 4, 2013. Trees were 'Rainier' on 'Gisela 3', planted in 2010 at the Clarksville Horticulture Research Center.

To simulate a frost damage event to blossoms, sterile scissors were used to wound the pistils and stamens of all blossoms of ~10 marked blossom clusters per tree but the number of blossoms per cluster was not quantified. All treatments except the uninoculated control were inoculated on May 5, 2013 with a cocktail of 4 *Pseudomonas syringae* pv. *syringae* (Table 2) strains at a concentration of ~$2x10^7$ CFU/mL (colony forming units per milliliter) by spraying to runoff with an atomizer. The branches were then bagged and the bags were removed after 2 days on May 7, 2013. Infection was assessed 16 days after inoculation on May 21, 2013. Clusters were counted as infected if they had one flower that was infected. Flowers were counted as infected if they were necrotic and shriveled, with necrosis spreading down the pedicel. Percent infection was calculated by dividing the number of infected clusters by the number of total treated flower clusters. Statistics were performed with SAS 9.3 and a significance level of 0.05. Data were analyzed using Anova with a Randomized Complete Block Design with six single tree replications blocked by location within the orchard.

Results and Discussion

Wire trial

The PSS inoculations did not always cause more infection, which could be due to native strains of PSS in the test orchard being more virulent than those used for inoculation. Steel wires caused the most infection across years with or without inoculation. The plastic wire reduced infection by at least 50%, and the plastic-coated steel wire reduced infection by 75%. Steel wires caused the most infection. In 2011, steel wires had a probability of becoming infected of 0.30 and 0.18 uninoculated and inoculated treatments, respectively. Buffer treatments only had probabilities of 0.14 for plastic wire and 0.06 for the coated wire and other treatments had a probability of 0.02. In 2012, steel wires the probability of infection was 0.52 and 0.39 for

uninoculated and inoculated treatments, respectively. Inoculated plastic wires had a 0.12 probability and all other treatments had 0.02 probability of becoming infected (Fig. A.1). There were no statistical differences with Tukey-Kramer minimum significant difference but all pairwise comparisons from Wald-tests are presented in Fig. A.1 but should be interpreted with caution because they do not control for experiment-wise error. The alternative wires have potential to reduce infections caused by standard steel trellis wires. Wounding by trellis wires is a potential problem for the adoption of new trellised training systems in areas affected by bacterial canker. By using plastic or coated trellis wires, infection could be reduced significantly, though perhaps not eliminated. This lower risk of bacterial canker infection may be a key factor for the widespread adoption of high density training systems such as the Upright Fruiting Offshoots and the Super Slender Axe that require trellising. From a practical point of view, the plastic-coated steel wires may be preferable to the high tensile plastic wires, which stretch more due to greater elasticity and are easier to cut accidentally with pruners.

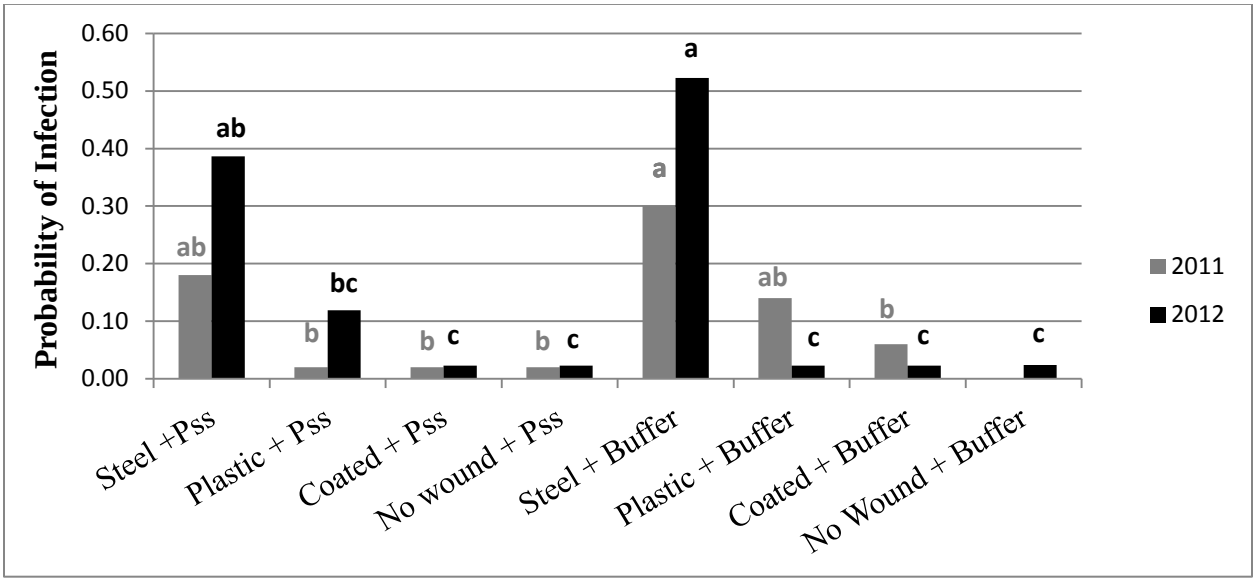

Figure A.1: Probability of wire wounded sweet cherry branches to become infected, by wire type (high-tensile plastic, polymer-coated steel, and high-tensile steel), following wounding and inoculation (PSS) or no inoculation (Buffer) conditions over 2 years (grey bars 2011 and black bars 2012). Statistical analysis was done with logistic regression with means separation using Wald-tests and data presented as probabilities. Using Tukey-Kramer minimum significant difference there was no statistical significance between treatments. Years were analyzed separately although there was no year affect. Bars with the same letter were not significantly different with a P-value of 0.05 from other treatments within the same year.

Blossom trial

Several of the prophylactic sprays demonstrated a potential to reduce bacterial infection. Cuprofix (copper), Fireline (oxytetracycline), and Blossom Protect reduced infection by 45 to 49%, and Kasumin reduced infection by 89% (Fig. A.2). The other spray treatments did not significantly reduce the amount of infection relative to the inoculated control. Copper resistance of PSS has been documented in the USA (Renick et al., 2008) and thus copper may not be as effective in many commercial orchards, although in this orchard trial the bacterial strains used were still sensitive. While the biocontrols tested in this study (other than Blossom Protect) were not very effective, *Bacillus subtilis* has been shown to reduce root infection by PSS in Arabidopsis (Bais et al., 2004). The biocontrols were applied only once in this study and did not have much time for colonization and competition; their performance may improve with multiple applications and/or a longer colonization time prior to the wound/infection event. The plant defense inducers were not significantly different from the control. Actigard has been shown to induce PR gene expression in apple two to five days after treatment and to potentially reduce fire blight (*Erwinia amylovora*) infection (Maxson-Stein et al., 2002). In this study, the short period between application and the wound/infection event may have reduced the potential effectiveness of these compounds because there was not sufficient time to up-regulate the key defense responses.

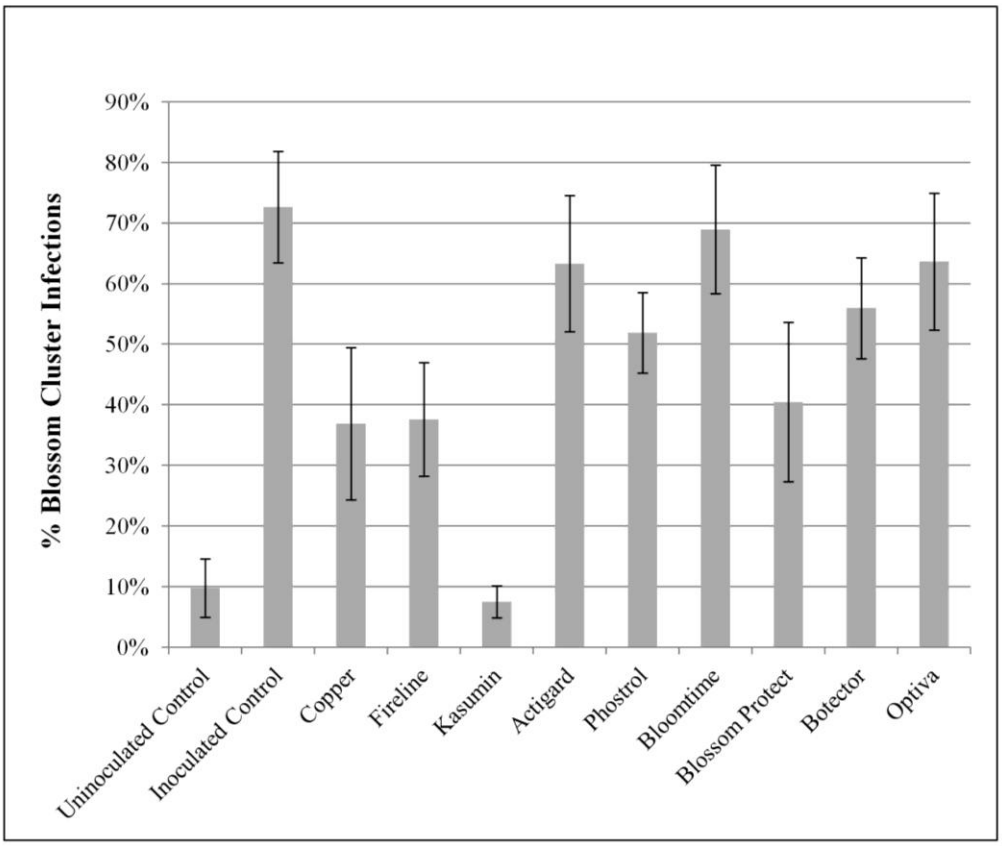

Figure A.2: Percent infected sweet cherry blossom clusters after simulated wounding and inoculation with Pseudomonas syringae pv. syringae, following prophylactic treatment with antibiotics (copper, Fireline and Kasumin), plant resistance inducers (Actigard and Phostrol), or biocontrols (Bloomtime, Blossom Protect, Botector and Optiva). Bars represent standard errors.

After causing blossom infections, PSS strains may migrate into wood, causing limb cankers that could result in the loss of fruiting or structural wood, and not just the spurs killed by the direct infection. The reduction of bacterial populations by applying prophylactic sprays of antibiotics one or two days before predicted infection conditions such as potential freeze events, would be likely to reduce infection and the loss of spurs. Multiple applications of biocontrol agents may have potential to provide similar reductions in PSS infection, with less concern about PSS resistance development, but further research will be needed to optimize and document consistent biocontrol effects. Spur loss can be devastating because it subsequently may take two

or three years to replace the lost canopy fruiting area. Through the use of weather forecast monitoring and prophylactic sprays, it could be feasible to reduce the severity of bacterial canker blossom blast infections and prevent limb cankers and/or significant losses of fruiting spurs.

Conclusions

Alternative wires have potential to reduce wire wound infections and which could be key for the expansion of new trellised training systems in areas prone to bacterial canker. Further long term testing of trellis wires is recommended to validate these results. Some spray products such as Kasumin, Blossom Protect, or Fireline also show promise and may be useful to reduce bacterial canker infection in the orchard. Further testing of these products is recommended at different infection points. Combining alternative wires with new potential spray products could help reduce bacterial canker infections in new trellised sweet cherry orchards.

APPENDIX B. PRODUCT TESTING FOR CONTROL OF BACTERIAL CANKER IN PRUNING WOUNDS

Introduction

With new potential spray controls being developed or tested (Braun-Kiewnick et al., 2000; Carroll et al., 2010; Maxson-Stein et al., 2002; McGhee and Sundin, 2011; Mikiciński et al., 2016) there is a need for product evaluation in sweet cherry. Blossom Protect and Kasumin were hypothesized to reduce infection of pruning wounds. Potted sweet cherry trees were pruned and sprayed with Blossom Protect or Kasumin to determine whether either is effective for reducing infection of pruning wounds. When little control was achieved at high inoculum loads, Kasumin was tested further at lower inoculum loads.

Materials and Methods

Testing of pruning wounds with Kasumin and Blossom Protect

Greenhouse-grown nursery trees of 'Bing' on 'Gisela 6' were used. A three factor treatment design was used to test Products, Spray timing, and Inoculum load. There were 18 treatments and four replications of each treatment. Kasumin 8L (Arysta Lifescience, Cary, NC) at a rate of 1.25 mL/L and Blossom Protect mixed with Buffer Protect (BP) (Westbridge Agricultural Products, Vista, CA) at a rate of 0.50 and 3.49 g/L respectively, were compared against an untreated control (Control). Different application times were tested by spraying either an hour before inoculation (B) or spraying both an hour before and an hour after inoculation (A). Inoculum loads were 0, 10^5, and 10^7 CFU/mL of four PSS strains. Strains used were rifampicin-resistant mutants generated in the lab of isolates 13-7, 19-6, 6-9, and 26-3 (original strains obtained from

G. Sundin). Strains were grown separately, then mixed in equal parts before concentration was determined using a spectrophotometer set at 600 nm. Dilutions with an absorbance of ~0.155 were considered to be ~2 x10^7 CFU/mL and further dilutions were made to attain desired inoculum load.

Pruning sites were surface sterilized by wiping with 70% alcohol wipes and pruners were surface sterilized between each cut with 10% bleach followed by 75% ethanol. Branches were cut and then sprayed with product for all treatments. About an hour later, surfaces were inoculated with 50 µL of PSS. An hour after inoculation, the "before and after" treatments were sprayed again. The experiment was started in June 2014 and the trees were maintained in a growth chamber at 20ºC. Re-isolation was attempted 2 weeks after treatments were imposed by mincing shavings of tissue from wound in phosphate buffered saline and then plating it on Kings B medium (King et al., 1954) amended with rifampicin at a rate of 75 µg/mL to select for inoculated strains and 50 µg/mL cycloheximide to inhibit fungal growth

Kasumin testing at different inoculum concentrations

Greenhouse-grown 'Bing' on 'Gisela 6' were divided into 8 treatments with 6 replicates each. Kasumin 2L at rate of 18.9 mL/gal and a water control were sprayed one hour before and one hour after inoculation. The first spray was completed right after pruning. Pruning sites and pruners were both sterilized as recorded above. Inoculum concentrations were 0, 10, 10^3, and 10^5 CFU/mL mixture of three of the rifampicin-resistant PSS strains as described above. Strain 26-3 was not used because there were concerns that it might be Kasumin resistant. Each pruning wound was inoculated with 50 µL of inoculum. The experiment was started in September 2014,

the trees were maintained in a growth chamber at 20ºC, and re-isolation was attempted 2 weeks after inoculation by the methods used above.

Results

Kasumin and Blossom Protect

One kasumin uninoculated control treatment had 50% infection, so the results of this experiment are inconclusive. For logistical regression analysis, only spray timing and inoculation significantly contributed to the model but product did not. Spray timing had a 0.91 and 0.43 probability of becoming infected for the before treatment and "before and after" treatments, respectively, and they were significantly different from each other by both Wald and Tukey-Kramer tests. Uninoculated treatments had a probability of 0.03-0.21 of becoming infected and the inoculated treatments probabilities of infection ranged from 0.79-0.97 and were not significantly different from each other (Table B.1).

Kasumin testing at different inoculum concentrations

Two-thirds of uninoculated water controls became infected and one kasumin uninoculated control was infected. From the logistic regression model that included inoculum level, product, and their interaction, none of the factors contributed significantly to the model. The probability of infection was statistically insignificant between all treatments with Tukey-Kramer adjustment. The Kasumin uninoculated control had a probability of 0.21 of becoming infected and was significantly different with Wald tests from water control treatments at 10 and 10^3 CFU/mL and Kasumin at 10^5, all of which had infection probabilities of 0.93. However, re-isolation of bacteria occurred from uninoculated controls, so the results of this experiment are inconclusive.

Discussion and Conclusion

There may be some benefit of the products tested at 10^5 CFU/mL or lower concentrations. However, due to the re-isolation of the rifampicin-resistant bacteria from uninoculated controls, results are inconclusive. This re-isolation could be due to insufficient rifampicin in the media to prevent non-resistant PSS from growing. It also could be due to contamination during inoculation or re-isolation. Work should be repeated to validate results.

Table B.1. Probability of pruning wounds becoming infected when treated with different products (Kasumin or Blossom Protect vs. an untreated control), at inoculum loads of 0, 105, and 107 CFU/mL, and spray timing before or after inoculation. Probabilities were generated for product and inoculum level combinations.

Treatment	Probability of becoming infected	Standard Error of the probability	Significance at 0.05 with Wald tests	Significance at 0.05 with Tukey adjustment
Control 0	0.03	0.05	c	a
Blossom Protect 0	0.03	0.05	c	a
Kasumin 0	0.21	0.16	bc	a
Control 10^5	0.97	0.05	a	a
Blossom Protect 10^5	0.90	0.10	a	a
Kasumin 10^5	0.79	0.16	ab	a
Control 10^7	0.97	0.05	a	a
Blossom Protect 10^7	0.97	0.05	a	a
Kasumin 10^7	0.97	0.05	a	a

[z]Probabilities were generated for product and inoculum load combinations. Probabilities are from a logistic regression model which included inoculation level, spray timing, product, and the product by inoculation interaction. Only inoculation and spray timing significantly contributed to the model.

[y]Results of Wald tests were reported because there was no significance with Tukey-Kramer adjustment. Results should be interpreted with caution because Wald tests do not control for experiment wise error and carry a risk of type I error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

[x]Comparisons with Tukey-Kramer adjustment to control for experiment wise error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

Table B.2 Probability of pruning wounds becoming infected when sprayed with Kasumin vs. an untreated control at inoculum levels of 0, 10, 10^3, and 10^5 CFU/mL.

Treatment	Probability of becoming infected	Standard Error of the probability	Significance at 0.05 with Wald tests	Significance at 0.05 with Tukey adjustment
Control 0	0.64[z]	0.20	ab[y]	a[x]
Kasumin 0	0.21	0.17	b	a
Control 10	0.93	0.10	a	a
Kasumin 10	0.50	0.20	ab	a
Control 10^3	0.93	0.10	a	a
Kasumin10^3	0.64	0.20	ab	a
Control 10^5	0.79	0.17	ab	a
Kasumin 10^5	0.93	0.10	a	a

[z]Probabilities from logistic regression model which included inoculation level, product, and their interaction. No factors significantly contributed to the model.

[y]Results of Wald tests were reported because there was no significance with Tukey-Kramer adjustment. Results should be interpreted with caution because Wald tests do not control for experiment wise error and carry a risk of type I error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

[x]Comparisons with Tukey-Kramer adjustment to control for experiment wise error. Probabilities followed by the same letter are not significantly different at a P-value < 0.05.

LITERATURE CITED

LITERATURE CITED

Bais, H., Fall, R., and Vivanco, J. 2004. Biocontrol of Bacillus subtilis against infection of Arabidopsis roots by Pseudomonas syringae is facilitated by biofilm formation and surfactin production. Plant Physiol. 134:307–319.

Beattie, G.A. and Lindow, S.E. 1999. Bacterial colonization of leaves: a spectrum of strategies. Phytopathology 89:353–359.

Braun-Kiewnick, A., B.J. Jacobsen, and D.C. Sands. 2000. Biological control of Pseudomonas syringae pv. syringae, the causal agent of basal kernel blight of barley, by antagonistic Pantoea agglomerans. Phytopathology 90:368–375.

Carroll, J., T. Robinson, T. Burr, S. Hoying, and K. Cox. 2010. Evaluation of pruning techniques and bactericides to manage bacterial canker of sweet cherry. New York Fruit Qrtly 18:9–15.

Hirano, S. and Upper, C. 1990. Population biology and epidemiology of Pseudomonas-syringae. Annu. Rev. Phytopathol. 28:155–177.

Kennelly, M.M., F.M. Cazorla, A. de Vicente, C. Ramose, and G.W. Sundin. 2007. Pseudomonas syringae diseases of fruit trees - Progress toward understanding and control. Plant Dis. 91:4–17.

King, E., M. Ward, and D. Raney. 1954. 2 simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clinical Medicine 44:301–307.

Latorre, B., Gonzalez, J., Cox, J. and 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 Dis. 69:409–412.

Maxson-Stein, K., S. He, R. Hammerschmidt, and A. Jones. 2002. Effect of treating apple trees with acibenzolar-S-methyl on fire blight and expression of pathogenesis-related protein genes. Plant Dis. 86:785–790.

McGhee, G.C. and G.W. Sundin. 2011. Evaluation of kasugamycin for fire blight management, effect on nontarget bacteria, and assessment of kasugamycin resistance potential in erwinia amylovora. Phytopathology 101:192–204.

Mikiciński A., P. Sobiczewski, J. Pulawska, R. Maciorowski. 2016. Control of fire blight (Erwinia amylovora) by a novel strain 49M of Pseudomonas graminis from the phyllosphere of apple (malus spp.). European J. Plant Pathol. 145:269–276.

Renick, L.J., A.G. Cogal, and G.W. Sundin. 2008. Phenotypic and genetic analysis of epiphytic Pseudomonas syringae populations from sweet cherry in Michigan. Plant Dis. 92:372–378.

Spotts, R.A., K.M. Wallis, M. Serdani, and A.N. Azarenko. 2010. Bacterial canker of sweet cherry in Oregon - Infection of horticultural and natural wounds, and resistance of cultivar and rootstock combinations. Plant Dis. 94:345–350.

Sundin, G., and C. Bender. 1993. Ecological and genetic-analysis of copper and streptomycin resistance in pseudomonas-syringae pv syringae. Appl. and Environmental Microbiology 59:1018–1024.

Young, J., Luketina, R. and Marshall, A. 1977. Effects on temperature on growth in vitro of Pseudomonas-syringae and Xanthomonas-pruni. J. Appl. Bacteriology 42:345–354.