WSU Tree Fruit Extension

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WSU New Product Efficacy for Control of *Erwinia Amylovora* Blossom Infections 2020

Note: New products without multi-year efficacy data are described by active ingredient. It is not recommended to use products without multi-year positive results.

SUMMARY

- Two applications of hydrogen peroxide (27%), peracetic acid (5%) products at 128 fl oz/ 100 gal resulted in blossom blight infections no different than water controls. Additional applications may be necessary to suppress infections. However, 2019 data showing extensive fruit marking from multiple post petal fall applications suggest that caution should be used with late applications of these products.
- Of plant essential oil/ extract products trialed ET91, an unlabeled experimental product provided control comparable to organic standard (Blossom Protect/Buffer + Previsto). Other plant essential oil/ extract products performed no better than the water-treated check.
- Of mineral products, the experimental copper sulfate product TDA-NC-1 provided control comparable to organic standard (Blossom Protect/Buffer + Previsto). The long-term average for Alum is 80% control, however control was no different than the water-treated check in 2020.
- The biological control phage product provided control no different than the water-treated check in 2020. In 2019 while strikes per 100 clusters were 50% of that from the water-treated check they were highly variable and not statistically different than the water-treated check.
- Acidification with citric acid or phosphoric acid did not significantly increase control from oxytetracycline in this treatment year. Multiple trials in Oregon have shown that acidification can increase efficacy due to suppression of Erwinia Amylovora replication at low pH in the flower and slowed antibiotic product degradation times.

METHODS BLOOM TRIAL

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 Gravely Sandy Loam with a 3-8% slope. The site has good air drainage and some wind protection.

Plots: Five blocks of 24 trees were designated for bloom trials. 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 18, 2020 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml. A 3-gallon backpack sprayer (solo) was used to lightly wet 100+ clusters per plot.

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). Bloom trial products were applied to wet, previously calibrated to equal 100 gal/A.

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2020 application dates were: April 14 (20% bloom), April 16 (50% bloom), April 17 (80% bloom) and April 18 (full bloom), April 19 (full bloom plus 1 day), April 22 (petal fall).

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. An organic standard (Blossom Protect/ Buffer Protect followed by soluble copper) was also included for comparison.

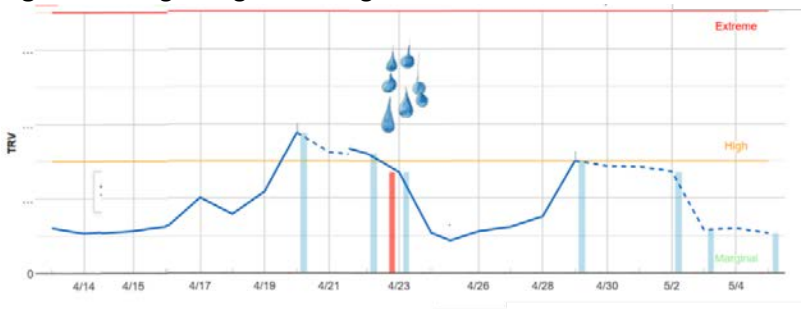
Environmental Conditions: Conditions during product application were as follows. The day of inoculation was overcast during the morning a very small amount of precipitation.

Table 3. Environmental Conditions

DATE	MIN AIR TEMP F	MAX AIR TEMP F	HUMIDITY	LEAF WETNESS	WIND SPEED
14-Apr	47.9	74	25.5	0	4.9
15-Apr	47.1	65.4	38.6	0	5.9
16-Apr	37	65.4	39.5	0	4.4
17-Apr	36	75.4	36.1	0	4.5
18-Apr	51.2	69.1	47.4	0	4.3
19-Apr	46.3	72.8	45.5	0	4.1
20-Apr	42.9	77.3	42	0	4
21-Apr	49.7	70	34.9	0	6.3
22-Apr	49.2	56.3	65.4	0.17	3.1
23-Apr	46.9	67.7	49	0.08	5.4
24-Apr	43.2	69.3	46.4	0	3.4
25-Apr	48.3	68.7	50.7	0	5.2
26-Apr	44	67.6	33.1	0	4.4
27-Apr	49.5	68.5	39.6	0	5.8
28-Apr	42.1	71.9	44.5	0	3.8
29-Apr	50.8	75.1	39	0	3.2
30-Apr	50.7	65.4	36.8	0	4.8
1-May	41.5	70.5	37.6	0	4.5
2-May	48.6	67.5	47.9	0.02	5.8
3-May	42.9	64.3	40.7	0	4.5
4-May	37.8	69.4	40.9	0	3.5
5-May	49.7	77.6	38.4	0	3.5
6-May	49.4	62.7	39.1	0.01	7.4
7-May	40.7	74.7	38.2	0	3.2
8-May	44.5	76.4	41.4	0	4.5

Fire Blight Risk and Pressure: During full bloom fire blight risk was moderate with warming temperatures right after full bloom. Petal fall sprays went on the evening before a significant rainfall event. See output from the Cougar Blight DAS model for full bloom and petal fall (Figure 1).

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 Jun 26, 2020. 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.

Erwinia enumeration: Additionally, *Erwinia Amylovora* was enumerated from flowers collected at full bloom, petal fall and one week after petal fall. Five flower clusters per tree were sampled at each time point. A bulk sample was immersed in sterile water and sonicated for three minutes. After sonication a 10- µl sample of the wash and two 1:100 dilutions will be spread on CCT medium amended with nalidixic acid (50 µg/ml) to selectively enumerate Ea153N.

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

RESULTS

Table 1. Effect of hydrogen peroxide, peracetic acid treatments applied to Red delicious apple trees on infection from *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	2.8	±	1.2	a
Oxytetracycline standard (Fireline 17) [z,y]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2	±	2	b
Organic standard (Blossom Protect/ Buffer Protect + Previsto)	1.24 lb 8.75 lb 3 qt	50% bloom, 80% bloom, 100% bloom, petal fall	9.5	±	1.3	b
hydrogen peroxide (26.5%), peracetic acid (4.9%) (J)	128 fl oz	Day after inoc and 3 days after inoc*	28	±	3.9	c
hydrogen peroxide (27%), peracetic acid (5%) (O)	128 fl oz	Day after inoc and 3 days after inoc	24	±	3.8	c
hydrogen peroxide (27%), peracetic acid (5%) (O)	50 fl oz	Day after inoc and 3 days after inoc	28	±	4.1	c
Untreated check	----	100% bloom, +1 day, petal fall	31	±	7.1	c

** Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown.

[y] Amended with Regulaid: 30 fl. oz. per 100 gallons.

[z] Buffered to 5.6 pH.

* Note inoculation is done at dusk. Day after spray is done early morning next day. 3 days after inoculation coincides with petal fall sprays.

[‡]Application dates were: April 15, pink, April 19 (20% bloom), April 21 (50% bloom), April 23 (full bloom), April 24 (full bloom plus 1 day), April 28 (petal fall). Inoculation was conducted on the evening of April 23, 2020 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 x10^6 CFU per ml.

Figure 1. Effect of treatments applied to Red delicious apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers at Full Bloom (FB), Petal Fall (PF) and Petal Fall + 1 week (PF+1).

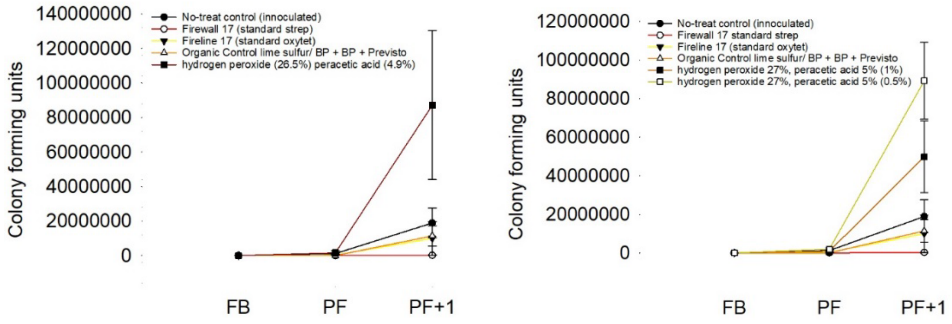


Table 2. Effect of Essential Oil/ Plant Extract Treatments on infection of *Erwinia Amylovora* in apple blossoms. [‡]

Treatment	Rate per 100 gal	Timing	strikes per 100 clusters	
Streptomycin standard (Firewall 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8 ± 1.2	a
Oxytetracycline standard [y] (Fireline 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2 ± 2	b
Organic Standard	1.24 lb			
(Blossom Protect/Buffer)	8.75 lb	50% bloom, 80% bloom,		
+ Soluble Copper (Previsto)	3 qt	100% bloom, petal fall	9.5 ± 1.3	bc
Thyme oil (23%) (TG)	2 qrt	80% bloom, 100% bloom +1 day, petal fall	17 ± 2.3	cd
Thymol (23%) (TX)	2 qrt	80% bloom, 100% bloom, petal fall	22 3.5	d
Cinnamon oil (60%) (C)	1 qt	50% bloom, morning after inoc, petal fall	19 ± 3.5	d
TS28	21.9 ml	100% bloom, +1 day, petal fall	23 ± 5.5	cd
TS108	25 ml	100% bloom, +1 day, petal fall	31 ± 5.8	d
ET91	38.4 oz	100% bloom, +1 day, petal fall	10 ± 6.6	b
Lupine[u] (P)	40 oz	50% bloom, morning after inoc, petal fall	22.6 ± 4.1	cd
Water-treated check	NA	100% bloom, +1 day, petal fall	31 ± 7.1	d

[y] Amended with Regulaid: 30 fl. oz. per 100 gallons.

[z] Buffered to 5.6 pH.

[‡] Application dates were: April 14 (20% bloom), April 16 (50% bloom), April 17 (80% bloom) and April 18 (full bloom), April 19 (full bloom plus 1 day), April 22 (petal fall). Inoculation was conducted on the evening of April 18, 2020 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml.

[u] Banda de Lupinus albus doce (20%).

Figure 2. Effect of essential oil/ plant extract treatments applied to Red delicious apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers at full bloom (FB), petal fall (PF) and petal fall + 1 week (PF+1).

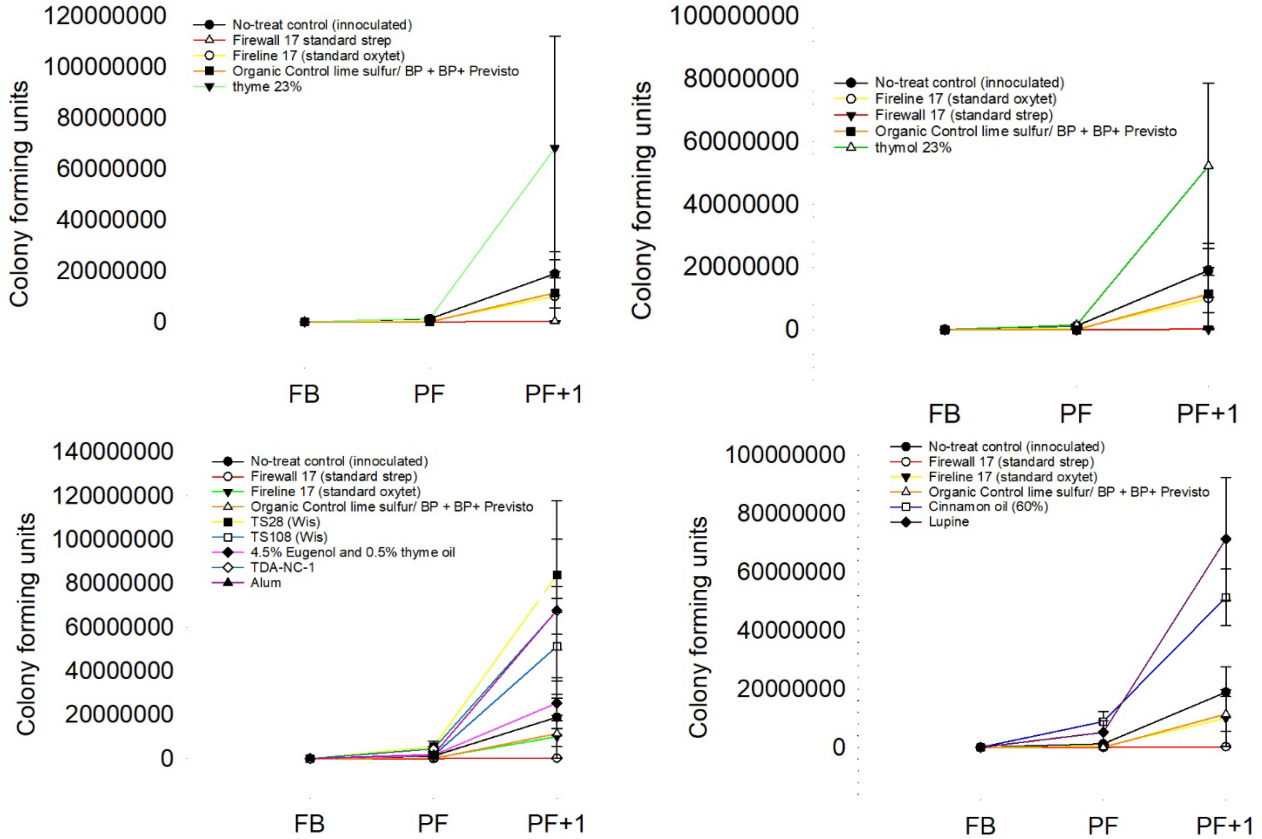


Table 3. Effect of Mineral Product Treatments on *Erwinia Amylovora* infection of apple blossoms.[‡]

Treatment	Rate per 100 gal	Timing	strikes per 100 clusters			
Streptomycin standard (Firewall 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8	±	1.2	a
Oxytetracycline standard [y] (Fireline 17) [yz]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2	±	2	b
Organic Standard	1.24 lb					
Blossom Protect/Buffer	8.75 lb	50% bloom, 80% bloom, 100% bloom, petal				
+ Soluble Copper (Previsto)	3 qt	fall	9.5	±	1.3	bc
Alum[y]	8 lb	100% bloom, petal fall	22	±	4.2	d
TDA-NC-1[x]	17.1 g	Tight cluster, 50% bloom, 100% bloom + 1 day, petal fall	13	±	2.3	bc
Water-treated check	NA	100% bloom, +1 day, petal fall	31	±	7.1	d

[y] Amended with Regulaid: 30 fl. oz. per 100 gallons.

[z] Buffered to 5.6 pH.

[x] Amended with Silwet oil at 0.0125%. Copper sulfate product.

[‡] Application dates were: April 14 (20% bloom), April 16 (50% bloom), April 17 (80% bloom) and April 18 (full bloom), April 19 (full bloom plus 1 day), April 22 (petal fall). Inoculation was conducted on the evening of April 18, 2020 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml.

Table 4. Effect of Biological Control Product Treatments on *Erwinia Amylovora* infection of apple blossoms.[‡]

Treatment	Rate per 100 gallons water	Timing	Strikes per 100 clusters**			
Untreated, Inoculated Check	water	100% bloom, +1 day, petal fall	31	±	7.1	c
Streptomycin standard (Firewall 17)[zy]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8	±	1.2	a
Oxytetracycline standard (Fireline 17) [zy]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2	±	2.0	b
Organic standard (Blossom Protect/Buffer Protect +Previsto)	1.24 lb 8.75 lb 3 qt	50% bloom, 80% bloom, 100% bloom, petal fall	9.5	±	1.3	b
Phage7 (Agriphage)	2 qt	100% bloom 12hr before ap, +1 day, +3 days	24	±	4.8	c
Phage7 (Agriphage +Surround)	2 qt + 0.1 lb	100% bloom 12hr before ap, +1 day, +3 days	31	±	3.7	c

** Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown.

[y] Amended with Regulaid: 30 fl. oz. per 100 gallons.

[z] Buffered to 5.6 pH.

[‡] Application dates were: April 14 (20% bloom), April 16 (50% bloom), April 17 (80% bloom) and April 18 (full bloom), April 19 (full bloom plus 1 day), April 22 (petal fall). Inoculation was conducted on the evening of April 18, 2020 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml.

Figure 3. Effect of Biological Control treatments applied to Red delicious apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers.

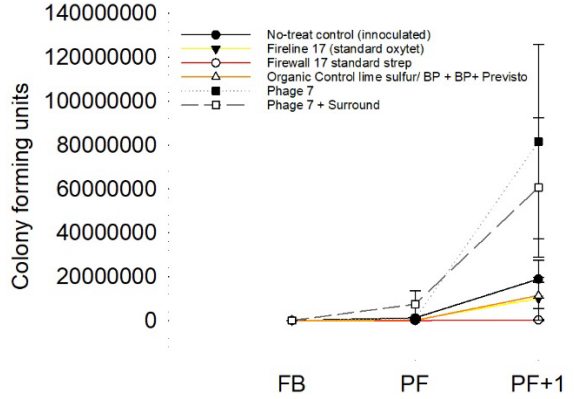


Table 5. Effect of acidification on antibiotic performance in the control of *Erwinia Amylovora* infection of apple blossoms.[‡]

Treatment	Rate per 100 gallons water	*pH*	Timing	Strikes per 100 clusters**			
Untreated, Inoculated Check	water		100% bloom, +1 day, petal fall	31	±	7.1	b
Oxytetracycline (Mycoshield) + Buffer Protect[y]	24 oz 4.3 lb	3.8	50% bloom, 100% bloom, petal fall	9.6	±	2.6	a
Oxytetracycline (Mycoshield) no acidifier[y]	24 oz	7.5	50% bloom, 100% bloom, petal fall	12	±	2.8	a
Oxytetracycline (Mycoshield) acidified to 3.8 (Tech Mg[u]) [y]	24 oz 21 oz	3.8	50% bloom, 100% bloom, petal fall	11	±	4	a
Oxytetracycline (Mycoshield) acidified to 5.6 (Tech Mg[u]) [y]	24 oz 15 ml	5.6	50% bloom, 100% bloom, petal fall	9.2	±	4.1	a

[y] Amended with Regulaid: 30 fl. oz. per 100 gallons.

[u] phosphoric acid

** Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown.

[‡]Application dates were: April 14 (20% bloom), April 16 (50% bloom), April 17 (80% bloom) and April 18 (full bloom), April 19 (full bloom plus 1 day), April 22 (petal fall). Inoculation was conducted on the evening of April 18, 2020 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml.

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