Evaluation of Delayed-Dormant Copper as a Component of a Fire Blight IPM Program

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Abstract

Introduction

There is a continuing need to test alternative tactics as components of fire blight control programs. Most recent research has justifiably focused on protecting flowers through the petal fall and rat-tail bloom period which comprises the major primary infection window. Prior to the widespread use of effective antibiotics, i.e. streptomycin and (to a lesser extent) terramycin, copper was heavily relied upon as a bactericide and was employed both in dormant and in-season. Late dormant copper (green tip) applications are still recommended in some locations, particularly in the eastern U.S. where fire blight conditions are extreme (Anon. 1972, Burr 1980, van der Zwet and Beer 1999, Wilcox 1995). The green tip timing, or just before bloom, was designated to ensure an adequate reservoir of intact copper when over-wintering cankers became active. Recommendations were apparently based on studies done in the early 1900s (Reimer, 1925) and 1950s (Powell, 1955?), the former using Bordeaux and the latter (unspecified) copper sulfate at 5 lbs. per 100 gallons of water. Conclusions from these earlier studies were derived primarily on the number of fire blight-infected shoots (“strikes”) in treated versus untreated plots rather than monitoring actual bacterial presence.

Late dormant copper applications have largely been discounted in the western U.S. as effective antibiotic bloom treatments have become predominant and risk management models perfected and utilized to predict likely infection periods. There is, however, interest in broadening fire blight control strategies to meet increasing limits placed on antibiotic use due to resistance, increased cost, and regulatory scrutiny.

Copper remains inexpensive relative to antibiotics and while only moderately effective, can supplement antibiotics if judiciously used. Besides limited efficacy, the main problem associated with copper is cosmetic russeting, especially problematic for pears destined for fresh market. Russet potential has largely removed copper from in-season fire blight control programs; however, there is renewed interest in using it prior to bloom when risk of russeting is low to nil.

In order to confidently recommend late dormant copper to enhance fire blight control, it is highly preferable to verify whether and to what extent it actually reduces inoculum level and hence initial disease risk at bloom, rather than to rely solely on counting fire bight strikes. Techniques have now been developed to rapidly quantify bacterial populations in the field; two examples are blossom rubs (Lindow, 1995) and more recently, loop-mediated amplification (LAMP) (Johnson and Stockwell, 2008). Both of these methods can be used to ascertain the level of bacteria within hours after sampling blossoms. LAMP is currently being refined by user groups in Oregon, Washington, and Utah, and work initiated in Lake County, California in 2009 (Temple and Johnson, 2009, Johnson, 2008). LAMP sample results can be verified by dilution plating and overlayed onto risk model output.

Initial experience with LAMP in 2009 inspired the concept of utilizing it to test whether delayed dormant applications of modern copper materials, e.g. Kocide 3000 (30% metallic copper equivalent copper hydroxide) could significantly reduce initial bacterial levels and hence delay and/or reduce the number of in-season antibiotic treatments. Russet evaluation methods developed at UC Berkeley could also be used to determine whether russeting would occur from this application timing.

Materials and Methods

Eight acre sections of five orchards in Lake County and three orchards in Sutter County with a history of fire blight were randomly divided into two 4-acre sections and either treated with 6 lbs. per acre of the 30% metallic copper equivalent copper hydroxide product Kocide 3000® (E.I. DuPont de Nemours and Co., Wilmington, Delaware) at bud swell – just prior to green tip (slightly earlier than the standard late dormant recommendation to avoid possible russeting and coincide better with oil timing for insect control), or left untreated. Treatments were applied at 125 gallons per acre by cooperating growers using commercial air blast sprayers. Copper treatments were combined with delayed-dormant oil applications for pear psylla and overwintering mites to avoid the cost of a separate application, thus untreated controls actually consisted of oil alone, not known to effect *E. amylovora* populations.

Five (Sutter County), three (Lake County), or one (Petal Fall 2, Lake County) samples of 100 flower clusters each (500 (Petal Fall 2) to 1000 clusters total per sample timing) were randomly collected into a 4- quart freezer bag from both treated and untreated sections according to a pre-determined walking pattern (1 to 5 'walks') at mid-bloom, full bloom, and petal fall (two petal fall timings in Lake County) to coincide with periods of building fire blight risk (Fig. 1). A total of 30 100-cluster samples were collected from each treatment section in each of the eight sample orchards plus the additional petal fall (Petal Fall 2) sample in the five Lake County orchards (for a total of 31 per treatment in these five orchards). Sample bags were labeled with date, location, bloom stage, and walk number and shipped overnight to Oregon State University, Corvallis, where they were analyzed for the presence of *Erwinia amylovora* bacteria using two techniques: loop-mediated isothermal amplification of DNA ('LAMP'),and to verify LAMP results, dilution plating. LAMP is a highly sensitive rapid pathogen detection protocol that targets and amplifies DNA of *E. amylovora*. 100-flower cluster samples were washed and the sample wash processed with LAMP to detect as little as a single epiphytically colonized flower in a 100 cluster sample (approximately 600 flowers). Cells of *E. amylovora* were boiled in a DNA extraction buffer (InstaGene matrix). A small sample of the extracted plasma DNA was then added to a tube containing a set of *E. amylovora*-specific LAMP primers (isolated in the Johnson laboratory), buffers and Bst DNA polymerase. Tubes were placed in a 65°C water bath for one hour at which time the presence of white magnesium pyrophosphate precipitate indicated a positive LAMP reaction. Samples were then subject to dilution plating to verify the number of CFUs per ml (5 to 25 CFUs corresponding with a positive LAMP sample).

Bloom sampling was followed by visual observation of fire blight strikes in July, as well as correlated with the Washington State University risk model, Cougarblight (Smith, 2010). Fruit

was also collected from each treatment section just prior to harvest and rated for russet presence and severity at UC Berkeley.

Results

There was a slight statistical trend (p=0.11) toward reduced inoculum presence in treated plots (10 of the 94 treated and 14 out of the 95 oil-alone). There was no significant difference in Log10 *E. amylovora* CFU between the treatments. There were four positive samples in mid-bloom in Sutter County and none thereafter, while positive samples occurred in Lake County only at petal fall, indicating warmer temperatures later in the season on the North Coast (Table 1). This pattern is reflected in the difference between Sutter and Lake Counties in the Cougarblight 4-day peak accumulated degree hour summations (Table 2). The overall correlation between positive LAMP samples and Cougarblight 4-day accumulated degree-hour summations is shown in Figure 1; LAMP assays detected inoculum presence on four out of five 4-day sum degree-hour peak sample dates in 2010, indicating its ability to predict pathogen presence. 2010 California results can be compared with other test sites and seasons from 2008-2010 in Table 3.

Actual disease occurrence in California was very low in 2010, thus there were no recorded fire blight strike counts other than immediately surrounding holdovers, and consequently no ability to correlate LAMP results with disease symptoms. Russet evaluations were negative for both treated and untreated fruit at all locations.

Conclusions and 2011 Plans

The LAMP assay successfully detected *E. amylovora* presence in blossom samples and thus can be used as a supplemental risk management tool in an integrated fire blight management program consisting of environmental (temperature, humidity), host (cultivar, vigor, holdover history), and pathogen (LAMP) monitoring. Whether LAMP will have a place in commercial IPM programs remains to be seen as degree-hour models, e.g. Zoller ‘California’, Maryblight, Cougarblight, have evolved as highly accurate in assessing conditions for inoculum presence and build-up. LAMP could replace commercial blossom sampling performed for many years by long-time Lake County pest control adviser John Sisevich, who no longer performs this service, and is now being considered for commercial adoption in Colorado, Utah, eastern Canada, and the Pacific Northwest.

LAMP and dilution plate results from this initial year of limited testing suggest that delayed-dormant copper applications may reduce the amount of *E. amylovora* inoculum and thus potentially lessen disease presence and/or severity, depending on orchard history and seasonal weather conditions. There were fewer positive LAMP samples in the copper treated sections, but no corresponding average reduction in average Log (CFU) per flower. While only preliminary, results suggest that the use of delayed-dormant copper should be further explored as a new tool in fire blight IPM programs to reduce initial inoculum. Russet evaluations performed at UC Berkeley revealed no difference between copper and untreated fruit (data not shown), thus it appears that delayed-dormant applications prior to green tip are safe for pears destined for fresh market. Kocide 3000® cost is $10 per lb., thus the dormant application at 6 lbs. per acre would cost $60 per acre. 2010 retail price for one every row application of antibiotics (e.g. 0.5 lbs.

Agristrep® plus 1 lb. Mycoshield®) costs $43.00. If two early season antibiotic applications could be eliminated, net material-alone savings would be $23.00. If only one antibiotic application is eliminated, material-alone cost would be $17.00 net. Thus, in order to be cost effective, users would need to see reduced in-season spray costs, including material and application. Orchards with existing resistance issues would likely benefit the most. The goal in 2011 is to double the number of treated orchards to increase replication and confirm or negate preliminary 2010 results. It would also be useful to compare bud swell and green tip timings on a smaller scale to determine if the latter is more effective, and more importantly, safe.

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Acknowledgements

The authors wish to thank cooperating growers and pest control advisers Joe Conant, Diane Henderson, Bill Oldham, Andy Scully, and David Weiss. We also thank research technicians Renee _____, Carolyn Shaffer, Daniel Suenram, and Makayla Rodrigues.

Fig. 1. Seasonal plots of a weather-based assessment of fire blight risk (Cougarblight model (30)) relative to positive and negative detection of *E. amylovora* by loop mediated isothermal amplification (LAMP) assay of washes of pear and apple flowers sampled from commercial orchards in the Pacific Northwest region of the United States. Open arrows indicate no detection of *E. amylovora* by LAMP on the corresponding date, and solid arrows indicate at least one of the 100-flower cluster samples assayed on that date were positive by LAMP assay for fire blight pathogen. Cougarblight is 4-day moving sum of degree hours > 15.5°C (60°F); values of this index are used to assess the likelihood that epiphytic populations of *E. amylovora* have developed in pear and apple flowers (30). The index values shown in each panel are a composite from temperature data measured at each of production areas sampled in that season: **A)** 2008, Rogue and Hood River valleys of Oregon; **B)** 2009, Rogue and Hood River valleys of Oregon; Lake County, California, and Yakima, Wenatchee, and Okanagan valleys of Washington. **C)** 2010, Rogue valley of Oregon; Lake and Sutter Counties, California, and Yakima and Okanagan valleys of Washington.

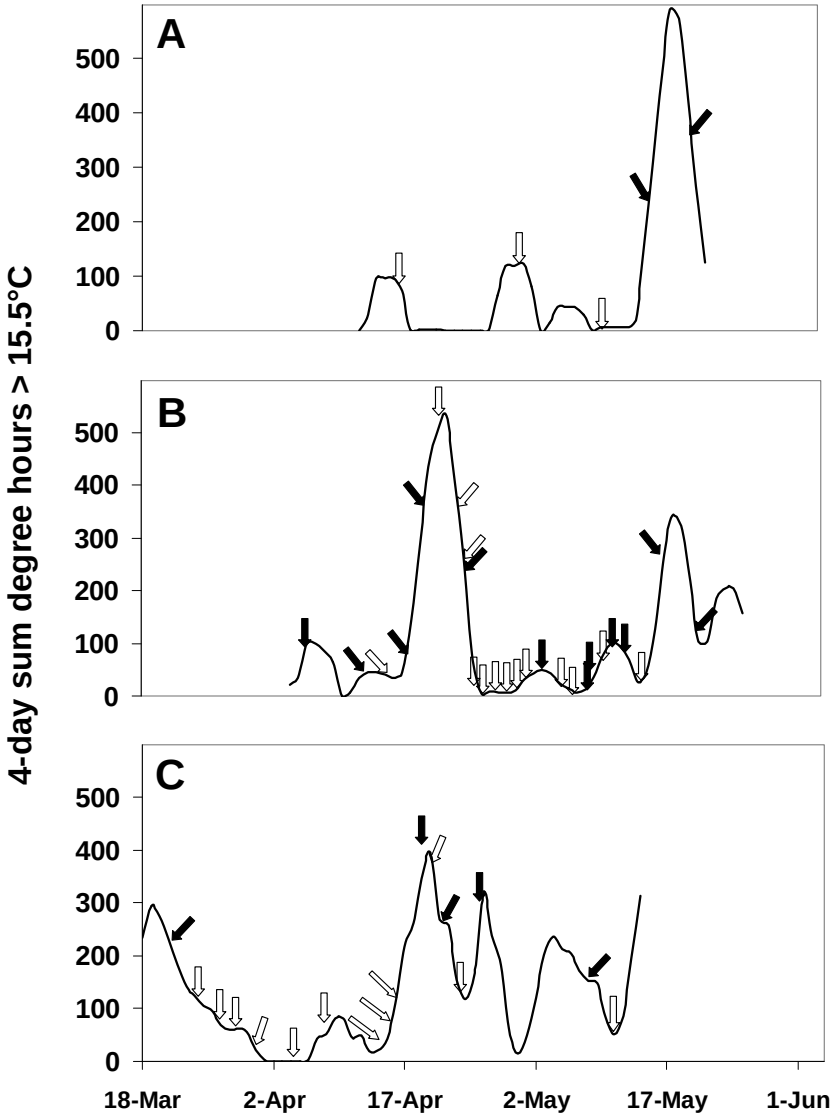

Table 1. Average number positive LAMP samples (100 flower clusters per 5 (Wheatland), 3 (Lake County), or 1 (Lake County, Petal Fall 2) samples and average Log10 *E. amylovora* per flower at mid-bloom, full bloom, and petal fall (n=30 per sample date except Petal Fall 2, n=5), 2010.

Treatment	Bloom Stage (Avg. no./30 samples)									
	Mid bloom 3/22-25/10		Full Bloom 3/29-4/1		Petal Fall 1 4/16-26		Petal Fall 2 5/12		Total	
	No.	Log^{10}	No.	Log^{10}	No.	Log^{10}	No.	Log^{10}	No.	Log^{10}
Copper + oil	1.0	1.2	0	0.0	1.1	1.5	1.2	1.9	1.0	1.6
Oil alone	1.0	1.7	0	0.0	1.1	1.4	1.4	2.2	1.1	1.7
P-value	0.54	(insufficient data)			0.20	0.28	0.14	0.15	0.11	0.69

[1] one additional positive sample not counted due to lack of dilution plate confirmation.

Table 2. Accumulated Cougarblight degree-hours in Sutter County versus Lake County, 2010.

Location	Bloom Stage			
	Mid Bloom	Full Bloom	Petal Fall 1	Petal Fall 2
Sutter County	330[1] (4.22)	163 (3/29)	124 (4/26)	-
Lake County	192 (3/25)	0 (4/1)	576[1] (4/26)	155 (5/12)

[1] LAMP detection of *E. amylovora*

Table 4. Summary of LAMP assay results from 100-flower cluster samples[a] collected from commercial pear and apple orchards in the Pacific Northwest region of the United States from 2008 to 2010**.**

					No. of positive LAMP of total samples						
Year	State	Production area	Host	No. of orchards	Mid-bloom	Full bloom	Petal fall	Media isolation[b]	Mean Log (CFU) per flower[c]	No. of orchards with fire blight	Disease severity in orchards with fire blight[d]
2008	OR	Rogue Valley	Pear	3	0 of 15	0 of 14	n.s.[e]	No	-	0	-
		Hood River Valley	Pear	3	0 of 15	3[f] of 15	7[f] of 15	Yes	1.6	2	Light to moderate
2009	OR	Rogue Valley	Pear	3	3 of 20	0 of 20	2 of 20	Yes	3.3	1	Light
		Hood River Valley	Pear	6	6 of 30	6 of 30	7 of 25	Yes	3.3	2	Light
		Hood River Valley	Apple	2	0 of 8	2 of 8	4 of 8	Yes	2.2	1	Light
		Walla Walla Valley	Apple	4	0 of 20	4 of 20	11 of 20	Yes	3.3	3	Light
	CA	Lake County	Pear	4	2 of 15	2 of 15	1 of 15	Yes	1.2	1	Light
	WA	Okanogan Valley	Pear	1	0 of 4	0 of 6	2 of 4	Yes	3.8	1	Light
		Wenatchee Valley	Pear	2	0 of 10	0 of 10	0 of 10	No	-	0	-
		Columbia Basin	Apple	3	0 of 15	0 of 15	0 of 10	No	-	3	Light to moderate
	UT	Utah County	Apple	6	11 of 19[f]	19 of 25[f]	10 of 18[g]	Yes	3.4	7	Moderate to heavy
2010	OR	Rogue Valley	Pear	2	0 of 12	0 of 12	0 of 12	No	1.5	0	-
	CA	Sutter County	Pear	6	4 of 30	0 of 30	0 of 30	Yes	2.0	0	-
	CA	Lake County	Pear	5	0 of 30	0 of 30	20 of 40	Yes	-	0	-
	WA	Okanogan Valley	Pear	1	2 of 3	0 of 5	n.s.	No	-	1	Light
		Yakima Valley	Apple	9	0 of 30	2 of 30	n.s.	Yes	1.6	6	Light
		Summary		60	28 of 276 10%	38 of 285 13%	64 of 227 28%		2.8	28	

[a] 100-flower clusters per sample were washed in 1.5 liter (2008) or 0.3 (2009 and 2010) of water in a re-sealable plastic bag.
[b] Whether or not *E. amylovora* was recovered on culture media from the wash of 100-flower cluster samples.
[c] Average $\log_{10}$ population size of *E. amylovora* (CFU per flower) recovered on culture media from the wash of 100-flower cluster samples (~600 flowers per sample).
[d] Whether or not fire blight developed in the orchards, and if yes, the range of disease ratings applied to the orchards: light = 1 strike per tree, moderate = 2 to 5 strikes per tree, and heavy $\geq$ 6 strikes per tree).
[e] Not sampled.
[f] Positive detection by LAMP required a four-fold speedvac concentration of extracted DNA.
[g] In Utah, one 100-flower cluster sample was collected from each orchard daily over a period to 10 to 12 days; dates were grouped into approximate stage of bloom.