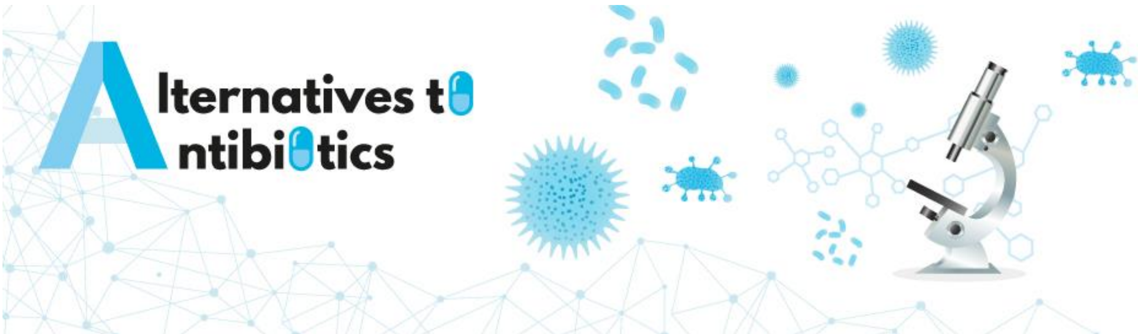

Research Gap Analysis Workshop

National Animal Disease Center
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Plant Working Group Report

Numerous bacterial diseases threaten the productivity of plants or quality of produce, yet antibiotics are used primarily to prevent disease only on tree fruits (apple and pear) and stone fruits (peach and nectarine). The diseases that are targeted with antibiotics share the following four characteristics: 1) occur on specific high-value perennial crops, 2) have a defined window of vulnerability, 3) are caused by pathogens sensitive to antibiotics, and 4) require pathogen growth on plant tissue surfaces prior to infection. Most bacterial diseases of plants fail to meet all four criteria, and thus antibiotics are not an affordable or viable option for controlling most bacterial plant diseases. The US Environmental Protection Agency regulates the use of antibiotics and all other materials, including biological pesticides, for the management of plant diseases. Registered materials for plant disease control must not present an unreasonable risk to the environment or human health; this includes by either direct or indirect exposure, including via residues on food. Currently, three antibiotics are permitted in plant agriculture: streptomycin (registered for plant use in 1958), oxytetracycline (1972), and kasugamycin (2014).

Streptomycin is used mainly on pear and apple trees for the prevention of fire blight caused by *Erwinia amylovora* (Stockwell & Duffy 2012). Streptomycin also is registered for minor uses, such as the prevention of rots and other diseases on seed potatoes, tomato and tobacco transplants, and stems of cut flowers. Oxytetracycline is used alone or in combination with streptomycin on apple and pear trees for fire blight prevention, especially in areas where streptomycin-resistant populations of *E. amylovora* are prevalent. Oxytetracycline is also used on peach and nectarine trees for protection against bacterial spot caused by *Xanthomonas arboricola* pv. *pruni*. Kasugamycin is the newest antibiotic registered for plant disease prevention. Kasugamycin, an aminoglycoside with a unique target site, has never been used clinically or for animal health. Use of kasugamycin is currently restricted in the US to pear and apple for fire blight prevention. On-farm chemical use data on plants are collected and available through the Agricultural Chemical Use Program of the National Agricultural Statistics Service (NASS, website: https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/index.php); these data include antibiotic use. The most recent data for chemical use on tree fruit are from 2011, but these exclude usage data on kasugamycin since it was registered in 2014. Growers applied approximately 6,080 kg of oxytetracycline to peach and nectarine trees to manage bacterial spot. Apple and pear growers used approximately 14,400 kg of streptomycin and 15,014 kg oxytetracycline in 2011 to manage fire blight. *Fire blight prevention accounts for >80% of the quantity of antibiotics used annually for plant disease control; therefore, management of fire blight has been the primary focus of efforts to develop antibiotic alternatives in plant agriculture.*

A short primer on fire blight within the context of antibiotic use. Blights are diseases that cause a rapid and progressive death of plant tissues. Fire blight begins with the infection of flowers (Thomson 2000). The pathogen survives the winter months in cankers, which are infections on the trunk and stems of trees. In the spring, pathogen cells are exuded from cankers and spread to open flowers by insects, wind and rain. The pathogen colonizes floral stigmas and develops population sizes exceeding 10^6 colony forming units per flower. Free moisture (light rain or heavy dew) facilitates migration of the pathogen to the base of the flower, i.e., the nectary. The pathogen invades the floral tissues via natural pores in the nectary that exude nectar. Once in the intercellular spaces of the plant, the pathogen spreads into branches and rapidly kills tissues. Although diseased tissues cannot be cured of fire blight, pathogen spread can be contained and further tissue infection prevented by removing diseased and surrounding asymptomatic tissues.

Fire blight control measures focus on 1) sanitation, which is the removal of infected tissues or entire trees to reduce populations of the pathogen in orchards, and 2) prevention of floral infections by suppressing growth of the pathogen on stigmas and/or in the nectary. Fire blight is a disease well-suited for control with antibiotics because the primary infection period is limited to one to four weeks when trees are blooming, and prior to infection, the pathogen grows on flower surfaces, which are accessible to antibiotic sprays. Antibiotics are not effective when symptoms are present and the pathogen is within tissues.

Antibiotic use for fire blight control. The quantities of antibiotics used for protection of apple and pear trees (29,414 kg total) are relatively modest considering the acreage of fruit orchards (337,000 acres for apple and 51,000 acres for pear) and the number of permitted applications of the antibiotics (up to 10 times per year for streptomycin and oxytetracycline). From the NASS database, we find that only 20% of the apple acreage in the US was treated with antibiotics an average of 1.5 times per year, and up to 45% of pear acreage was treated an average of 3 times with antibiotics per year. Given that fire blight is a serious disease, it may be surprising that the majority of orchards are not sprayed annually. The lack of extensive use of antibiotics in pear and apple orchards is due, in part, to knowledge of the epidemiology of fire blight and the use of decision aids (disease risk models) by growers to anticipate the potential for a severe outbreak of fire blight in their orchard that year. The decision aids predict the disease potential based on bloom stage and conducive environmental conditions that support rapid growth of the pathogen on flowers and subsequent infection of flower clusters (Billing 2000). A grower may decide to not spray antibiotics if the disease potential is low, for example due to little disease in orchard in prior years or cool weather during bloom. Growers use the decision aids to target antibiotic treatments to times when they will be effective for disease control.

Grower interest in alternatives to antibiotics increased with the widespread emergence of streptomycin-resistant populations of *E. amylovora* in apple and pear orchards in the western states (Loper *et al*. 1991; Jones & Schnabel 2000). The emergence of streptomycin-resistance destabilized antibiotic-based disease management programs and resulted in periodic epidemics, which cost growers millions of dollars. Research scientists screened thousands of microbes isolated from orchards for their ability to suppress growth of *E. amylovora* on flowers, thereby interrupting a key stage in the disease cycle (Johnson & Stockwell 1998; Lindow 1985, Pusey *et al*. 2009). Additional studies focused on the mechanisms of disease control of potential biological control agents and possible adverse effects of using biological control agents (Pusey *et al*. 2008; Stockwell *et al*. 2002; Wilson & Lindow 1993). The outcome of these studies and many others resulted in the development of commercial products to aid in the management of fire blight of pear and apple.

Alternatives to antibiotic products for plant disease prevention and challenges to adoption.

For many bacterial plant diseases, host resistance has been effective for disease control. However, commercial cultivars of peach and nectarine are susceptible to bacterial spot. Similarly, although Red Delicious apple is fairly tolerant of fire blight, this cultivar has been largely replaced with newer, consumer-desirable cultivars (e.g., Braeburn, Gala, Fuji, Honeycrisp, and others) that are very sensitive to fire blight. All commercial pear cultivars are very susceptible to fire blight. Plant breeders have developed fire blight resistant rootstocks, but integrating host resistance into desirable fruit cultivars for grafting onto these rootstocks has been a long and challenging process, as these are slow-growing plants (Norelli *et al*. 2003). Current technologies, such as genomic sequencing, marker-assisted breeding and genome editing, could hasten the development of resistant tree fruits and stone fruits (Norelli *et al*. 2003; Yang *et al*. 2012). Genetic modification for disease resistance is promising technology, but may not be adopted for orchard crops if consumers will not purchase the fruit.

As indicated in Table X, antibiotic alternatives for plant disease prevention consist of chemical products, biological control agents, and cultural practices. Each of these approaches is used for fire blight management and the alternatives will be discussed in the context of fire blight management.

Chemical control: Copper has long been used as a general pesticide in plant agriculture. Although copper-resistant bacteria are present in the environment, there are no published reports of copper-resistance in the pathogens that cause fire blight of pear and apple or bacterial spot of peach and nectarine. A challenge for growers using copper for plant disease control is the potential for phytotoxicity, i.e., the killing of plant tissues exposed to copper ions. On pear and apple, copper is generally used on dormant plants or during early bloom (Psallidas & Tsiantos 2000, Elkins *et al*. 2015). If copper is applied on trees with young developing fruit, the fruit surfaces can be damaged, resulting in spotted or misshapen fruit. These fruits have a reduced market value; thus, copper is used more commonly in orchards with fruit destined for processing, e.g. canning or juice. Recently, advances in the formulation of copper bactericides have reduced the incidence of phytotoxicity, and these newer products may be used during late bloom for fire blight control with less potential for marring fruit finish. For peach and nectarine, the phytotoxic response is mainly seen on leaf tissues and the fruit finish is not affected (Richie 1995 and 1999). In addition to the potential for copper phytotoxicity, the potential for accumulation of this heavy metal in the environment is a concern (Nagajyoti *et al*. 2010).

The product OxiDate 2.0 (BioSafe Systems, East Hartford, CT), which is a combination of hydrogen dioxide and peroxyacetic acid, is registered for numerous crops to control fungal and bacterial diseases including fire blight. OxiDate 2.0 applications start prior to the appearance of fire blight and can continue to harvest. Two additional products, which are not bactericidal, are registered for fire blight management. Apogee (Prohexadione calcium, BASF Crop Protection, Research Triangle Park, NC) is a plant growth regulator that is registered for apple. Apogee reduces shoot growth and thus can reduce damaging secondary infections of succulent shoots by the fire blight pathogen; this damage is common in orchards exposed to humid summers and frequent rain such as the eastern US (Norelli *et al*. 2003). Actigard 50WG (Acibenzolar-S-methyl, Syngenta Crop Protection, Greensboro, NC) can reduce disease severity by inducing a natural process called systemic activated plant resistance. Actigard 50WG may be used therapeutically on infected trees by drenching the soil or painting the material on infected branches or trunks to reduce canker expansion (Johnson & Temple 2016, Johnson *et al*. 2016).

Bacterial biological control agents: Currently, four bacterial biological control agents are registered for prevention of fire blight. *Bacillus amyloliquefaciens* strain D747 (DoubleNickel LC, Certis, Columbia, MD) is registered for control of fungal and bacterial diseases on numerous crops, including pear and apple. *Bacillus subtilis* strain QST 713 (Serenade Max WDG or Serenade Opti, Bayer Crop Science LP, Research Triangle Park, NC) is sold as a spray-dried fermentation product containing the live organism and a mixture of lipopeptides produced in culture. The lipopeptides are essential for efficacy; growth of the bacterium on plant surfaces is not required for disease control. Serenade is applied just prior to predicted infection periods, similar to antibiotic applications, but numerous applications are recommended for disease control.

The efficacy of the other two bacterial biological control agents depends on effective colonization of flowers, namely achieving population sizes ca. 10^6 colony forming units per flower. The product BlightBan A506 (*Pseudomonas fluorescens* strain A506, NuFarm Americas, Burr Ridge, IL) was isolated from pear in California (Lindow 1985) and A506 was the first registered biological control agent for fire blight. The mechanism of action of A506 is preemptive exclusion (Wilson & Lindow 1993). The bacterium colonizes and forms large populations on floral stigmas, excluding *E. amylovora* from sites for colonization and growth. A506 also was demonstrated to reduce microbial induced-russet and frost injury of pear (Lindow 1985). Bloomtime FD (*Pantoea agglomerans* strain E325, NuFarm Americas, Burr Ridge IL) is the other commercial bacterial biological control agent. Like A506, the bacterium colonizes stigmas and excludes the pathogen (Pusey *et al*. 2008). In addition to competition, E325 produces an uncharacterized antibiotic on flowers that is toxic to *E. amylovora*. Growth of E325 also reduces the pH of floral stigmas, which, in turn, reduces the growth of the fire blight pathogen. Neither A506 nor E325 causes damage to fruit finish. An advantage of biological control agents is that they grow and spread, unlike antibiotics; that is, the biocontrol bacteria spread from colonized flowers to newly opened flowers that may not have been protected by earlier chemical sprays (Johnson *et al*. 2000; Lindow & Suslow 2003). Well-timed applications of the bacterial biological control agents during bloom can significantly reduce the incidence of fire blight under low to moderate disease pressure (Johnson *et al*. 1993; Lindow 1985; Stockwell *et al.* 2010).

Use of BlightBan A506 and Bloomtime FD requires grower education and changes in how they approach fire blight management. Instead of using traditional decision aids to determine the need for disease control measures and the timing of intervention, growers need to commit during early bloom to a biologically-based disease control program to permit establishment and growth of the biological control agents prior to arrival of the pathogen to flowers. Furthermore, growers need to apply the biological control agents during conditions that support growth of the bacteria. A decision-aid for use of biological control agents was developed to guide the timing of applications to maximize the potential for successful establishment and growth prior to migration of the pathogen to flowers (Johnson *et al*. 2004).

Bacterial biological control agents work best in the western states where bloom progresses over one to three weeks and conditions are moderately warm to support bacterial growth. In other regions of the US, bloom occurs rapidly and environmental conditions during early bloom are often too cold to support rapid growth of bacterial biological control agents, which may decrease control efficacy (Sundin *et al*. 2009).

Variability in consistency in performance of biological control agents across environments is an impediment to widespread adoption of this technology (Johnson & Temple 2013; Sundin *et al*. 2009). An integrated approach to disease control consisting of a biological control agent application in mid to near full bloom and a single antibiotic application in late bloom reduces the variability associated with biological control (Johnson & Temple, 2013; Stockwell *et al.* 2008). All of the commercial biological control agents are resistant to streptomycin and tolerate oxytetracycline (Lindow *et al*. 1996; Stockwell *et al*. 1996). The ability to integrate bacterial biological control agents with antibiotics provides growers options for effective disease control, especially under conditions with moderate to high disease pressure.

Yeast biological control agent: A mixture of *Aureobasidium pullulans* strains DSM 14940 and DSM 14941 suspended in an acidic buffer (Blossom Protect, Westbridge Agricultural Products, Vista, CA) was approved for fire blight management in 2012. Like other biocontrol agents, this colonizes the flower stigma and provides the best control if applied before infection. The mechanism of action is proposed to be competitive inhibition but it has not been critically identified yet. Unlike the bacterial biological control agents, the yeast is osmotolerant and thrives on the sugar-rich nectar, thus likely excluding *E. amylovora* from the nectar-secretion pores through which the pathogen invades plant tissues. The addition of the acidic buffer in the formulated product augments disease control. Acidic conditions support growth of yeast, but are inhibitory to *E. amylovora*. Excellent disease control has been reported with Blossom Protect (Johnson & Temple, 2013). Nonetheless, yeasts, including those in Blossom Protect, are known to cause russet or mark fruit finish on certain cultivars of pear and apple during cool, wet environmental conditions (Johnson & Temple, 2013). Russet mars the fruit finish and can decrease the fresh market value of the fruit. Consequently, some growers hesitate to use Blossom Protect, especially in orchards in regions with cool, wet spring weather. Additionally, the yeasts in Blossom Protect are sensitive to copper and many of the fungicides used to control scab, powdery mildew and other fungal diseases in orchards. The incompatibility of Blossom Protect with many fungicides adds an extra level of complexity for management of fruit orchards during bloom to fruit development (Johnson & Temple, 2013; Sundin *et al*. 2009)

Bacteriophage technology: Bacteriophages have been under development for plant disease management for decades (Balogh *et al*. 2010; Boulé *et al*. 2011; Frampton *et al*. 2012; Jones *et al*. 2008; Lehman 2007; Nagy *et al*. 2012; Svircev *et al*. 2010). Deploying and sustaining high titers of bacteriophages in orchard environments has been challenging due to their sensitivity to UV radiation, temperature, and desiccation (Iriarte *et al*. 2007). Additional concerns in phage-mediated biological control include the development of bacterial resistance to single phages (Roach 2011; Vidaver 1976) and the inherent potential for the transfer of bacterial genes by temperate phages (Roach *et al*. 2015). To overcome the obstacles inherent in bacteriophage technology, A. Svircev (Agiculture and Agri-Food Canada) has developed a system using the bacterial biological control agent of Bloomtime FD (*Pantoea agglomerans* E325) and other *Pantoea* spp. as a carrier of several families of lytic bacteriophages with specific activity against *E. amylovora* (Gill *et al*. 2003; Lehman 2007). Conceptually, the phages are integrated into the *Pantoea* spp. host (or carrier) during the industrial production. The biological product, namely the *Pantoea* spp. cells carrying the phage, is sprayed on trees during bloom, colonizes flowers, and competes with *E. amylovora* for sites and nutrients. The *P. agglomerans* and the released bacteriophages reach high population numbers on the sites where *E. amylovora* grows. Laboratory-based flower assays and 5 years of orchard trials show promise for this innovative approach for disease management that may be commercialized in the near future. Work towards expediting the commercialization of the phages is in progress based on the 2014 (Svircev unpublished) and 2016 (Ken Johnson, personal communication) field trials with Bloomtime and phage mixtures.

Summary: Antibiotics have been used for decades for control of two serious plant diseases, fire blight of pear and apple and bacterial spot of peach and nectarine without deleterious effects to the environment or animal and human health (McManus 2014). Nonetheless, the development of resistance of *E. amylovora* to streptomycin led to the need for alternatives to antibiotics for disease control. Ultimately, host resistance is the most desirable antibiotic alternative disease management tool. For fruit crops, breeding for host resistance is a slow process but is likely to be hastened by marker-assisted breeding. It may also be accomplished through genetic modification, but modified trees could not be used by certified organic growers. Furthermore, it is expensive to establish a new orchard and growers may not plant genetically-modified tree fruit cultivars without assurance that the fruit will be marketable and acceptable to consumers into the future.

Industry survey: We conducted an informal nationwide survey of researchers and extension personnel involved with disease management of tree fruits for their input on the future need for antibiotics and the potential use of alternative products. Each of them responded that antibiotics are important tools for management of fire blight and bacterial spot of stone fruits. The respondents shared three major reasons that antibiotics are important materials: 1) consistent efficacy, 2) reasonable cost, and 3) safety to crops.

Each year, the majority of orchards in the US are *not* treated with antibiotics. The orchard managers monitor conditions in their orchard and intervene with antibiotics to protect the crop only if the risk of fire blight is high. The need for high and consistent efficacy was summarized by a respondent that wrote "Antibiotics are used for fire blight control during the infrequent instance when the host, the pathogen, and the weather all align correctly for the disease to occur. When this occurs, antibiotics are the best tool to prevent damage. …If an orchard manager does not spray when they should have, fire blight can cause sporadic damage to complete death of the orchard." Growers have relied on the high efficacy and consistency of antibiotics to control fire blight. Even with antibiotics, fire blight is a notoriously difficult disease to control. Growers are interested in using antibiotic alternative products, but there are challenges associated with these technologies. For example, the biologically-based alternatives to antibiotics can be plagued with inconsistencies; they can significantly reduce the incidence of disease by 50 to 90%, but the level of control can vary from year to year and orchard to orchard and they sometimes fail completely.

Expenses associated with programs based on use of alternative products may be greater than programs using antibiotics only. The expenses of alternative products includes not only the price for the materials, which may or may not exceed that of antibiotics, but also extra costs of fuel and personnel operating sprayers to apply additional applications of the alternative products. Additionally, some of the antibiotic alternative products need to be applied proactively during early bloom, before growers can predict if weather will support an outbreak of the disease in their orchard. If the conditions for fire blight do not develop in late bloom, then the alternative products applied proactively may be considered an unnecessary production expense. Finally, beautiful fruit finish on pear and apple is expected and valued by consumers. Some of the alternative products, such as copper or yeasts, tend to mark the surface of developing fruit during wet weather, which severely downgrades the fruit and lowers the price compared to premium fresh market produce.

Many respondents wrote that there is an increase in the complexity of methods needed for disease control if antibiotics are not used. For example, they indicated that the orchard manager would be required to apply two or four additional sprays of less effective disease control measures in an average year, during a time when management of other issues is at a critical point. Both organic and conventional fruit growers often need to apply several sprays during bloom that are critical for the suppression of key insects and other diseases. The materials used are often not compatible with biological control agents when applied in the same spray tank, or near each other in time. Some critical fruit thinning sprays, such as lime sulfur that inhibits growth of biological control agents, must be applied to assure yearly cropping.

Organic-certified growers are at the forefront of testing antibiotic-free fruit production because antibiotic registrations for organic pear and apple production were withdrawn in October 2014. Currently, about 10% of the Washington apple industry is certified organic and the organic acreage is increasing. University researchers have developed fire blight control programs without the use of antibiotics (Johnson & Temple, 2013). There has been little commercial orchard experience with the new alternative products, so there are valid questions if the products that perform well in experimental research orchards will be effective or consistent as antibiotics have been in commercial orchards. Epidemics of fire blight generally occur every 5 to 10 years, thus, the capacity of antibiotic alternative products to control fire blight effectively likely will be subjected to serious real-world tests within the decade.

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Table 1. Plants

Primary Agent (disease)	Crop	Relative antibiotic use and antibiotic used	Current alternatives to antibiotic products	Research needed to improve tools and adoption	Priority given for antibiotic alternatives
Erwinia amylovora (Fire blight)	Apple, pear, and other related hosts	**High** streptomycin, oxytetracycline, kasugamycin	**Chemicals**: Copper formulations, sanitizers (e.g. OxiDate), plant growth regulator (e.g. Apogee), induced resistance promotor (e.g. Actigard). **Biologicals:** BlightBan A506, Bloomtime FD, Blossom Protect, Serenade Opti, Double Nickel	• Improve efficacy and consistency of biological control agents across US, especially considering impact of site-specific environments (e.g. climates, vectors) on efficacy. • Compatibility with other products used in crop protection. • Extension and demonstration programs in grower fields. •Identification of genetic resistance in hosts and use of biotechnology tools for host resistance (and public education/acceptance of these tools).	High
Xanthomonas arbicola pv. *pruni* (Bacterial spot)	Peach and nectarine	**High** oxytetracycline	Copper	• Development of additional alternatives to antibiotics. • Improved deployment of copper. • Identification of host resistance.	High
Pectobacterium and *Dickeya* spp.	Potato seed pieces	**Low** streptomycin	Sanitation, broad spectrum pesticides, and sanitizers		Low
Pseudomonas spp. and *Xanthomonas* spp.	Tomato, tobacco, and pepper seedlings for transplants.	**Low** streptomycin	Plant Host resistance, sanitation, broad spectrum pesticides, and cultural management.		Low
Various	Ornamentals and cut flowers	**Low** streptomycin	Sanitation and host resistance.		Low

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