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The Use of Bactericides in Plant Agriculture with Reference to Use in Citrus to Mitigate HLB

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The purpose of this communication is to discuss the use of bactericides in plant agriculture to control disease epidemics and the approaches that have been tested to control Huanglongbing (HLB) in citrus trees. The Citrus Research and Development Foundation (CRDF) has made this topic a priority in responding to HLB in Florida citrus. HLB is a disease devastating the citrus industry in Florida and throughout the world. Presently, no chemical treatment or resistant plant is available that will control the disease. For the Florida citrus industry to survive this epidemic, a chemical control will be necessary to suppress the disease, keeping the trees in production until groves can be replanted with resistant or tolerant varieties. This is similar to approaches taken during plant disease epidemics in other crops, which were eventually controlled by the planting of resistant varieties. Oxytetracycline, streptomycin sulfate and copper have been the main chemicals available to treat bacterial plant diseases in the US. The use of copper is limited to foliar diseases in regions where copper resistance is not widespread. Streptomycin and oxytetracycline are routinely used on some crops when copper is inadequate. Oxytetracycline has also often been used in the past to help manage plant disease epidemics. Research using bactericides on citrus suggests that chemical control may improve citrus tree health and contribute to sustaining the citrus industry in the current epidemic.

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Introduction

The disease HLB has been known in many regions of the world for over a century. This disease was only introduced into Florida a decade ago, but has since reached epidemic proportions. HLB in Florida is presumably caused by the bacterium *Candidatus* Liberibacter asiaticus (*C*Las), which is transmitted by an insect, the Asian citrus psyllid (ACP, *Diaphorina citri* Kuwayama). The bacterium is injected directly into the vascular system of the tree by the vector and can spread systemically down to the roots and throughout the tree canopy. The number of Florida citrus groves still in production has shrunk dramatically because of HLB due to a decrease in marketable fruit and significantly higher than previous production costs resulting from tree decline. The citrus industry employs around 62,000 people and has an economic impact of approximately 10.8 billion dollars[1]. The January 2016 all-orange crop estimate of only 69 million boxes was less than half of the pre-epidemic numbers. With the rising costs to keep the diseased trees in production, the sustainability of the industry is in question. What will ultimately ensure the continued existence of the industry are resistant citrus varieties, but no resistant variety is presently being propagated in nurseries. Even if a resistant variety were identified today, it would take many years to replant more than 500,000 acres of citrus and for those trees to reach maturity. Plant disease epidemics like HLB are relatively common, and while ultimately epidemics are controlled using resistant or tolerant plant varieties, a chemical control is often used as a stopgap.

Agricultural Bactericides

Plant diseases caused by bacteria are very difficult to control, especially when a bacteria like *C*Las resides in the phloem of the plant vascular system, and few bactericides are available to treat bacterial diseases. Although copper has been used in agriculture for several centuries as a foliar treatment to protect plants from fungal and bacterial diseases, when used systemically at bactericidal levels, copper is toxic thus, copper has not been demonstrated to be an effective treatment for HLB. A type of bactericides that has been found to provide a level of control of HLB and many other bacterial diseases is antibiotics. The two main antibiotics labeled for use in agriculture are streptomycin sulfate and oxytetracycline. Two forms of oxytetracycline are used, oxytetracycline hydrochloride and oxytetracycline calcium, but once in solution the molecules are identical. These materials have been used since the 1950s and are also important in human and animal medicine, but presently only a fraction of one percent of total domestic antibiotic use is for plant agriculture[2].

The main uses for both oxytetracycline and streptomycin sulfate in agriculture are for prevention of fire blight (*Erwinia amylovera*) in apples and pears. Streptomycin is the preferred bactericide, since it is kills the bacteria on contact (bactericidal), whereas oxytetracycline is bacteriostatic, suppressing bacterial growth. Bacteriostatic bactericides require a longer duration of exposure to suppress growth long enough to reduce the bacterial titer. Alternating chemicals with different modes of action is the primary strategy for resistance management. Streptomycin-resistant *E. amylovera* strains now occur in some areas because streptomycin was the only chemical available in the past. Oxytetracycline has been labeled for use against fire blight to manage resistance in some regions and recently, kasugamycin has been approved for use in most US states for the same purpose.

Oxytetracycline is also used against bacterial spot of peach and nectarine (*Xanthomonas arboricola* pv. *pruni*) and in non-bearing plants, where it is mainly applied by injection to prevent infection or to treat important landscape or specimen plants. Oxytetracycline has been used historically to control important bacterial and phytoplasma diseases in emergency situations, such as coconut lethal yellowing disease, X-disease of peaches and cherries, peach yellow leaf roll and pear decline. These diseases now are mainly controlled using tolerant or resistant varieties combined with good management practices.

Bactericide Research Against HLB

The use of oxytetracycline has been evaluated in the past to control HLB and has been shown to have some efficacy. Most of the studies using tetracyclines and similar bactericides to treat the symptoms of HLB were conducted in the 1960s through the 1980s in areas of long-established infection by the disease. Several members of the tetracycline group were investigated including tetracycline hydrochloride, chlortetracycline, demethylchlortetracycline and oxytetracycline. These studies mainly evaluated performance of bactericides applied

by foliar application or by trunk injection, although some earlier studies immersed budwood in a bactericide solution before grafting or immersed the roots before transplanting[3, 4]. Molecular methods for detection of the HLB bacterium were not available when these early studies were performed and thus the studies evaluated the timing of visible symptom development as a measure of efficacy. Results varied by experiment, but tetracycline was found to be effective in most cases with the suppression of symptoms lasting from months to years[3-16]. In one experiment evaluating both injection and foliar applications of tetracycline, more trees recovered and symptoms were suppressed for a longer period of time when tetracycline was injected compared with foliar applications[8]. Other studies focused on either trunk injection or foliar applications. Some of the tetracyclines used in these experiments were found to be toxic to the plant, causing symptoms such as yellowing, smaller leaves and discoloration at trunk injection sites. Demethylchlortetracycline and oxytetracycline did not cause the same symptoms, possibly because of the greater solubility of these materials. One study also found that the pH of the tetracycline solution influenced the amount of toxicity[15], providing further evidence that the formulation chemistry can be adjusted to prevent toxicity.

Penicillin was included in a few of the early studies, and results ranged from having little to no effect on symptoms[5, 10, 13], having a lesser, but more sustained effect over time when compared to oxytetracycline[9], to having a similar effect to tetracycline HCl[6]. A more recent study conducted in Florida tested a combination of a high dose of penicillin G and streptomycin along with several other chemicals including a high dose of oxytetracycline and kasugamycin, using a budwood immersion followed by grafting technique[17]. The results were inconclusive for oxytetracycline because it was phytotoxic at the high rate and the grafts were unsuccessful. The combination of penicillin and streptomycin successfully reduced the number of bacteria to a low, but detectable level, but how this compared with oxytetracycline was not determined, nor was the efficacy of the individual compounds. This study demonstrated that kasugamycin was ineffective against *C*Las. In a study examining these treatments applied to the foliage of periwinkle plants infected with *C*Las, both oxytetracycline and the combination treatment reduced the amount of the bacteria to a statistically similar level[18]. Penicillin does not currently have any registered use in plant agriculture. The timeline for registering this new active ingredient would be many years as with any new active ingredient. This, along with the health risks and lack of evidence of superiority to other chemicals currently used in plant agriculture, suggest that penicillin is not a viable treatment for HLB.

Discussion

Despite showing some efficacy, oxytetracycline was not widely used in the past to treat HLB. This is partially because of the phytotoxicity of early formulations and also because the most efficient method for introducing the material into the plant was by trunk injection. While effective to some degree, trunk injection historically has not been found to be cost effective for treating many acres of citrus trees. Authors of a review of citrus greening in South Africa explained that trunk injections of tetracycline in South Africa did not become common because of phytotoxicity, residues in the fruit, production of small fruit and because disease suppression was not sustained[19]. An additional explanation for not relying heavily on antibiotics that was not discussed in the review was that the *Liberibacter* species found in Africa (*Candidatus* Liberibacter africanus), along with the vector psyllid species, have a greater sensitivity to heat than *C*Las and ACP, and shifting the industry to a more tropical region in South Africa has reduced the disease incidence. This may be why methods for trunk injection treatments were never optimized. In other citrus growing regions effected by HLB, relocating the citrus production to areas with periods of cooler and/or drier weather that allow for a dormancy period, or geographically isolated areas, have helped to reduce the psyllid pressure and slow the spread of the disease.

Not having the options available to other regions with this disease, the Florida citrus industry has relied on other aspects of citrus culture including nutrition, soil chemistry and pruning in order to manage HLB. While these methods can contribute to keeping trees in economical production, they do not reduce the amount of infection once a tree develops the disease. Good psyllid control will always be an important aspect of disease management. Psyllid control will only help to prevent new infections, but if a chemical is found to suppress the bacteria in the tree, psyllid control will be important to maintain this suppression. Few methods are available to suppress or eliminate the bacterium in the tree. Heat

therapy is one of the earliest methods used in the history of plant pathology to eliminate pathogens from plant material. This method has been adapted for larger-scale use in citrus groves and provides some disease suppression, but is still in experimental trials.

Recently, industry interest has grown for the potential use of antibiotics to treat HLB. This is based on past research and also from the Section 18 for emergency use of streptomycin sulfate to treat another important disease in citrus, citrus canker (*Xanthomonas citri* subsp. *citri*). From this interest, several studies of the efficacy of foliar applications of oxytetracycline and streptomycin sulfate against HLB have been funded by CRDF since mid-2014. Results from these studies, along with results produced independently by the registrants, are part of the 2015 petition to EPA for a Section 18 emergency exemption allowing use of these materials on citrus in Florida. The decision on this petition is forthcoming. Approval of the Section 18 petition will allow growers to apply foliar applications of oxytetracycline and streptomycin sulfate.

Foliar application is the most common method of applying pesticides to citrus groves. This is a main reason why the research for the Section 18 application has initially focused on this method. Adjuvants are available that increase the ability of the bactericides to move through the leaf surface. However, there may be more efficient methods to introduce the material into the tree and have a greater impact on the bacterial population in the phloem. Trunk injection is an example of an alternative method. Trunk injection may be economically viable if equipment and methods were to be developed to allow growers to inject a large number of trees in a short period of time similar to how injections are conducted to prevent infection of avocado by the laurel wilt pathogen in southern Florida. A proof-of-concept experiment is currently in place, funded by CRDF, to evaluate the effect of trunk injections of oxytetracycline and streptomycin on tree health and fruit quality. If successful, this study may lead to the development of cost-effective injection techniques for growers. Although the work has been initiated to make this technique an option for growers, expansion of current labels will be required to include trunk application.

The use of chemicals alone to control plant diseases is not sustainable, but a stopgap solution. When confronting an epidemic, chemical control is often the first line of defense, followed by the development of resistant plants. Citrus plant breeders are working towards developing tolerant and resistant citrus varieties to sustain the industry over the long term, but until these varieties are available, short-term solutions are necessary. The use of oxytetracycline and streptomycin with effective adjuvants may suppress the bacteria in the tree, allowing the tree to produce a stable crop of adequate quantity and quality to sustain current production. Further advances in application, coupled with additional bactericides, may be a vital step towards managing the HLB epidemic in Florida.

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