

## **Fire Blight Focus: Resistance to Bactericides and Managing Blight in 2016**

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Fire blight in 2015 was catastrophic in many Sacramento Delta pear orchards. Substantial numbers of scaffold branches were killed or severely cut back, which will reduce production for at least two years, and many trees were killed. The problem started in 2014, when weather was conducive to severe blight outbreaks after several years of little blight. That resulted in more holdover cankers in early 2015, coupled with warm temperatures in late winter and early spring. Favorable conditions were further created by low winter chilling that increased the length of bloom, as well as by fewer drying winds.

Few products are available for controlling blight, and resistance of the blight bacterium (*Erwinia amylovora*) to antibiotics and copper are a major concern.

### **Streptomycin Resistance**

A high level of resistance to streptomycin (Agri-Mycin, Firewall, Agri-Strep) was originally found in California in the 1970s. At the original discovery site of streptomycin resistance, where more than 95% of the population was resistant and where streptomycin was then no longer applied, the incidence of resistance declined gradually over the years, and was present at less than 5% after 10 years. But resistance problems in several Delta orchards have again been a concern for over a decade, so many growers have used little or no streptomycin in recent years and instead used oxytetracycline and/or copper products.

Since 2006, blight samples from the California pear production districts have been sent to the Adaskaveg lab at UC Riverside for testing for resistance to antibiotics. Except for one orchard in one year, no resistance to oxytetracycline (Mycoshield) has been found in the Sacramento Delta.

A recent paper describes the results of resistance testing for streptomycin from 2006 to 2014 (Förster et al., 2015). Over the nine years of sampling, 993 strains of *E. amylovora* were isolated from separate blight strikes collected from seven counties. Sensitivity against streptomycin was evaluated using the “spiral gradient dilution” method, in which different radial concentration gradients are created in 15-cm nutrient agar plates by spirally plating an aqueous stock concentration of streptomycin using a spiral plater. Suspensions of the test bacteria were streaked out (like spokes on a bike) along the radial concentration gradient. Measurements were taken for “minimum inhibitory concentration” (MIC) by measuring the distance of inhibition, measured from the center of the plate outward. The minimum inhibitory concentration of each strain (in mg/L or ppm) was determined:  $\leq 2.5$  ppm = sensitive,  $>2.5$  to 37 ppm = moderately resistant, and  $>50$  ppm = highly resistant.

The incidence of streptomycin resistance in North Coast orchards was very low, with only two resistant strains found in one orchard (Table 1). Nearly all the moderately resistant strains were isolated from orchards in Sacramento County. In 2006 and 2007 the far majority of these orchards had moderately resistant strains, but in 2013 and 2014 very low levels of this resistance were found. Highly resistant strains were found in Sutter-Yuba counties in three of the five years tested.

Table 1. Summary of surveys for streptomycin resistance in *Erwinia amylovora* populations in California (El Dorado and Solano not shown).

County	Year	No. of orchards	No. of strains	% of strains that were MR <sup>1</sup>	% of orchards with MR strains	% of orchards with HR <sup>1</sup> strains
Lake	2008	11	30	0	0	0
	2009	7	7	0	0	0
	2012	5	10	0	0	0
	2013	21	44	0	0	0
	2014	6	19	0	0	0
Mendocino	2006	1	4	50	100	0
	2008	3	14	0	0	0
	2009	1	3	0	0	0
	2014	1	7	0	0	0
Sacramento	2006	14	67	76	86	0
	2007	17	172	67	94	0
	2008	7	57	5	43	0
	2009	21	132	33	62	0
	2010	10	56	46	80	0
	2011	9	39	33	44	0
	2013	13	105	7	15	0
	2014	18	129	2	6	0
San Joaquin	2008	1	5	0	0	100
Sutter-Yuba	2006	2	30	0	0	0
	2007	2	12	0	0	100
	2008	1	3	0	0	0
	2009	7	24	0	0	43
	2012	3	11	0	0	67

<sup>1</sup>MR = Moderately resistant

<sup>2</sup>HR = Highly resistant

In 2015, statewide resistance testing showed that only two orchards had streptomycin resistance, both in Sacramento County. In one orchard only one of five samples was resistant, and in the other orchard all eight samples were resistant, with one being highly resistant. In the latter orchard, streptomycin was alternated with oxytetracycline for multiple years.

In the North Coast, the use of tank mixes of antibiotics, combined with lower temperatures during bloom than in the Central Valley (the treatment threshold is 100 degree hours greater than the Central Valley), have likely led to less selection for resistance than in the less favorable for disease development in the North Coast, treatment frequency and antibiotic amounts used per application are also lower. Antibiotic use continues as a tank mix of streptomycin (40-60 ppm) and oxytetracycline (200 ppm) at much lower overall antibiotic rates than in the Central Valley districts (Zoller, 2011). This tank mix is used to slow the development of resistance to

oxytetracycline and streptomycin. It had been postulated in the early 1970s that perhaps the development of resistance to streptomycin could be attributed, at least in part, to the cancellation in 1960 of a 15% streptomycin/1.5% oxytetracycline premixture (Agrimycin 100, Pfizer) that was used in the 1950s. This premixture was replaced with a product containing only streptomycin. This combined use is an attempt to extend this reasoning to prolong oxytetracycline use in areas with little streptomycin resistance.

This strategy will also be strongly recommended for the use of the new antibiotic Kasumin. This antibiotic is registered federally and is pending registration in California (Adaskaveg et al. 2011). There are no known resistant strains to kasugamycin, the active ingredient in Kasumin. This new antibiotic has similar performance to streptomycin before resistance developed to it. Mixtures of kasugamycin with streptomycin or oxytetracycline, in rotation with streptomycin-oxytetracycline mixtures should provide excellent disease control and greatly reduce the likelihood of selecting for resistance to any one of the three unique modes of action.

### **Copper Resistance**

The UC Riverside team led by J. Adaskaveg also evaluated most of the *E. amylovora* strains from blight strikes collected in spring 2015 for copper sensitivity. Based on the results obtained, it was concluded that most strains tested (including populations from Delta and North Coast orchards) are moderately copper-resistant. However, blight strikes from 14 additional orchards were tested with different methodology by Steve Lindow (UC Berkeley) in fall 2015, and no resistance was found. Still, strains that he tested grew on CYE medium amended with 15 ppm MCE. Perhaps there may be some issue with the definitions of the researchers, but Adaskaveg explains that in other bacterial systems, strains that cannot grow at equal to or less than 10 ppm MCE are sensitive. Strains growing at higher concentrations are considered less sensitive or moderately resistant, whereas strains capable of growing at concentrations more than 50 ppm are considered resistant. Additionally, Adaskaveg in 2014 showed that pear flowers treated with a registered copper formulation that were immediately inoculated using a strain that can grow at 15 ppm MCE on CYE medium did not completely inhibit the pathogen and some disease resulted.

On both rich (NA) and poor (CYE) culture media, "spontaneous mutants" were commonly seen in the Adaskaveg lab. These grew as larger colonies over the weaker mixed bacterial growth on copper-amended media. These spontaneous mutants were previously reported by others. Sub-culturing these strains onto copper media showed higher growth ratings. Some researchers call this "adaptation" and "not true resistance". But if copper is present in the orchard and these spontaneous mutations occur as they do in the laboratory, then disease can result.

Regardless, copper was less effective than antibiotics in controlling blight during the epidemic of 2015. In 2015 field trials conducted by J. Adaskaveg, copper failed to control naturally occurring blight, and moderate resistance (reduced sensitivity) to copper in the bacterial population was found in that orchard. Many growers will back off using copper in the future, although organic growers have a far smaller toolbox of products.

Copper is a contact material and is suppressive to growth of the pathogen. It appears that after exposure to copper, the pathogen is still viable and can cause disease when copper levels decrease. Our ratings in the UC IPM Fungicide and Bactericide Tables in 2013 on the

performance of copper were +/+++ and these were revised to +/++ in 2014. This rating indicates low to moderate activity under highly favorable to less conducive conditions for disease development, respectively. Multiple ratings such as +/++ also indicate inconsistent performance. The issue with copper on pears is that it is registered at low rates (Kocide 0.5 lb/A \* 30% MCE = 0.15 MCE lb/A). If 100 gal/A are being applied, then this translates to 68 g MCE/378.5 L or 180 ppm. This seems like it would be sufficient when we have growth on CYE and NA amended at 15 and 30 ppm, respectively, but this rate is very low compared to other bacterial diseases where we use 2400 ppm in a 100 gal spray tank to provide disease control.

The bacterium lives in cankers, which are dead host tissue, and the bacteria ooze out in droplets from cankers. Various fire blight models estimate temperatures that favor growth of the bacterium and some relate this to phenological stages of the crop such as bloom stage. The 180 ppm copper application is diluted during redistribution on the plant surface by 10X or more during rain or plant tissue growth, and this would put the rate of copper on the surface of the tree at 18 ppm or less. This is why when we see any growth at 15 ppm or 30 ppm on CYE or NA media, we are describing the bacterium as moderately resistant and that there is not enough copper to protect the plant from disease. Copper, like antibiotics, does not eradicate the pathogen from cankers, and the bacteria will keep oozing out with warm temperatures and tree growth.

Adaskaveg and Lindow both agree that commercially registered fixed coppers are relatively insoluble in water and only a small fraction is soluble upon wetting. This adds to the problem that only low labeled rates of copper are registered on pears. Actually, less copper is available than the concentration in the spray tank due to dilution effects and copper interactions with other materials on the plant surface. In the lab, Adaskaveg uses copper sulfate, which is fully soluble and provides an absolute value for copper sensitivity.

Several factors likely have contributed to the failure of copper applications to control fire blight in the spring 2015 season:

1. Highly conducive disease conditions
2. Low labeled rates of copper used
3. Moderate copper resistance in *E. amylovora*
4. Selection of populations (spontaneous mutants) with higher copper resistance after repeated applications. This was further exasperated by the fact that copper is bacteriostatic and does not kill the pathogen (We plated out *E. amylovora* on 50 ppm MCE and the pathogen did not grow. When we re-transferred the bacteria onto copper-free medium, growth occurred).

The conclusion is that copper at the concentration labeled for pear cannot be given more than a single "+" (out of +++) in future guidelines. In other years up to 2013, under low disease pressure, it appeared to perform better. In trials in 2015 conducted by J. Adaskaveg, four weekly applications of copper did not provide control under highly favorable conditions for disease, whereas other treatments with similar timing did. More resistance testing will be conducted in 2016.

### **Moving Forward**

Blight has the potential to be severe again in 2016. Although we have received adequate chill, at least through early January, the presence of large numbers of holdover cankers and a

potentially strong El Niño spring (i.e., warm and wet) could lead to severe blight outbreaks again, but only if heat unit accumulations develop to sufficient levels prior to these rainfall events. Below are some important tips to consider for achieving control.

1. Consider delayed dormant copper treatments where holdovers are especially prevalent. Research in the North Coast by Rachel Elkins (UCCE Lake & Mendocino Counties) showed that this single high-rate application with surfactant reduced the level of *E. amylovora* in the flowers in three of four years, especially later in the primary bloom period (Elkins et al., 2015). In the same study, Sacramento sites in 2012-13 had very low levels of *E. amylovora* so no determination of a reduction was possible.
2. Do not use copper in repeated in-season applications.
3. Monitor the accumulated heat units prior to wetting events, paying attention to the predictive models coupled with weather forecasts (see Table 2).
4. Be prepared to spray more often than twice weekly when conditions warrant more frequent treatments. This could be particularly important where holdovers are more prevalent than usual. Also, some sprays may be unjustified if weather conditions are not conducive to infection.
5. With the likelihood of more holdover cankers going into bloom, it is essential to continually monitor for clusters of new strikes and cut new strikes and cankers as soon and often as possible. It is not uncommon for growers to let late spring strikes remain until after harvest to minimize dropping of fruit, then cutting blight after harvest. With so much blight, as well as labor shortages, cutting in many blocks continued into late fall, and new strikes developed.
6. Consider the use of streptomycin if the antibiotic has not been used in recent years and no resistance was detected in surveyed orchards nearby. If streptomycin is used, if possible limit the number of applications to no more than one or two per season in a rotational program.
7. Tank mixing blight treatment products rather than alternating them. This practice likely reduces the risk of resistance buildup. But by all means use multiple chemistries through the season. Kasumin can be used in mixtures and rotations with streptomycin and oxytetracycline. It is federally registered but is still pending registration in California.
8. Consider the use of Actigard (not yet registered in California). Actigard is a systemic compound that stimulates or induces the plant's natural defenses, turning on the genes that are involved in fighting off infections. According to the label, in pears Actigard is applied 2-3 times between 20% bloom and petal fall, at least 7 days apart to help manage blight. Research in Oregon has shown improved blight protection when Actigard is used in combination with antibiotics. In 2013, West Coast trials in 25 orchards found that Actigard + grower program had 37% fewer strikes than the adjoining area that received only the grower program (Johnson et al., 2014). In California grower and research trials conducted by J. Adaskaveg, however, Actigard applications were inconsistent in their performance against fire blight.
9. Consider the use of the product Blossom Protect in antibiotic rotational programs. Antibiotics do not affect the growth of this fungal organism in the biocontrol product, however, sulfur is inhibitory. Russetting is a concern with this product, especially in wet springs.

10. Late season applications of potassium phosphite may help reduce fire blight on rat-tail bloom and reduced the severity or size of cankers. Research is ongoing on this strategy. This is shaping up to be a wet winter, so pre-bloom irrigation to wet deeper soil layers will likely be unnecessary. Of course, irrigation can sometimes take place during the rattail bloom period and it must be realized that early irrigations can increase the severity of warm dew infection periods. Delaying the first irrigation can sometimes result in avoidance of some of the later infection periods during the spring.

Optimal blight management can be virtually impossible to implement at times, considering weather extremes, labor availability, and labor skill. Years like 2015 are a reminder of how damaging blight can be if all available resources aren't used to fight it.

Table 2. Sacramento Valley degree hour system action thresholds (<http://www.ipm.ucdavis.edu/MODELS/FBEA/aboutfireblight.html>).

Degree-Hours	Weather	Action
0	Not relevant	None
1-150	Rain predicted within 24 hours	Spray in the 24 hour period prior to rain
150-500	Predicted rain or warm, humid weather where the temperature is at least 57F and humidity is at least 90%	Repeat treatment every 3-4 days with treatment in the 24 hours prior to predicted conducive weather
Over 500	Predicted rain or warm, humid weather where the temperature is at least 57F and humidity is at least 90%	Treat every other day during major bloom

Treatments are half treatments applied every other row. Higher thresholds (+ 100 added to each threshold) are used in the North Coastal Mountain districts as long as dormant season chilling has been typically greater than in the Sacramento Valley districts.

## References

- Adaskaveg, J. E., H. Förster, and M.L. Wade. 2011. Effectiveness of kasugamycin against *Erwinia amylovora* and its potential use for managing fire blight of pear. *Plant Dis.* 95:448-454. <http://dx.doi.org/10.1094/PDIS-09-10-0679>.
- Elkins, R.B., T.N. Temple, C.A. Shaffer, C.A. Ingels, S.B. Lindow, B.G. Zoller, and K. Johnson. 2015. [Evaluation of dormant-stage inoculum sanitation as a component of a fire blight management program for fresh market Bartlett pear](#). *Plant Dis.* (online), Vol. 0, No. ja
- Förster, H., G.C. McGhee, G.W. Sundin, and J.E. Adaskaveg. 2015. Characterization of streptomycin resistance in isolates of *Erwinia amylovora* in California. *Phytopathology* 105: 1302-1310.
- Johnson, K., R. Elkins, S. Castagnoli, T. Smith, and D. Sugar. 2014. Fire blight epidemiology and improved post-infection control. Report to Washington Tree Fruit Research Commission, [http://jenny.tfrec.wsu.edu/wtfr/PDFfinalReports/2014FinalReports/K\\_Johnson\\_PEAR\\_fire\\_blight\\_final.pdf](http://jenny.tfrec.wsu.edu/wtfr/PDFfinalReports/2014FinalReports/K_Johnson_PEAR_fire_blight_final.pdf).
- Zoller, B. 2011. Use of oxytetracycline and streptomycin for fire blight management in conventional and organic pear production in California. <http://www.tfrec.wsu.edu/pdfs/P2399.pdf>.