



WSU Efficacy of New Products for Control of *Erwinia Amylovora* Blossom Infections 2019

SUMMARY

- Oxytetracycline followed by four applications of hydrogen peroxide (26.5%), peracetic acid (4.9%) products had control no better than oxytetracycline products alone and had significant fruit marking compared to oxytetracycline alone.
- Seven applications of the thyme oil product (23%) provided 50% blossom blight control comparable to the organic standard Cueva/Previsto with three applications but resulted in significant fruit marking. Additional testing with fewer applications and faster drying times is needed to reduce marking risk and show cost effectiveness.
- The phage product did not perform significantly different from the untreated inoculated check in this trial.
- Copper product Instill provided 75% relative control and Mastercop (2.5 pt per 100 gal) 57% control comparable to copper standards across multiple years.
- Alum (Potassium aluminum sulfate) at 8 to 10 lbs/ 100 gal has provided consistent positive results with an average control of 75% statistically similar to the oxytetracycline check (82% control) across 3 years.

METHODS

Site: A two-acre research block of mature Red Delicious apples at WSU Columbia View Orchard 48 Longview Rd. East Wenatchee, WA 98802-8283 was used for the trial. Soils are a Cashmont Gravelly Sandy Loam with a 3-8% slope. The site has good air drainage and some wind protection.

Plots: Four blocks of 40 trees were designated (1-2 tree rows each). Individual trees were marked as plots in a randomized complete block where suitable trees were selected based on sufficient bloom (100+ flowers on lower branches).

Inoculum: Frozen-preserved cultures of the *Erwinia amylovora* 153 (streptomycin sensitive fireblight strain) were grown for 72 hours 28°C in NYDA agar to propagate dormant colonies. Subsequent inoculations were made transferring cultures to fresh NYDA plates every 24 hours to ensure fresh (<48 hrs old) plates.

Cluster Inoculation: Inoculation was conducted on the evening of April 27, 2019 at full bloom (of king blooms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1×10^6 CFU per ml. A 3-gallon backpack sprayer (solo) was used to lightly wet 100 clusters per plot. On May 1, 2019 at petal fall a second inoculation was conducted using live culture due to lower than projected numbers of live cells in freeze dried culture and extremely slow growth of fire blight in cold temperatures (cold, windy nights) during full bloom in order to ensure adequate infection. Fresh cultures were diluted to 1×10^7 CFU per ml and verified using an optical density spectrometer. A 1:9 dilution of the 1×10^7 CFU ml⁻¹ solution was used to obtain the 1×10^6 CFU ml⁻¹ solution used in field inoculation.

Treatments: Products were applied by tree to the area of the tree to be inoculated according to manufacturer recommendations using a Stihl SR420 blow mister backpack sprayer with a wetting agent (Biolink, organic; Regulaid, conventional). Products were applied to wet, previously calibrated to equal 100 gal/A. 2019 application dates were: April 21 (pink), April 23 (20% bloom), April 24 and 25 (50% bloom), April 26 (full bloom minus 1 day), April 27 (full bloom), April 28 (full bloom plus 1 day), May 1, 2019 (petal fall), May 2, May 4 and



May 6, and May 10, 2019. Treatment dates were adjusted to account for petal fall inoculations (adding an additional spray).

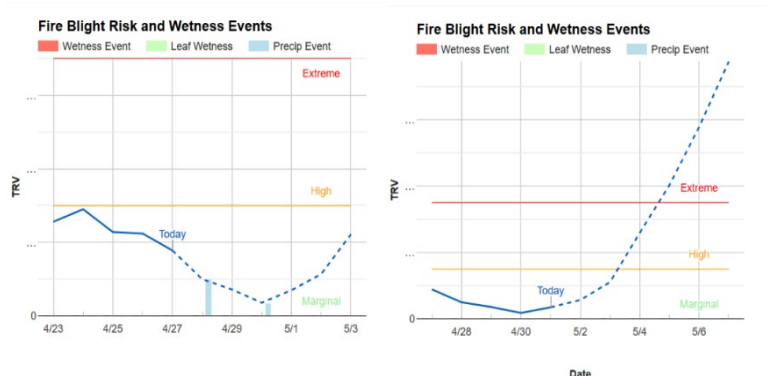
Included in this trial as a comparison and as “treated checks” were FireLine (oxytetracycline 17%) at 1.5 lbs. / 100 gal. / A and FireWall (streptomycin sulfate 17%), at 1.5 lbs. / 100 gal. / A, both antibiotics from AgroSource, Inc., and critical for comparisons as long-term standards). An untreated and inoculated check treatment and an untreated non-inoculated check treatment were included. Standard organic controls including coppers (Cueva and Previsto) are also included for comparison.

Table 2. Environmental Conditions

DATE	MIN AIR TEMP F	MAX AIR TEMP F	HUMIDITY	LEAF WETNESS
21-Apr	41	74.2	42.9	0
22-Apr	43.4	72	46.3	0
23-Apr	47.6	71	48.7	0
24-Apr	47.7	73.8	29.2	0
25-Apr	39.7	69.5	36.2	0
26-Apr	50.5	70	31.2	0
27-Apr	45.5	55.6	32.7	0
28-Apr	38.6	61.6	42.1	0
29-Apr	40.6	62.3	48.9	0
30-Apr	34.3	67.3	35.1	0
1-May	43.9	70.1	27.8	0
2-May	49	70.5	32	0
3-May	42.9	74.7	37.9	0
4-May	45.7	79.8	39.7	0
5-May	49.2	79.7	36.2	0
6-May	49.6	82.1	40.1	0

Fire Blight Risk and Pressure: During full bloom fire blight risk was low due to cold overnight temperatures. Warming after petal fall increased risk to extreme. See output from the Cougar Blight DAS model for full bloom and petal fall (Figure 2).

Figure 1. Cougar Blight Fire Blight Risk.



Evaluation: Trees were visually evaluated for flower cluster infection every week following treatment. Symptoms became visible 13 days after inoculation. Strikes were counted for 4 weeks. Blighted clusters were removed immediately after counting. Cluster infection counts were summed across all dates. Fruit were evaluated for russet fruit skin marking on June 12, 2019. 25 fruit per tree were rated. Russet ratings were on a 1 to 15 scale with individual values lower than 3 consider insignificant for commercial packing.

Analysis: Statistical analysis was performed using an analysis of variance ANOVA and multiple means comparison t tests (LSD) (SAS).

RESULTS

Table 2. Effect of hydrogen peroxide, peracetic acid treatments applied to Red delicious apple trees on infection *Erwinia Amylovora* in apple blossoms. [‡]

	Rate per 100 gal	Timing	strikes per 100 clusters			
Streptomycin standard (Firewall 17) ^{z,y}	28.8 oz	50% bloom, 100% bloom, petal fall	4.6	±	2.7	a
Oxytetracycline standard (Fireline 17) ^{z,y}	24 oz	50% bloom, 100% bloom, petal fall	5.8	±	3.2	a
Organic standard (lime sulfur, Blossom Protect+ Buffer Protect, Previsto)	6 gal 1.24 +8.75 lb 3 qt	LS: 70% bloom BP: 20% bloom, 80% bloom PR: 100% bloom, petal fall	7.8	±	3.1	a
oxytet (Fireline 17) ^{zy} + hydrogen peroxide (26.5%), peracetic acid (4.9%) (Jet Ag)	24 oz + 128 oz	Fireline at: 50% bloom, 100% bloom, PF; Jet Ag at 5, 7, 10, 14 days after full bloom	4.0		2.5	a
oxytet (Fireline 17) ^{zy} + hydrogen peroxide (27%), peracetic acid (5%) (Oxdate T&V)	24 oz + 128 fl oz	Fireline at: 50% bloom, 100% bloom, PF; Oxdate T&V at 5, 7, 10, 14 days after full bloom	4.7	±	1.6	a
Untreated, Inoculated check	----	100% bloom	20.9	±	11.1	b

^y Amended with Regulaid: 32 fl. oz. per 100 gallons. ^z Buffered to 5.6 pH.

[‡] Application dates were: April 21 (pink), April 23 (20% bloom), April 24 and 25 (50% bloom), April 26 (full bloom minus 1 day), April 27 (full bloom), April 28 (full bloom plus 1 day), May 1, 2019 (petal fall), May 2, May 4 and May 6, and May 10, 2019. Inoculation was conducted on the evening of April 27, 2019 at full bloom (of king blooms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1.3 x10⁶ CFU per ml and on May 1, 2019 using live culture prepared at 1x10⁶ CFU ml⁻¹.

Table 3. Russet fruit marking of hydrogen peroxide treatments applied to Red delicious apple trees.

	russet fruit marking, average of 25 fruit on a 1 to 15 scale		
Untreated, Inoculated check	0	±	0
Streptomycin standard (Firewall 17)	0	±	0
Oxytetracycline standard (Fireline 17)	0	±	0
oxytet (Fireline 17) + Oxdate F&V	8.2	±	0.7

Figure 2. Russet fruit marking of Oxdate treatment.

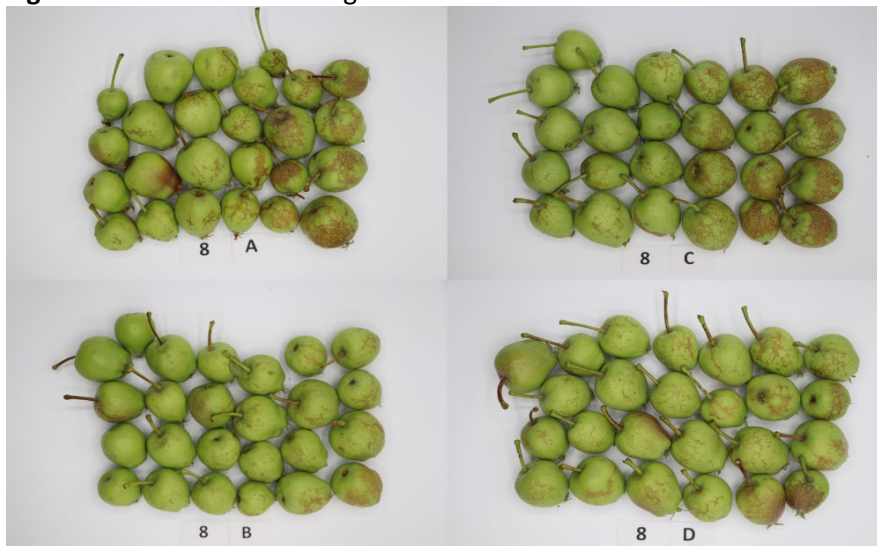


Table 4. Effect of Essential Oil/ Plant Extract Treatments on infection of *Erwinia Amylovora* in apple blossoms. †

Treatment	Rate per 100 gal	Timing	strikes per 100 clusters**
Streptomycin standard (Firewall 17) ^{yz}	28.8 oz	50% bloom, 100% bloom, petal fall	4.6 ± 2.7 a
Oxytetracycline standard (Fireline 17) ^{yz}	24 oz	50% bloom, 100% bloom, petal fall	5.8 ± 3.2 a
Organic standard (lime sulfur, Blossom Protect+ Buffer Protect, Previsto)	6 gal	LS: 70% bloom	6.1 ± 1.2 a
Cueva/ Previsto	1.24+8.75 lb	BP: 20% bloom, 80% bloom	
	3 qt	PR: 100% bloom, petal fall	
Thyme oil (23%) (Thyme Gard 0.5%)	4qt/3qt	day before and day after 100% bloom, petal fall	9.7 ± 2.7 a
	2 qrt	50%, 100% bloom, petal fall, + 4 post petal fall apps	9.2 ± 5.3 a
Untreated, Inoculated check	NA	100% bloom	20.9 ± 11.1 b

^zBuffered to 5.6 pH. ^y Amended with Regulaid: 32 fl. oz. per 100 gallons.

† Application dates were: April 21 (pink), April 23 (20% bloom), April 24 and 25 (50% bloom), April 26 (full bloom minus 1 day), April 27 (full bloom), April 28 (full bloom plus 1 day), May 1, 2019 (petal fall), May 2, May 4 and May 6, and May 10, 2019. Inoculation was conducted on the evening of April 27, 2019 at full bloom (of king blooms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1.3×10^6 CFU per ml and on May 1, 2019 using live culture prepared at 1×10^6 CFU ml⁻¹.

Table 5. Russet fruit marking of essential oil/plant extract treatments applied to Red delicious apple trees.

	russet fruit marking, average of 25 fruit on a 1 to 15 scale		
Untreated, Inoculated check	0 ± 0	a	
Streptomycin standard (Firewall 17)	0 ± 0	a	
Oxytetracycline standard (Fireline 17)	0 ± 0	a	
Organic control	0 ± 0	a	
Thyme oil (23%) (Thyme Gard 0.5%)	4.1 ± 0.9	b	

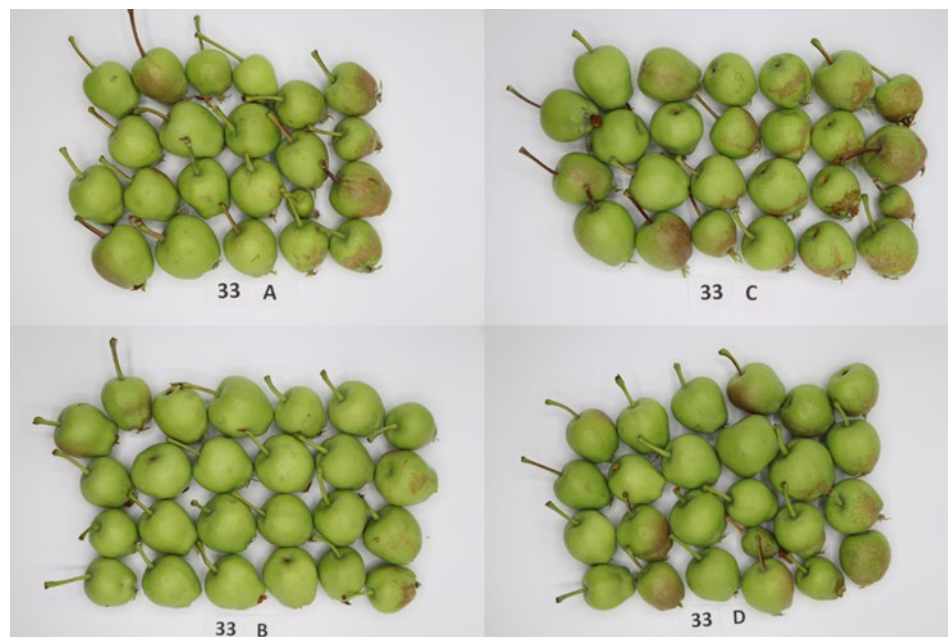
Figure 3. Russet fruit marking of Thyme Gard treatment.

Table 6. Effect of Mineral Product Treatments on *Erwinia Amylovora* infection of apple blossoms.**

Treatment	Rate per 100 gal	Timing	strikes per 100 clusters
Streptomycin standard (Firewall 17) ^y ^z	28.8 oz	50% bloom, 100% bloom, petal fall	4.8 ± 2.8 a
Oxytetracycline standard (Fireline 17) ^y ^z	24 oz	50% bloom, 100% bloom, petal fall	5.7 ± 3.1 a
	6 gal	LS: 70% bloom	
Organic standard (lime sulfur, Blossom Protect+ Buffer Protect, Previsto)	1.24+8.75 lb	BP: 20% bloom, 80% bloom	
	3 qt	PR: 100% bloom, petal fall	6.1 ± 1.1 a
Alum (0.5%)	4 lb	100% bloom, petal fall	8.3 ± 4.7 a
Alum (0.75%)	6 lb	100 % bloom, petal fall	9.0 ± 3.5 a
Alum (1%)	8 lb	100% bloom, petal fall	4.3 ± 2.7 a
Alum (1.25%)	10 lb	100% bloom, petal fall	4.5 ± 2.3 a
Untreated, Inoculated check	NA	100% bloom	21.0 ± 11.1 b

^y Amended with Regulaid: 30 fl. oz. per 100 gallons.^zBuffered to 5.6 pH.

[‡] Application dates were: April 21 (pink), April 23 (20% bloom), April 24 and 25 (50% bloom), April 26 (full bloom minus 1 day), April 27 (full bloom), April 28 (full bloom plus 1 day), May 1, 2019 (petal fall), May 2, May 4 and May 6, and May 10, 2019. Inoculation was conducted on the evening of April 27, 2019 at full bloom (of king blossoms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1.3 x10⁶ CFU per ml and on May 1, 2019 using live culture prepared at 1x10⁶ CFU ml⁻¹.

*No significant fruit marking found for any treatments.

Table 7. Effect of Biological Control Product Treatments on *Erwinia Amylovora* infection of apple blossoms.*

Treatment	Rate per 100 gallons water	Timing	Strikes per 100 clusters
Standard strep (Firewall 17) ^{zy}	28.8 oz	50% bloom, 100% bloom, petal fall	4.6 ± 2.7 a
Standard oxytet (Fireline 17) ^{zy}	24 oz	50% bloom, 100% bloom, petal fall	5.8 ± 3.2 ab
	6 gal	LS: 70% bloom	
Organic standard (lime sulfur, Blossom Protect+ Buffer Protect/ Previsto)	1.24 lb/8.75 lb	BP: 20% bloom, 80% bloom	
	3 qt	PR: 100% bloom, petal fall	6.1 ± 1.1 ab
Cueva/ Previsto	4qt/3qt	day before and day after 100% bloom, petal fall	9.7 ± 2.7 abc
Phage7 (Agriphage) ^y	1 qt	50% bloom, 100% bloom, petal fall	17.3 ± 3.6 bc
Phage7 (Agriphage) +oxytet (Fireline) ^y	1 qt + 0.1 lb	50% bloom, 100% bloom, petal fall	12.4 ± 3.4 abc
Bacillus Subtilis (Aviv)	30 oz	50% bloom, 100% bloom, petal fall	22.5 ± 7.1 c
Bacillus Subtilis QST 713 strain (Serenade)	20 oz	day before and day after 100% bloom, petal fall	16.0 ± 3.2 abc
Untreated, Inoculated Check	water	100% bloom	20.9 ± 11.1 c

^yAmended with Regulaid: 32 fl. oz. per 100 gallons.^zBuffered to 5.6 pH.

[‡] Application dates were: April 21 (pink), April 23 (20% bloom), April 24 and 25 (50% bloom), April 26 (full bloom minus 1 day), April 27 (full bloom), April 28 (full bloom plus 1 day), May 1, 2019 (petal fall), May 2, May 4 and May 6, and May 10, 2019. Inoculation was conducted on the evening of April 27, 2019 at full bloom (of king blossoms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1.3 x10⁶ CFU per ml and on May 1, 2019 using live culture prepared at 1x10⁶ CFU ml⁻¹.

Table 8. Effect of Copper Product Treatments in the control of *Erwinia Amylovora* infection of apple blossoms.*

Treatment	Rate per 100 gallons water	Timing	Strikes per 100 clusters
Standard strep (Firewall 17) ^{zy}	28.8 oz	50% bloom, 100% bloom, petal fall	4.6 ± 2.7 a
Standard oxytet (Fireline 17) ^{zy}	24 oz	50% bloom, 100% bloom, petal fall	5.8 ± 3.2 a
	6 gal	LS: 70% bloom	
Organic standard (lime sulfur, Blossom Protect+ Buffer Protect, Previsto)	1.24 lb+8.75 lb	BP: 20% bloom, 80% bloom	
	3 qt	PR: 100% bloom, petal fall	7.8 ± 3.1 ab
Copper Octanoate (Cueva)		Day before and day after 100% bloom, petal fall	
Metallic copper eq 1.8%	4 qt		11.5 ± 4.1 abcd
Copper hydroxide (Previsto)		Day before and day after 100% bloom, petal fall	
Metallic copper eq 3.3%	3 qt		7.8 ± 3.7 ab

Copper Sulfate Pentahydrate (Mastercop)		Day before and day after 100% bloom,				
Metallic copper eq. 5.4%	2.5 pt	petal fall	9.9	±	2.6	abc
Copper Sulfate Pentahydrate (Instill)		Day before and day after 100% bloom,				
Metallic copper eq. 5.4%	30 oz	petal fall	10.5	±	4.6	abcd
Untreated, Inoculated Check	water	100% bloom	20.9	±	11.1	cde

^y Amended with Regulaid: 32 fl. oz. per 100 gallons.

^zBuffered to 5.6 pH.

[‡]Application dates were: April 21 (pink), April 23 (20% bloom), April 24 and 25 (50% bloom), April 26 (full bloom minus 1 day), April 27 (full bloom), April 28 (full bloom plus 1 day), May 1, 2019 (petal fall), May 2, May 4 and May 6, and May 10, 2019. Inoculation was conducted on the evening of April 27, 2019 at full bloom (of king blooms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1.3 x10⁶ CFU per ml and on May 1, 2019 using live culture prepared at 1x10⁶ CFU ml⁻¹.

Table 9. Russet fruit marking of copper treatments applied to Red delicious apple trees.

russet fruit marking,
average of 25 fruit on a 1 to
15 scale

Untreated, Inoculated check	0	±	0
Streptomycin standard (Firewall 17) ^{z,y}	0	±	0
Oxytetracycline standard (Fireline 17) ^{z,y}	0	±	0
Organic Control	0.04	±	0.04
Cueva (3 qt)	0.11	±	0.06
Previsto (4 qt)	0.04	±	0.03
Mastercop	0.01	±	0.01
Instill	0.04	±	0.03