Strategies to minimize bacterial canker in high density sweet cherry orchards

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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 bio-controls (such as Optiva), can reduce flower infections. Antibiotics were most effective, reducing infection 48 to 90% compared to the inoculated control. The bio-controls and plant defense inducers were less effective and more variable, ranging from a 45% reduction (Blossom Protect bio-control) to little or no apparent effect. Further research on application parameters (e.g., timing) may improve the efficacy of these materials.

Keywords: *Pseudomonas syringae*, *Prunus avium*, blossom blast, wound, flower, antibiotics, plant defense inducers

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 bio-controls 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 s to smaller branches (less than 2.5 cm) and 2 s 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, 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 $tree^{-1}$. In 2012, there were seven single tree replications with three wounds $tree^{-1}$.

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. 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. Trees were 'Rainier' on 'Gisela 3', planted in 2010 at the Clarksville Horticulture Research Center. To simulate a frost damage event to blossoms, we adapted a technique from University of California-Riverside (J.E. Adaskaveg, pers. commun.) in which sterile scissors were used to cut the pistils and stamens of all blossoms of ~10 marked blossom clusters per tree. All treatments except the uninoculated control were inoculated with a cocktail of four PSS strains at 10^8 cfu mL^{-1} by spraying to runoff with an atomizer. Wounding and inoculations were done in clear, warm

weather in spring 2012. The branches were then bagged with opaque white plastic bags that were tied closed. Bags were removed after 2 days and infection was assessed 16 days after inoculation. 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

Wounding by trellis wires is a potential problem for the adoption of new trellised training systems in areas affected by bacterial canker. The PSS inoculations did not always results in more infections, 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% (Figure 1). Therefore, the use of plastic or coated trellis wires can reduce infection potential significantly, though perhaps not eliminate it. 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 plasticcoated
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.

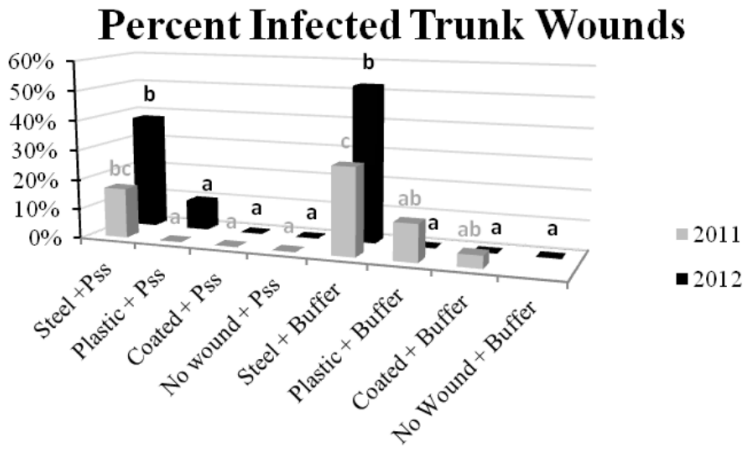

Figure 1. Percent infected sweet cherry trunk wounds, by wire type (high tensile plastic, plastic-coated steel, and high tensile steel), following wounding and inoculation (PSS) or no inoculation (buffer) conditions over 2 years (grey bars 2011 and black bars 2012). Years were analyzed separately for statistics. Bars with the same letter were not significantly different from each other with a P-value of 0.05.

Blossom trial

Several of the prophylactic sprays demonstrated a potential to reduce bacterial infection. Cuprofix (copper), Fireline (oxytetracycline), and Blossom Protect reduced
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infection by 45 to 49%, and Kasumin reduced infection by 90% (Figure 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 bio-controls 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 bio-controls 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.

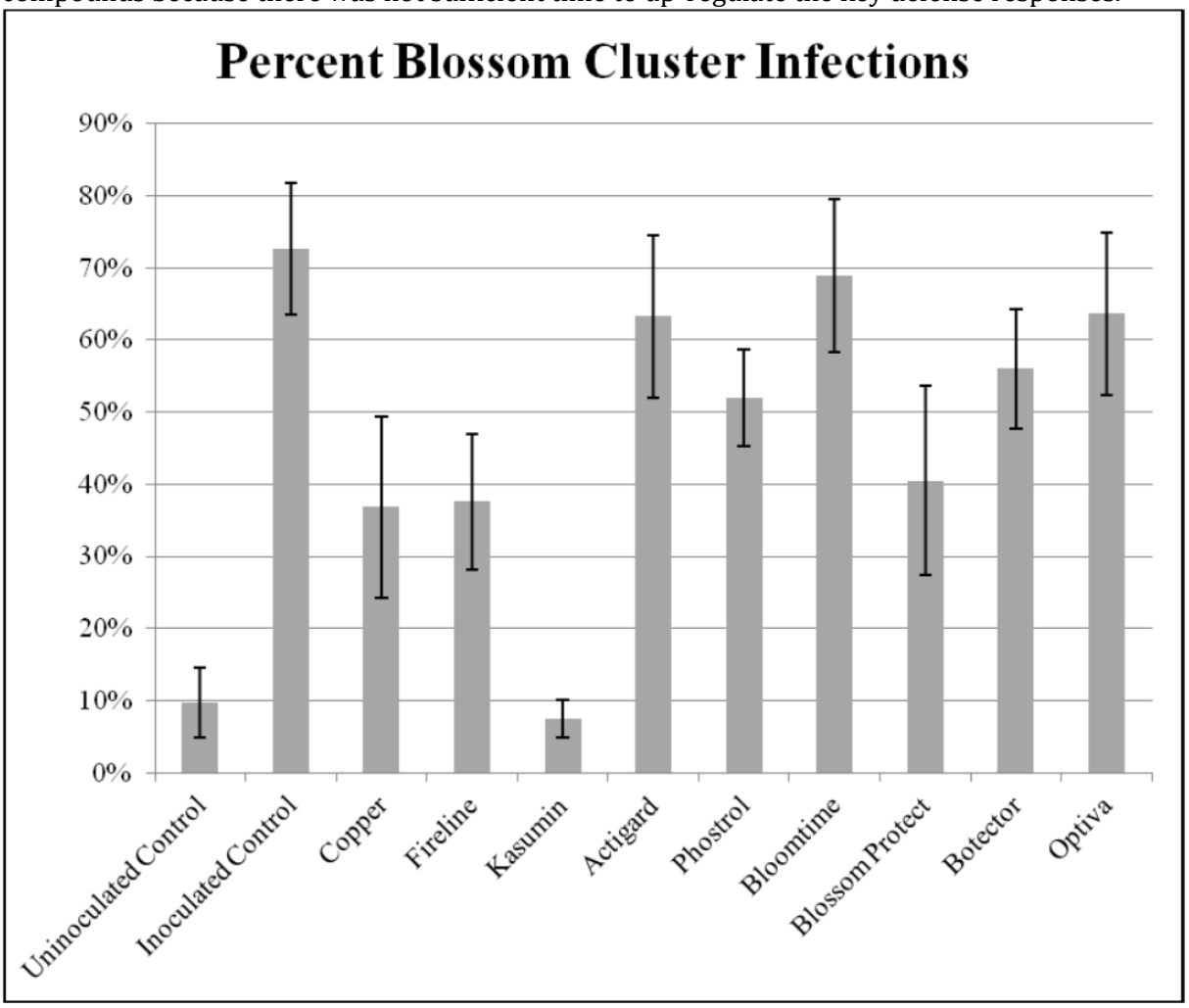

Figure 2. Percent infected sweet cherry blossom clusters after simulated wounding and inoculation with *Pseudomonas syringae* pv. *syringae*, following prophylactic treatment with antibiotics (copper, Fireline and Kasumin), plant resistance inducers (Actigard and Phostrol), or bio-controls (Bloomtime, 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
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of bio-control 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 bio-control 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.

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