FINAL PROJECT REPORT **YEAR**: 3 of 3

Project Title: Refinement of practical fire blight control: Buffered oxytetracycline

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Budget

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Item	**2018-19**	**2019-20**	**2020-21**
Salaries Faculty Res. Assist. 2 mo	9,908	10,106	No-cost
Benefits OPE 61%	6,044	6,165	
Undergraduate labor (&OPE 12%)	1,000	1,020	
Equipment	0	0	
Materials and Supplies	750	765	
Local Travel	750	765	
Plot Fees	750	765	
Medford russet trials	5,000	5,100	
Total	**$24,202**	**$24,686**	

OBJECTIVES:

1) Evaluate rate of pH-buffering on oxytetracycline-mediated suppression of fire blight pathogen populations on flowers and incidence of fire blight infection (Corvallis).
2) Evaluate effect of pH-buffering on finish quality of Comice and Bartlett pear fruit (Medford).
3) Evaluate if oxytetracycline formulation ('-hydrochloride' (FireLine) or '-calcium complex' (Mycoshield)) influences the pH-buffering enhancement of oxytetracycline.

SIGNIFICANT FINDINGS:

- In both apple and pear, a lowered pH improved the efficacy of oxytetracycline for fire blight suppression
- Pathogen populations on flowers were suppressed to a greater degree with acidified oxytetracycline compared non-acidified oxytetracycline.
- pH-adjustments of oxytetracycline caused negligible to slight effects to fruit finish quality (russeting).
- A prolonged inhibitory residual is the likely reason that fire blight control is improved by acidification of oxytetracycline.
- In more limited trialing, acidifying an alternative antibiotic, kasugamycin, also improved fire blight control.

RESULTS:

Summary of orchard trials. Fire blight suppression trials (8 total) were arranged in randomized complete block designs of 4 to 12 treatments applied to single-tree plots replicated four times. The number of flower clusters on individual trees were counted prebloom and this count as well as tree location were considered in the assignment of trees to blocks in the plot design. Experimental protocols were similar among trials with the fire blight pathogen inoculated onto the trees near full bloom followed by sprayed treatments at full bloom and prior to petal fall. Trial-specific dates of inoculation and sprayed treatments are summarized in Table 1.

Table 1. Mean number of flower clusters per tree and dates of experimental actions related to evaluation of acidified oxytetracycline treatments for fire blight control in experimental pear and apple orchards located near Corvallis, OR.

				Date of experimental action			
Year	Orchard	Cultivar	Mean flower clusters per tree [x]	Pathogen [y] inoculation	Full bloom treatment	Treatment prior to petal fall	Post-petal fall sample [z]
2017	Pear	Bartlett	171	18 April	20 April	22 April	1 May
	Apple	Golden Delicious	399	28 April	29 April	3 May	10 May
2018	Pear	Bartlett	1012	12 April	14 April	20 April	27 April
	Apple	Gala	375	24 April	25 April	1 May	8 May
2019	Pear	Bartlett	156	18 April	20 April	26 April	2 May
	Apple	Gala	218	24 April	26 April	2 May	9 May
2020	Pear	Bartlett	455	8 April	10 April	14 April	21 April
	Apple	Gala	431	15 April	17 April	21 April	28 April

[x] Mean number of flower clusters per replicate tree. Each treatment was replicated four times. [y] The pathogen, *E. amylovora* stain Ea153N, was misted onto the trees (1 x 10^6 CFU/ml) 12 to 36 h prior to the full bloom treatment. [z] Flower clusters were sampled to measure epiphytic *E. amylovora* populations on the day after the 'full bloom' and 'prior to petal fall' treatments, and at 6 to 9 days after petal fall.

Objective 1:

Infection suppression. As a result of pathogen inoculation, fire blight developed in the water-treated control trees of each trial with infection incidences ranging from 6% (pear 2018) to 73% (apple 2018) of total flower clusters; among the eight trials, mean incidence of infection for the water control was 26% (Table 2). The acidifier controls, Buffer Protect or citric acid only, reduced fire blight incidence significantly ($P \leq 0.05$) in five of eight trials, but averaged over trials, this reduction in infection incidence averaged only 15% (Fig. 1).

In all eight orchard trials, relative to the water-treated control, treatment of pear or apple with a non-acidified antibiotic resulted in a significant reduction ($P \leq 0.05$) of fire blight incidence (Table 2). Moreover, also relative to the water-treated control, acidified antibiotics always suppressed fire blight significantly ($P \leq 0.05$). Within-trials, the differences in fire blight suppression by acidified oxytetracycline (OTC) compared to OTC by itself was frequently smaller than least significant difference for the trial, and therefore, many of the direct comparisons of acidified to non-acidified materials did not differ significantly ($P > 0.05$). Nonetheless, for 26 of 30 within-trial comparisons, acidified OTC showed better suppression than OTC only.

Averaged across orchard trials, relative infection suppression from Mycoshield (OTC-calcium complex) was improved from a mean of 57% (± (standard error) 13.5) without acidification to 80% (± 4.9%) when amended with citric acid or Buffer Protect (Fig. 1). Acidified FireLine (OTC-hydrochloride formulation), in contrast, was improved to a lesser degree with a relative infection suppression of 88.8% ± 1.7 compared to 79.7% ± 3.7 for the antibiotic only. In more limited trialing, acidifying kasugamycin (Kasumin 2L) increased relative infection suppression to 94% (± 2.7%) compared to 83% (± 5.7%) for the non-acidified kasugamycin.

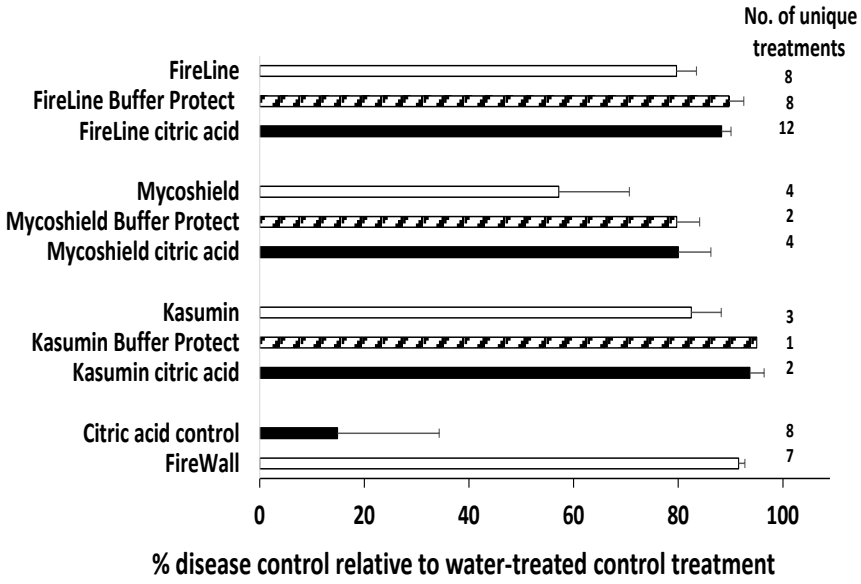

Fig. 1. Effect of acidified oxytetrcycline materials for fire blight control in Bartlett pear and Golden Delicious or Gala apple expressed as 'percent infection suppression relative to the water-treated control'. Data are from eight orchard trials conducted from 2017 to 2020 and depict the mean and standard error from the number of unique treatments within each material subheading on the y-axis. Each unique treatment was replicated on four trees in each trial. For the 'FireLine citric acid' and Mycoshield citric acid' treatments, the amount of citric acid in the unique treatments ranged among trials from 16 to 32 oz./100 gal and amount of Na_2HPO_4 raged between 0 and 16 oz./100 gal (see Table 2). The citric acid control was Buffer Protect in 2017, and citric acid with Na_2HPO_4 (2018), K_2HPO_4 (2019), or no buffer amendment (2020). Rates of antibiotic materials were held constant among trials and are shown in Table 2.

Table 2. Incidence of fire blight on pear and apple flower clusters as affected by oxytetracycline and various materials used to acidify the sprays applied the trees in eight orchard trials [t] conducted near Corvallis, Oregon from 2017 to 2020.

Treatment	Rate [u] oz./100 gal	Incidence (%) [v] 2017 Pear		2017 Apple		2018 Pear		2018 Apple		2019 Pear		2019 Apple		2020 Pear		2020 Apple	
Water control		**6.0** (13)	**a** [w]	**13.6** (62)	**a**	**44.0** (440)	**a**	**73.4** (223)	**a**	**8.5** (15)	**ab**	**15.0** (30)	**a**	**14.5** (67)	**a**	**30.7** (126)	**a**
Buffer Protect	75	4.9	b	15.8	a	---		---		---		---		---		---	
Citric acid buffer [x]	16 8	--- [y]		---		23.9	b	15.1	b	16.5	a	8.0	b	16.4	a	14.6	b
FireWall	8	0.7	c	---		5.5	d	3.3	c	0.8	e	1.4	c	1.0	e	1.5	cd
FireWall Citric acid	8 16	---		---		---		---		---		---		1.0	e	1.1	d
FireLine	16	1.6	c	1.4	b	10.9	c	11.1	b	3.6	cd	1.5	c	2.9	de	4.1	c
FireLine plus Buffer Protect	16 75	0.3	c	0.8	b	6.4	d	2.2	c	1.8	de	1.8	c	---		---	
FireLine Citric acid	16 8	---		---		---		---		---		---		2.3	e	4.2	c
FireLine Citric acid	16 16	---		---		---		---		1.5	de	1.6	c	1.6	e	1.3	cd
FireLine Citric acid $Na2HpO_4$	16 32 16	---		---		5.3	d	2.3	c	---		---		---		---	
FireLine Citric acid $Na2HpO_4$	16 16 8	---		---		8.9	c	3.9	c	---		---		---		---	
FireLine Citric acid $Na2HpO_4$	16 12 12	---		---		9.6	c	3.9	c	---		---		---		---	
FireLine LI70	16 64 [z]	---		---		---		---		2.5	cde	1.2	c	2.1	e	2.1	cd
FireLine TRIFOL	16 64 [z]	---		---		---		---		2.6	cde	1.6	c	---		---	
Mycoshield	16	---		---		18.4	b	---		6.3	bc	---		6.8	bcd	2.6	cd
Mycoshield Buffer Protect	16 75	---		---		7.0	cd	---		2.1	de	---		---			
Mycoshield Citric acid	16 16	---		---		---		---		3.0	cde	---		3.3	de	1.7	cd
Mycoshield Citric acid $Na2HpO_4$	16 16 8	---		---		7.2	cd	---		---	cde	---		---			
Kasumin	64 [z]	0.2	c	---		---		---		---		---		2.7	de	2.2	cd
Kasumin Buffer Protect	64 [z] 75	0.3	c	---		---		---		---		---		---			
Kasumin Citric acid	64 [z] 16	---		---		---		---		---		---		1.3	e	1.1	d

[t] Single-tree plots were arranged in a complete randomized block design with four replications per treatment. All treatments applied twice except FireWall, which was applied once at full bloom. Dates of pathogen inoculation and treatment applications are provided in Table 1.

[u] Approximated an orchard spray volume of 100 gallons per acre.

[v] Infected flower clusters divided by total number of clusters per tree. Proportional incidence data transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown.

[w] Means within a column followed by same letter do not differ significantly ($P = 0.05$) based on Fischer's protected least significance difference.

[x] Na_2HPO_4 in 2018, K_2HPO_4 in 2019, and no buffer amendment in 2020.

[y] '---' indicates treatment was not included in that specific experiment.

[z] Fluid ounces per 100 gallons.

Pathogen populations in flowers. Inoculation of the pear and apple trees near full bloom with the fire blight pathogen resulted in measureable populations of *E. amylovora* for all sampling dates of all trials. Across trials, the full bloom sample from water-treated trees averaged 4.0 $\log_{10}$ (CFU/flower) with mean population size increasing to > 5.0 $\log_{10}$ (CFU/flower) at petal fall and exceeding 6.0 $\log_{10}$ (CFU/flower) at the post-petal fall sample (Fig. 2A).

Over all trials, relative to the water control, FireLine reduced the measured *E. amylovora* population size by 2.0 to 2.5 log units through petal fall, with this difference decreasing to one log unit by the post-petal sample (Fig. 2A). Also relative to the water treatment, acidifying FireLine increased the magnitude of the population reduction at all sampling dates with the greatest differences occurring at petal fall (3 log units). Similarly, relative to the water control, Mycoshield reduced *E. amylovora* population size by 1.5 to 2 log units from full bloom to petal fall (Fig. 2B). For the petal fall sampling date, the addition of Buffer Protect or citric acid to Mycoshield further reduced the pathogen's population size by an additional 1 to 1.5 log units compared to the suppression obtained by Mycoshield only.

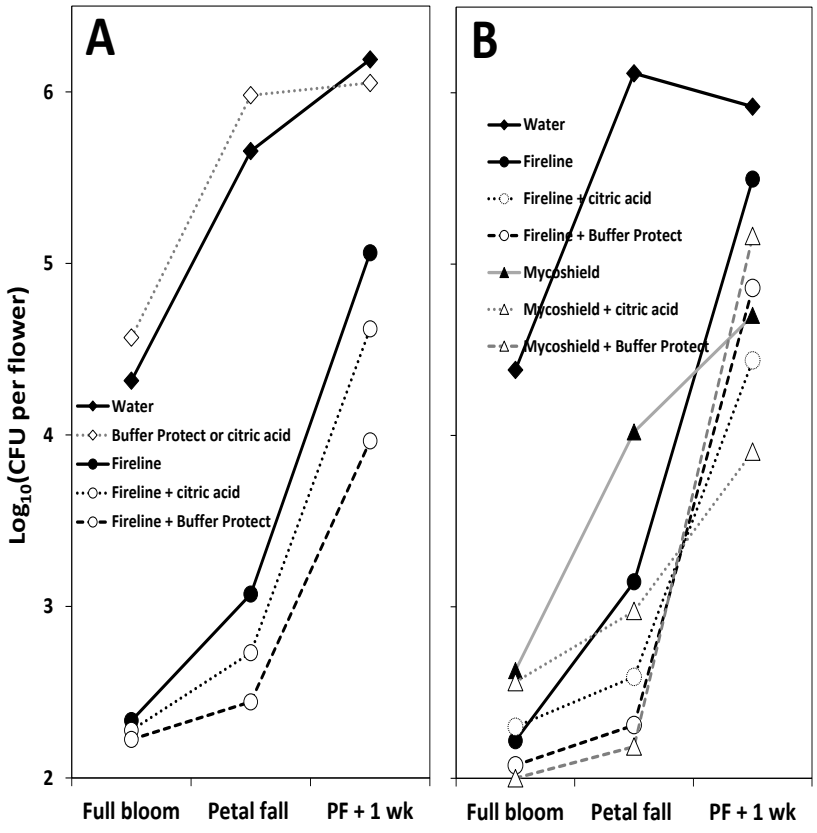

Fig. 2. Effect of acidified oxytetracycline materials on epiphytic populations of *Erwinia amylovora* on Bartlett pear and Gala apple flowers sampled from research orchards located near Corvallis, OR. A) Data depict across-trial means from up to eight pear and apple trials conducted from 2017 to 2020 (see Table 1). B) Data depict across trial means from three pear trials and one apple trial conducted from 2018 to 2020. Each treatment was applied to four replicate trees in each trial. Pathogen populations were determined by washing five flower clusters (~25 flowers, bulked) from each replicate tree, and plating the wash onto a selective culture medium. The Y-axis is on log scale for which a value of '2.0' is 100 pathogen CFU/flower (the detection limit) and a value of '6.0' is one million CFU per flower.

Pathogen population size data were further summarized with the statistic 'relative area under the population size curve' (A_{pop}), which represents the average population size ($\log_{10}$ (CFU/flower)) from full bloom to one week past petal fall weighted for length of time between each sampling date:

$$A_{pop} = \sum_{i=1}^{\eta - 1} \{[(y_i + y_{i+1})/2] \bullet (t_{i+1} - t_i)\}/(t_n - t_1)$$

where y is $\log_{10}$ (CFU/flower) for a sample and t is days after inoculation for the ith sample date, and n is the total number of sample dates. Variation in A_{pop} was summarized utilizing individual trials as

replicates with means of common treatments across trials as the summary statistic. These means characterized the impact of treatments on the pathogen's epiphytic populations on flowers (Table 3). For example, citric acid alone had an overall A_{pop} of 5.71 $\log_{10}$ (CFU/flower), which was slightly greater than the water-treated control. In contrast, streptomycin, which was applied to trees only at full bloom, had an A_{pop} of 2.67 $\log_{10}$ (CFU/flower).

A_{pop}-values for OTC formulations or kasugamycin (Kasumin) by themselves were in the range of 3.13 to 3.89 $\log_{10}$ (CFU/flower) (Table 3). The addition of an acidifying amendment to either OTC formulation or kasugamycin reduced A_{pop} compared to the antibiotic only. The smallest changes in A_{pop} attributable to an acidifier was observed when citric acid or LI-700 was added to FireLine (0.13 to 0.16 log units), and the largest reduction occurred when Buffer Protect was added to Mycoshield (0.70 log units).

Table 3. Values of relative area under the population size curve, A_{pop}, for epiphytic populations of *Erwinia amylovora* measured on pear and apple flowers at stages of full bloom, petal fall, and 1-wk post-petal fall as affected by antibiotic sprays and the materials used to acidify the sprays in orchard trials [t] conducted near Corvallis, Oregon from 2017 to 2020.

Treatment	Number of times treatment was represented over the eight orchard trials	Mean A_{pop} value [u]	Standard error of A_{pop}
Water	8	5.57	0.33
Citric acid [v]	6	5.62	0.18
FireWall	7	2.67	0.14
FireLine	8	3.26	0.18
" with Buffer Protect	6	2.77	0.21
" with citric acid [w]	10	3.10	0.15
" with LI 700	4	3.13	0.30
Mycoshield	4	3.89	0.53
" with acidifier [x]	4	3.09	0.30
Oxytetracycline [y]	12	3.47	0.22
" with acidifier	24	2.96	0.10
Kasumin	3	3.13	0.35
" with acidifier [z]	3	2.43	0.06

[t] In each trial, single-tree plots were arranged in a complete randomized block design with four replications per treatment. All materials applied twice except FireWall, which was applied once at full bloom. Dates of pathogen inoculation and treatment applications are provided in Table 1. Rates of materials are provided in Table 2.

[u] Mean and standard error of $A_{pop\ values}$ obtained by averaging the number of times a treatment was represented over the eight orchard trials.

[v] Includes treatments of citric acid only (2019 and 2020) and those where the antibiotic was amended with citric acid plus Na_2HSO_4 at a ratio of 2:1 (2018).

[w] Includes FireLine treatments amended with citric acid only (2019 and 2020) and those where the antibiotic was amended with citric acid plus Na_2HSO_4 at a ratio of 2:1 (2018).

[x] Includes Mycoshield treatments amended with Buffer Protect (2018 and 2019) or citric acid (2020).

[y] Includes all FireLine and Mycoshield treatments; following line includes any OTC-treatment amended with an acidifier.

[z] Includes Kasumin treatments amended with Buffer Protect (2017) or citric acid (2020).

pH of sprayed materials and floral pH as a result of treatment. Well water (pH 6.3) amended with citric acid (16 oz./100 gal) had a pH of 3.0 (Table 4). The addition of di-sodium phosphate or di-potassium phosphate (8 to 16 oz./100 gal) to citric acid (12 to 32 oz./100 gal) raised the pH to a range of 3.3 to 4.6. The commercial formulations of OTC in well water had pH-values closer to neutral (pH 6.1 to 6.6). OTC formulations with citric acid (± phosphate) had pH-values between 3.0 and 4.0. Also in well water, the pH of commercial acidifying surfactants, LI 700 (5 ml/liter) and TRI-FOL (5 ml/liter), measured 3.6 and 2.6, respectively.

Among orchard trials, the pH of the floral wash for the well water-treated control and antibiotic only treatments averaged between 5.9 and 6.0 (± (s.e.) 0.01) and declined slightly through the bloom period to 5.7 (± 0.12) (Fig. 3). In contrast, near full bloom, the pH of flower clusters treated with a citric acid or the citric acid-based Buffer Protect, or with an OTC-formulation mixed with citric acid had pH-values that averaged between 5.7 and 5.8. At petal fall, floral pH for treatments that included citric acid declined to a range of 5.2 to 5.5, but then increased to 5.6 (± 0.04) at 7 days after petal fall. Within individual orchard trials, variation in pH measurements were influenced partly by tree species with apple flowers becoming more acidic over time (e.g. water-treated apple flowers decreased to 5.5 to 5.6 at one week post-petal fall whereas water-treated pear flowers averaged 5.9). In addition, pH measurement for trials with the less rain during bloom were 0.1 to 0.3 units lower than those with more rain (data not shown).

Table 4. pH of well water, commercial oxytetracycline formulations and materials used to acidify antibiotic sprays for fire blight control.

Treatment	Acidifying amendment	Rate oz./100 gal.	pH [v]
Well water		-	6.7 ± 0.1
+	Buffer Protect	75	3.7 ± 0.0
+	citric acid	16	2.9 ± 0.1
+	citric acid plus $Na2HpO_4$	32, 16	3.4 ± 0.1[w]
+	citric acid plus $Na2HpO_4$	16, 8	3.5 ± 0.1
+	citric acid plus $Na2HpO_4$	12, 1	4.6 ± 0.0
+	citric acid plus K_2HPO_4	16, 8	4.1 ± 0.3[w]
+	LI-700		3.6 ± 0.1[w]
+	TRI-FOL		2.6 ± 0.0[w]
+ FireLine [y]		16	6.6 ± 0.1
+	Buffer Protect	75	3.6 ± 0.2
+	citric acid	15	3.2 ± 0.1
+	LI-700		3.5 [x]
+ Mycoshield [z]		16	6.1 ± 0.4
+	Buffer Protect	75	3.9 ± 0.2
+	citric acid	16	3.5 ± 0.2
+ Kasumin		64 fl. oz.	6.5 [x]
+	citric acid	16	2.8 [x]

[v] Means of measurement taken in springs of 2018 to 2020.
[w] Not measured in 2018.
[x] Measured in 2020 only
[y] '-hydrochloride' formulation
[z] '-calcium complex' formulation

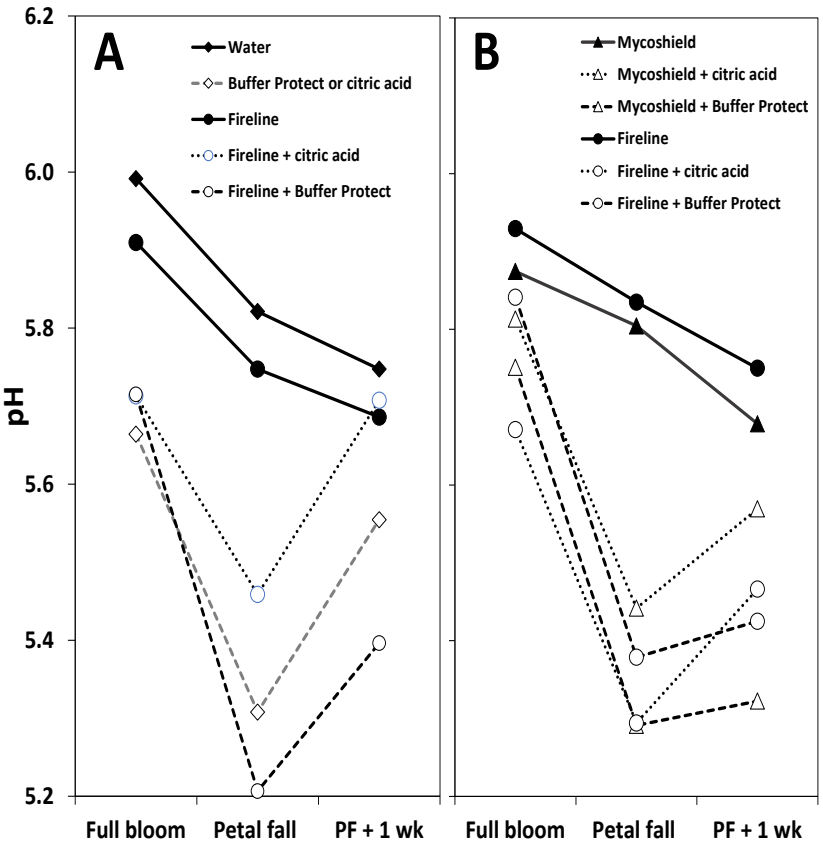

Fig. 13. Measured pH of washed Bartlett pear and Gala apple flowers after treatment with acidified oxytetrcycline in research orchards located near Corvallis, OR. Data depict across-trial means from up to six pear and apple trials conducted from 2018 to 2020 (see Table 1). At the bloom stages indicated, a pH-probe was placed in a deionized-water wash of five flower clusters (~25 flowers, bulked in 25 ml of water) from each replicate tree in each trial. A), data are from three pear trial and three apple trials conducted from 2018 to 2020. B), data are from three pear trials and one apple trial conducted from 2018 to 2020. Because panel B is mostly pear trials, the pH values for FireLine treatments are slightly higher than for comparative treatments in panel A where pear and apple trials are equally represented.

Treatment effects on fruit russeting. For both Bartlett and Comice pear, application of citric acid-based acidifying treatments at full bloom and petal fall resulted in negligible to slight effects on percent russeting of fruit surfaces (Fig. 2). For Bartlett pear, the mean difference between the most russeted treatments and the least russeted treatments was < 0.7%; for Comice pear, this difference was < 1%. Statistically, significant effects of treatment on percent fruit russeting resulted only for Bartlett pear in 2018 (Fig. 2A). Treatments with significantly ($P \leq 0.05$) greater russeting than the water-treated control included 'Buffer Protect', 'citric acid (32 oz./100 gal) plus disodium phosphate (16 oz./100 gal)', and 'citric acid (32 oz./100 gal) plus disodium phosphate (8 oz./100 gal)'. The same trends of treatment effects observed in Bartlett pear 2018 were also observed in Comice pear 2018 and Bartlett pear 2019 with the highest rates of citric acid combined with di-sodium phosphate (2018) or with di-potassium phosphate (2019) showing slightly more fruit russeting (up to 0.9%) than the water-treated control. In contrast, 'citric acid only', which had a relatively low pH among the sprayed treatments (Table 2), had the lowest percent fruit russeting in three of the four trials ((Fig. 2B-D); for the fourth trial (Bartlett pear 2018), citric acid only was the treatment most similar to the water-treated control (Fig. 2A).

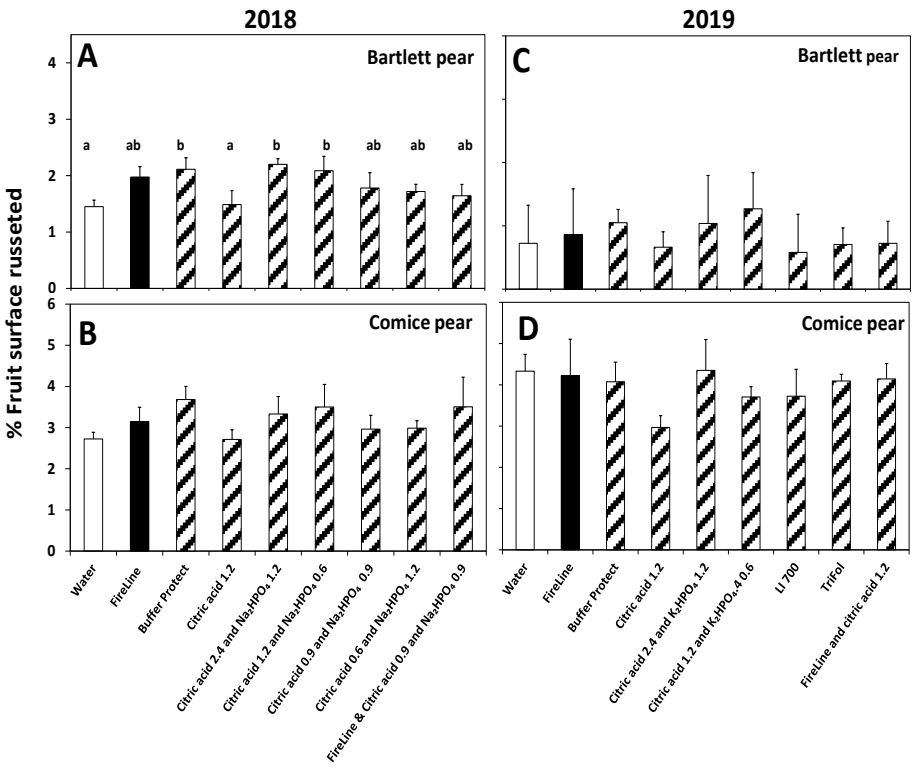

Fig. 4. Effect of citric acid-based buffers and oxytetracycline applied to A, C) Bartlett and B, D) Comice pear trees on severity of fruit russeting (%) in research orchards located near Medford, OR. Treatments were applied at full bloom and at petal fall (April 2018 and 2019). In late August, 30 fruit from each replicate tree were rated for russeting severity. Data depict mean and standard error from four replicate trees that received each treatment. X-axis: numbers in treatment labels indicate the rate of citric acid or phosphate buffer in grams per liter (Conversions: 1.2 g/l = 16 oz per 100 gal., 0.6 g/l = 8 oz per 100 gal., and 0.9 g/l = 12 oz per 100 gal.). Rates of other materials are shown in Table 2. In panel A, bars labeled with same letter are not significantly different according to Fischer's protected LSD at $P = 0.05$; in the other panels, differences among treatments did not differ significantly ($P > 0.05$).

Discussion

Antibiotics are regarded highly in conventional fire blight control because of their ability to suppress the pathogen's rate of growth on flowers and to protect flowers from infection. They also provide a longer-term benefit of reducing the amount of epiphytic inoculum available for secondary infection phases of the disease (e.g., late or secondary flowers and new vegetative shoots). In achieving these goals, acidifying oxytetracycline (and kasugamycin) enhanced infection suppression, although the observed enhancement was incremental to the degree of control achieved by the antibiotic alone. Nonetheless, we view the incremental improvement in suppression achieved with acidifiers as valuable to fire blight control as it is inexpensive and easy to implement. Moreover, because secondary phases of fire blight can be both very damaging to trees and time-consuming to clean-up, excellent infection suppression during primary bloom is considered by orchardists to be vastly superior to only good/very good infection suppression. At a minimum, the data generated by this research should result in closer monitoring of pH of antibiotics in spray tanks, and consequently, potentially improve the quality of sprays used for fire blight management.

As to why more acidic conditions enhances the efficacy of oxytetracycline on pome flowers, we hypothesized that the stability of OTC in a spray tank might be improved or the effective residual of OTC on floral surfaces could be prolonged. Although we did not measure OTC residuals directly, pathogen populations on flowers treated with acidified OTC increased more slowly than on flowers treated with OTC only (Fig. 3), which we believe reflects a prolonged half-life as a result of the more acidic conditions. In general, pear and apple flowers are not susceptible to infection after petal fall (Thomson, 2000). Therefore, on acidified OTC-treated flowers, the smaller pathogen population sizes

observed as the primary bloom period approached petal fall is likely where the benefits of acidification occur; this also was the bloom stage where we measured the lowest floral pH. The rapid increase in pathogen populations after petal fall represents inoculum that has mostly missed the window of susceptibility offered by individual flowers. Nonetheless, this inoculum may be available for later phases of infection. In this regard, we were disappointed that acidifying OTC did not extend a suppressive residual farther into the post-petal fall period.

Various researchers (McManus and Stockwell, McManus and Jones (1994), Stockwell et al. (1996a), and Stockwell et al. (2008)) have characterized oxytetracycline for fire blight suppression as being "bacteriostatic", meaning that the antibiotic slows the rate of pathogen reproduction but does not kill existing cells. To a degree, our data refutes this characterization because shortly after inoculation pathogen populations on OTC-treated flowers were so much smaller than on water-treated flowers. Our view is that OTC is best understood in terms of its effective residual (half-life), which in addition to pH sensitivity is a concept that becomes a more focused rationale for additional investigations (e.g., Christiano et al., 2010). An increased half-life as a result of a pH-adjustment appeared to be independent of OTC formulation, although, in more limited trialing, the calcium-complex formulation (Mycoshield) benefited more from a lower pH than the hydrochloride formulation (FireLine). In contrast, our hypothesis that acidification of spray tank-water (without OTC) could affect *E. amylovora* directly was not well supported by the data. This was shown by poor infection suppression with either citric acid or Buffer Protect by themselves. Because *E. amylovora* cannot grow at pH < 5, a possible explanation for ineffectiveness of citric acid by itself is that on a micro-scale, the floral surfaces on which a lower pH was achieved differs from surfaces where *E. amylovora* populations increase epiphytically (Wilson et al., 1999).

With regard to fruit russeting, moderately-sensitive Bartlett pear and highly-sensitive Comice pear provided an indication of the relative safety of a pH-lowering adjustment to the spray suspension. Surprisingly, for russeting-sensitive Comice pear, no significant effects of the treatments were observed, but in 2018 a few treatments slightly increased fruit russeting on Bartlett pear. Across all the russeting trials, compared to the low pH treatment of citric acid by itself, those with higher amounts of buffering salts in the spray suspension also showed slightly higher amounts of fruit russeting. This result was unexpected as initially we hypothesized that low pH would be a greater risk to fruit finish than the material load in the spray suspension. Consequently, the fire blight trials in 2018 utilized citric acid with Na_2HPO_4 (which is also in Buffer Protect) but then shifted to citric acid alone in 2019 and 2020. Also, in using buffering salts, a problem encountered at the time of treatment applications was that Na_2HPO_4 dissolved very slowly in the cold, well water used for spraying. Consequently, in 2019, fruit russeting treatments utilized K_2HPO_4, which buffers similar to Na_2HPO_4 but dissolves more readily in cold water; however, after the second season of fruit russeting trials, we concluded that a buffering material was unnecessary.

Compared to citric acid, the commercial acidifiers, LI 700 and TRE-FOL, also reduced pH and in limited trialing, also appeared improve fire blight control when used to acidify OTC. Some commercial pome fruit growers who have followed this research have been experimenting with phosphorus acid (and other) amendments to adjust the pH of OTC in spray tanks but we have not collected any data on potential benefits of these material(s).

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References

Christiano, R. S. C., Reilly, C. C., Miller, W. P., and Scherm, H. 2010. Oxytetracycline dynamics on peach leaves in relation to temperature, sunlight, and simulated rain. Plant Dis. 94:1213-1218.

Doi, A. M., and Stoskopf, M. K. 2000. The kinetics of oxytetracycline degradation in deionized water under varying temperature, pH, light, substrate, and organic matter. J. Aquatic Animal Health 12:246-253.

Elkins, R. B., Temple, T. N., Shaffer, C. A., Ingels, C. A., Lindow, S. B., Zoller, B. G., and Johnson, K. B. 2015. Evaluation of dormant-stage inoculum sanitation as a component of a fire blight management program for fresh-market Bartlett pear. Plant Dis. 99:1147-1152

Loftin, K. A., Adams, C. D., Meyer, M. T., and Surampalli, R. 2008. Effects of ionic strength, temperature, and pH on degradation of selected antibiotics. J. Environ. Qual. 37:378-386

McManus, P. S., Stockwell, V. O., Sundin, G. W., and Jones, A. L. 2002. Antibiotic use in plant agriculture. Annu. Rev. Phytopathol. 40:443-465.

McManus, P. S., and Jones, A. L. 1994. Epidemiology and genetic analysis of streptomycin-resistant *Erwinia amylovora* from Michigan and evaluation of oxytetracycline for control. Phytopathology 84:627-633.

Musacchi, S., and Sera, S. 2018. Apple fruit quality: Overview on pre-harvest factors. Scientia Hortic. 234:409-430.

Shrestha, R., Lee, S.-H., Hur, J.-H., and Lim, C.-K. 2005. The effects of temperature, pH, and bactericides on the growth of *Erwinia pyrifoliae* and Erwinia *amylovora*. Plant Pathol. J. (Korea) 21:127-131.

Stockwell, V. O., and B. Duffy. 2012. Use of antibiotics in plant agriculture. Revue Sci-entifique Et Technique-Office International Des Epizooties 31:199–210.

Wilson, M., Hirano, S. S., and Lindow, S. E. 1999. Location and survival of leaf-associated bacteria in relation to pathogenicity and potential for growth within the leaf. Appl. Environ. Microbiol. 65:1435-1443.

Executive summary

Project title: Refinement of practical fire blight control: Buffered oxytetracycline

Keywords: fire blight control, *Erwinia amylovora*, antibiotics, oxytetracycline

Abstract

The half-life of the fire blight control material, oxytetracycline, is strongly affected by pH, increasing with increasing acidity. From 2017 to 2020, pear and apple orchard trials were conducted to evaluate if citric acid-based amendments to oxytetracycline sprays improve fire blight control. Over four seasons, acidified oxytetracycline resulted in better infection suppression than oxytetracycline by itself for 26 of 30 within-trial comparisons. Acidified oxytetracycline also suppressed epiphytic populations of *E. amylovora* on flowers to a greater degree than the antibiotic only. As sprays, commercial oxytetracycline formulations at label rate and amended with citric acid (16 oz./100 gal) in well water had pH-values near 4.0, and after spraying, the pH of flowers washed in distilled water (1 ml/flower) was reduced to a range of 5.2 to 5.5 compared to a pH near 6.0 after a treatment of oxytetracycline only. In fruit finish trials in pear orchards, sprays acidified with citric-acid based materials had negligible to slight effects on the finish quality (percent russeting) of fruit surfaces. Overall, compared to the water-treated control, infection suppression after two bloom applications of an acidified commercial oxytetracycline formulation averaged 85.9% $\pm$ 0.4 compared to 72.2% $\pm$ 1.7 without an acidifier.