EVALUATION OF DELAYED-DORMANT COPPER AS A COMPONENT OF A FIRE BLIGHT IPM PROGRAM

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ABSTRACT

Copper applied at green tip to reduce populations of fire blight (*Erwinia amylovora*) associated with overwintering ("holdover") cankers is a component of some fire blight management programs, but is not currently a standard practice in California. Resistance, increasing costs, and regulatory pressures has led to renewed interest in this tactic. Orchards treated in 2010 and 2011, with positive results, were again treated. The presence of *E. amylovora* in blooms was measured using loop-mediated amplification (LAMP), and the number of fire blight strikes, russet presence and severity, and frost damage evaluated. In contrast to 2010 and 2011, populations of *E. amylovora* remained very low through the sampling period and there was no statistical difference between treated and untreated plots. There were significantly fewer fire blight strikes in treated plots of one orchard. There were no differences in russet or frost damage. Unlike 2011, the long extended period of cool and wet weather between the copper application and infection period likely diminished the amount of copper on treated buds. A fourth year of testing is desirable to confirm the usefulness of the delayed dormant application strategy.

INTRODUCTION

There is a continuing need to test alternative tactics as components of programs to control fire blight disease caused by the bacterial pathogen *Erwinia amylovora*. 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) oxytetracycline, 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 and Reinhardt 1955, Powell 1965), the former using Bordeaux and the latter (unspecified) copper sulfate at 5 lbs. per 100 gallons of water. More recently, copper sulfate added to a dormant oil spray has been shown to improve fire blight control in apples in conjunction with bloom sprays (Ellis 1980, Ellis 1981).

Late dormant copper applications were largely discounted in the western U.S. as effective antibiotic bloom treatments became predominant and risk management models perfected and utilized to predict likely infection periods. However, there is renewed 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 on the North Coast and northern Sacramento Valley; however, low rates of copper hydroxide (0.50 lb. per acre) have been used in-season in the Sacramento Delta in recent years, reportedly with few incidences of fruit russeting. Where there is concern about fruit russet, as well as resistance to antibiotics, there is renewed interest in using it prior to bloom when risk of russeting is low to nil.

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. 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) (Temple and Johnson 2010). 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 was initiated in Lake County, California in 2009 (Temple and Johnson 2008, Johnson 2009). The British company OptiGene Limited has shown positive results in a field trial measuring *E. amylovora* using their commercially-available portable Genie II unit (Optigene, 2011). 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.

The project began in 2010, and has continued through 2012 to obtain robust verification of using this strategy to reduce initial *E. amylovora* inoculum. The focus in 2012 was to repeat delayed dormant copper treatments in those orchards treated in previous years to confirm whether inoculum levels could be further suppressed and fire blight strikes further reduced. Three new comparison orchards (6 orchard blocks) were also added in the Sacramento Delta.

MATERIALS AND METHODS

8-acre sections of seven orchard blocks in Lake County and six orchard blocks in Yuba County, all treated with delayed dormant copper in 2010 and 2011, were randomly divided into two 4-acre sections and either treated with 6 lbs. per acre of the 25% copper oxychloride/23% copper hydroxide (28% metallic copper equivalent) product Badge X_2 (Isagro USA, Inc., Morrisville, North Carolina) 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. Four orchard blocks in Sacramento County not treated in previous years were also similarly divided and treated as in the Lake and Yuba locations. 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.

Three samples of 100 flower clusters each (total of 300 clusters per treatment per timing per plot) 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 and/or rattail to coincide with periods of building fire blight risk (Fig. 1). A total of 9 to 39 100-cluster samples were collected from each treatment from the sample orchards in mid-bloom and at petal fall and/or rattail, for a total of 110-111 samples per treatment in Lake, Yuba, and Sacramento Counties. 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 *E. 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 65ºC 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 late May-early June (Lake and Yuba Counties only), as well as correlated with the Zoller "California" risk model (Gubler, 2007). Fruit was also collected from each treatment section just prior to harvest and rated for russet presence and severity and frost damage at UC Berkeley.

RESULTS

LAMP results (Tables 1-6) – Very little fire blight inoculum was detected during the sampling period from March 30-April 30. Inoculum was first detected at petal fall in Sacramento County (April 4), rattail in Yuba County (April 24), and mid-bloom in Lake County (April 19). As in previous years, highest levels were detected in rattail samples (average 36% across all orchards). While the pattern of detection was similar to previous years, particularly in Lake County (Table 5), there was no overall significant difference between treated and untreated plots, either in incidence (number of positive LAMP samples) or amount of inoculum per positive sample (Log_{10} CFU). Results were the same in both previously- and newly-treated orchards.

Fire blight strikes (Tables 7-8) – Complete data was collected from seven paired plots from May 11 – June 1. Data was analyzed using Spearman Rank Correlation Coefficient. Similar to 2011, there was no significant difference between treatments in Lake and Yuba orchards combined, but as in 2011, there were again significantly fewer strikes in the Yuba Dantoni orchard.

Fruit russeting and frost damage (Table 9-13)

Russeting – There was no difference in russeting between treatments in Lake County. In contrast to 2010 and 2011, there was a trend toward less russeting in copper-treated sections in Yuba County orchards in 2012. There was significantly less russet in the Dantoni plots, mainly due to a greater percentage of fruit with 3% or less russet. There was no significant difference in the average number of russeted fruit in the Dole plots, however, russet severity was significantly greater in the control fruit.

Frost damage – Unsurprisingly, damage differed significantly between Lake and Yuba Counties (about 14% versus none), however, there was no difference between treatments in Lake County.

DISCUSSION

LAMP and dilution plate results from this third year of testing contrasted with 2010 and 2011, in that they failed to show that delayed dormant copper applications significantly reduced the amount of *E. amylovora* inoculum. While the overall pattern of inoculum build up was similar to past years, the overall level was negligible through the sampling period. Weather pattern relevant to inoculum development differed in 2012 versus 2011. Degree-hour accumulation in 2012 was negligible prior to bloom the third week of April due to prolonged cold weather, then increased steadily through May, well after sampling ended (Figures 1 and 3). This pattern contrasts with 2011, in which sampling coincided with discreet periods (albeit interrupted with cool spells) of degree-hour accumulation during early bloom at the end of March and first half of April (Figures 2 and 4). Thus, in 2012, though total rainfall was one-third less from February through April 1 than in 2011, and the amount from March 1 through May 30 about the same in both years, the amount of total active copper remaining at the time of bloom in 2012 was conceivably less than in 2011 due to the longer period between application and timing of flower opening and critical degree hour accumulation threshold. This would result in less intact material due to natural weather-related degradation and subsequently less protection at the onset of rapid inoculum build up. The bloom sampling interval was also shorter in 2012, for example, April 19-30 versus March 31-May 28 in Lake County in 2011.

While LAMP samples were insignificant, there was some blossom infection observed and many California orchards did exhibit infection later in the season due to ideal conditions as indicated in Figure 1. There was no difference in the number of fire blight strikes between treatments in Lake County; however, there were significantly fewer strikes in one Yuba County orchard versus 2010-2011 (Table 7). Russet evaluations performed at UC Berkeley revealed significant difference in favor of the treatment. In general, it appears that delayed-dormant copper applications prior to green tip are safe for pears destined for fresh market, and may be beneficial in reducing russet-forming bacteria in very cold, wet springs such as 2012 (Tables 3 to 13).

Badge X2 cost was about $7.50 per lb. in 2012, or $45 per acre for the delayed-dormant spray ($42 in 2011). 2013 retail price for one every row (two every other row) applications of antibiotics (assuming 0.3 lbs. Agristrep® plus 1 lb. Mycoshield®; individual costs may be lower) costs will likely cost about $30. If one early season antibiotic application could be eliminated, there would be a net material-alone cost of $15. If two antibiotic applications are eliminated, net saving for material alone would be $15. Lower antibiotic rates incurred on a part per million basis (reduced gallonage) would lead to further cost reduction. Thus, if effective, the delayed-dormant copper application could feasibly reduce the overall cost of a fire blight IPM program at the current price of Badge X2 or similarly priced copper. In any case, orchards with severe fire blight history, indicated antibiotic resistance, as well as organic orchards not marketing under IFOAM (which disallows copper) will benefit the most from this strategy. Three years of results, 2010-2012 suggest that the treatment has been effective in two of three years. An additional year is needed to show if the chance of success remains greater that 50%.

Despite very low inoculum levels during the sampling interval, LAMP results again correlated well with early season risk model output 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.

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Table 1. Average number positive LAMP samples per 300 flower clusters and average Log_{10} *E. amylovora* per flower at mid-bloom, full bloom, petal fall and rattail in orchards treated with and without delayed dormant copper from 2010-2012 in Lake, Sacramento and Yuba Counties, California. 2012.

	Bloom Stage									
	Mid Bloom		Full Bloom		Petal Fall		Rat Tail		Total	
Treatment[1]	3/22-4/19		3/29-4/16		4/9-30		4/20-30			
	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}
Copper + oil	0.03	<0.01	0.00	0.00	0.00	0.00	0.36	0.51	0.14	0.18
Oil alone	0.00	0.00	0.00	0.00	0.11	0.06	0.36	0.43	0.14	0.16
P-value[2]	0.32	0.32	--	--	0.33	0.33	1.00	0.63	0.98	0.76

Treated	n=39	n=39	n=24	n=24	n=9	n=9	n=39	n=39	n=111	n=111
Untreated	n=39	n=39	n=23	n=23	n=9	n=9	n=39	n=39	n=110	n=110

[1] Additional positive LAMP samples (treated: mid-bloom=3, petal fall=1, rattail=1 and untreated: mid-bloom=1, petal fall=1, rattail=1) not included due to lack of dilution plate confirmation.
[2] Means analyzed using T-test, P<0.05. Data normalized with (SQRT+1) transformation.

Table 2. Average number positive LAMP samples per 300 flower clusters and average Log_{10}, *E. amylovora* per flower at mid-bloom, full bloom, petal fall and rattail in McCormack North and McCormack South, and Hemly East and West combined, separate and combined three orchards in Sacramento County, California. 2012.

	Bloom Stage									
Orchard and Treatment[1]	Mid Bloom		Full Bloom		Petal Fall		Rat Tail		Total	
	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}
McCormack North	3/20/12		3/21/12		4/2/12		4/23/12			
Copper + oil	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.10	0.25	0.27
Oil alone	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.43	0.25	0.11
P-value[2]	--	--	--	--	--	--	~	0.14	1.00	0.36
McCormack South	3/21/12		3/26/12		4/4/12		4/19/12			
Copper + oil	0.00	0.00	0.00	0.00	0.00	0.00	0.67	<0.01	0.17	<0.01
Oil alone	0.00	0.00	0.00	0.00	0.33	<0.01	0.33	<0.01	0.17	<0.01
P-value[2]	--	--	--	--	0.37	0.37	0.52	0.52	1.00	1.00
McCormack North and South Combined										
Copper + oil	0.00	0.00	0.00	0.00	0.17	0.00	0.83	0.37	0.21	0.09
Oil alone	0.00	0.00	0.00	0.00	0.00	<0.01	0.67	0.13	0.21	0.01
P-value[2]	--	--	--	--	0.34	0.34	0.55	0.56	1.00	0.43

~*No E amylovora*
[1] Additional positive LAMP samples (McCormack South treated: petal fall=2, rattail=1 and untreated: rattail -2) not included due to lack of dilution plate confirmation.
[2] Means analyzed using T-test, P<0.05. Data normalized with (SQRT+1) transformation.
McCormack North: treated and untreated, n=3, total, n=12. McCormack South: treated and untreated, n=3, total, n=12.
~ All values equal

Table 3. Average number positive LAMP samples per 300 flower clusters and average Log_{10} *E. amylovora* per flower at mid-bloom, full bloom, petal fall and rat tail in McCormack North and McCormack South, separate and combined orchards in Sacramento County, CA. 2012

	Bloom Stage									
	Mid Bloom		Full Bloom		Petal Fall		Rat Tail		Total	
McCormack North	3/20/2012		3/21/2012		4/2/2012		4/23/2012			
	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}
Copper + oil	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.10	0.25	0.27
Oil alone	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.43	0.25	0.11
P-value[2]	--	--	--	--	--	--	~	0.14	1.00	0.36
McCormack South	3/21/2012		3/26/2012		4/4/2012		4/19/2012			
	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}
Copper + oil	0.00	0.00	0.00	0.00	0.00	0.00	0.67	<0.01	0.17	<0.01
Oil alone	0.00	0.00	0.00	0.00	0.33	<0.01	0.33	<0.01	0.17	<0.01
P-value[2]	--	--	--	--	0.37	0.37	0.52	0.52	1.00	1.00
Hemly East and West Combined										
Copper + oil	0.00	0.00	0.00	0.00	0.17	0.00	0.83	0.37	0.21	0.09
Oil alone	0.00	0.00	0.00	0.00	0.00	<0.01	0.67	0.13	0.21	0.01
P-value[2]	--	--	--	--	0.34	0.34	0.55	0.56	1.00	0.43

~*No E amylovora*

[1] Additional positive LAMP samples (McCormack South treated: PF=2, rat tail=1 and untreated: rat tail -2) not included due to lack of dilution plate confirmation.

[2] Means analyzed using T-test, P<0.05. Data normalized with (SQRT+1) transformation.
McCormack North: treated and untreated, n=3, total, n=12. McCormack South: treated and untreated, n=3, total, n=12.

~ All values equal

Table 4. Average number positive LAMP samples per 300 flower clusters and average Log_{10} *E. amylovora* per flower at mid-bloom, full bloom, petal fall and rattail from orchard blocks treated with delayed dormant copper from 2010-2012 in Lake and Yuba Counties, California. 2012

	Bloom Stage									
	Mid Bloom		Full Bloom		Petal Fall		Rattail		Total	
Treatment[1]	3/22-4/19		3/29-4/16		4/9-30		4/20-30			
	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}
Copper + oil	0.03	<0.01	0.00	0.00	0.00	0.00	0.24	0.35	0.10	0.10
Oil alone	0.00	0.00	0.00	0.00	0.33	0.17	0.24	0.24	0.10	0.14
P-value[2]	0.32	0.32	--	--	0.37	0.37	1.00	0.51	0.98	0.68

Treated	n=33	n=33	n=18	n=18	n=3	n=3	n=33	n=33	n=87	n=87
Untreated	n=33	n=33	n=17	n=17	n=3	n=3	n=33	n=33	n=86	n=86

[1] Additional positive LAMP samples (treated: mid-bloom=3, petal fall=1, rattail=1 and untreated: mid-bloom=1, rattail=1) not included due to lack of dilution plate confirmation.

[2] Means analyzed using T-test, P<0.05. Data normalized with (SQRT+1) transformation

Table 5. Average number positive LAMP samples per 300 flower clusters and average Log $_{10}$ *E. amylovora* per flower at mid-bloom, full bloom, petal fall and rattail in Lake County, California. 2012.

	Bloom Stage									
	Mid Bloom		Full Bloom		Petal Fall		Rattail		Total	
Treatment[1]	4/19		No data		4/30		4/27-30			
	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}
Copper + oil	0.07	<0.01	----	----	0.00	0.00	0.00	0.00	0.03	<0.01
Oil alone	0.00	0.00	----	----	0.33	0.17	0.00	0.00	0.03	0.01
P-value[2]	0.33	0.33	----	----	0.37	0.37	--	--	1.00	0.19
Treated	n=15	n=15	----	----	n=3	n=3	n=15	n=15	n=33	n=33
Untreated	n=15	n=15	----	----	n=3	n=3	n=15	n=15	n=33	n=33

[1] Additional positive LAMP samples (treated: mid-bloom=3, petal fall=1 and untreated: mid-bloom=1) not included due to lack of dilution plate confirmation.
[2] Means analyzed using T-test, P<0.05. Data normalized with (SQRT+1) transformation.

Table 6. Average number positive LAMP samples per 300 flower clusters and average Log $_{10}$ *E. amylovora* per flower at mid-bloom, full bloom, petal fall and rattail in Dantoni and Dole separate and combined orchards in Yuba County, California. 2012.

	Bloom Stage							
	Mid Bloom		Full Bloom		Rattail[3]		Total	
Treatment[1]	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}	No.	Log_{10}
Dantoni Orchard	4/6		4/16		4/24			
Copper + oil	0.00	0.00	0.00	0.00	0.89	1.03	0.30	0.34
Oil alone	0.00	0.00	0.00	0.00	0.89	1.28	0.31	0.44
P-value[2]	--	--	--	--	1.00	0.67	0.93	0.73
Dole Orchard	4/3		4/7		4/20			
Copper + oil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Oil alone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P-value[2]	--	--	--	--	--	--	--	--
Dantoni and Dole Combined								
Copper + oil	0.00	0.00	0.00	0.00	0.44	0.52	0.15	0.17
Oil alone	0.00	0.00	0.00	0.00	0.44	0.64	0.15	0.22
P-value[2]	--	--	--	--	1.00	0.78	0.97	0.78

~*No E amylovora*
[1] Additional positive LAMP samples (treated: rattail=1 and untreated: rattail-1) not included due to lack of dilution plate confirmation.
[2] Means analyzed using T-test, P<0.05. Data normalized with (SQRT+1) transformation.
Dantoni: treated and untreated, n=9 per sample timing, Total data: n=54. Dole: treated and untreated, n=9 per sample timing, Total data: n=54. Dantoni and Dole combined: n=18 per sample timing, Total data: n=108.
[3] No petal fall data collected

Table 7. Average number of fire blight strikes in Dole, Dantoni and Henderson orchards, Lake and Yuba Counties, California, 2012.

Treatment	Dole (5/16-24)	Dantoni (5/11-6/1)	Combined Dole and Dantoni (5/11-6/1)	Combined Dole, Dantoni and Henderson
Treated	62	42	49	52
Control	28	54	44	42.5
P-value[1]	NS (0.93)	*(0.02)	NS (0.18)	NS (0.61)
Sample size (complete cases)	Treated n=18	Treated n=29	Treated n=47	Treated n=49
	Control n=18	Control n=29	Control n=47	Control n=49

[1] Indicates significance at P≤ 0.05, NS indicates not significant P>0.05 (Multiple-Variable analysis with Spearman Rank Correlation test).

Table 8. Average number of fire blight strikes in Dole and Dantoni Orchards, Yuba County, California, 2012.

Treatment	Dole (5/26-24)	Dantoni (5/11-6/1)	Combined Dole and Dantoni
Treated	62	42	49
Control	28	54	44
P-value[1]	NS (0.93)	* (0.02)	NS (0.18)
Sample size (complete cases)	Treated n=18	Treated n=29	Treated n=47
	Control n=18	Control n=29	Control n=47

[1] * Indicates significance at p≤0.05, NS indicates not significant P>0.05 (Multiple-Variable analysis with Spearman Rank Correlation test).

Table 9. Comparison of average fruit russeting, percent russet severity and percent frost damage in Bartlett pears harvested in Lake and Yuba Counties, California, 2012.

	Average Russeting		Russet Severity (7% or greater)		(3% or less)		Frost Damage (%)	
Treatment[1]	Copper + oil	Oil alone	Copper + oil	Oil alone	Copper + oil	Oil alone	Copper + oil	Oil alone
Lake County	1.2	1.6	3.2	6.3	87.2	83.7	15.1	13.7
Yuba/Sutter Counties	1.6	1.9	2.5	4.4	89.5	84.1	0.0	0.00
P-value[2]	0.39	0.55	0.76	0.63	0.67	0.95	*0.01	0.01

[1] Lake County, treated and control: n=5, Yuba County, treated and control: n=6
[2] Means analyzed using T-test; *indicates significance at P≤ 0.05.

Table 10. Average fruit russeting, percent russet severity and percent frost damage in Bartlett pears harvested in Lake County, California, 2012.

Treatment[1]	Average Russeting	Russet Severity (7% or greater)	(3% or less)	Frost Damage (%)
Copper + oil	1.2	3.2	87.2	15.1
Oil alone	1.6	6.3	83.7	13.7
P-value[2]	0.62	0.49	0.72	0.84

[1] Treated and control: n=5.
[2] Means analyzed using T-test, P≤ 0.05.

Table 11. Average fruit russeting, percent russet severity and percent frost damage in Bartlett pears harvested in Dole and Dantoni orchards in Yuba County, California, 2012

Treatment[1]	Average Russeting	Russet Severity (7% or greater)	(3% or less)	Frost Damage (%)
Copper + oil	1.6	2.5	89.5	0.0
Oil alone	1.9	4.4	84.1	0.0
P-value[2]	0.29	0.29	0.12	~

[1] Treated and control: n=6.
[2] Means analyzed using T-test, P≤ 0.05.
~ All values equal.

Table 12. Average fruit russeting, percent russet severity and percent frost damage in Bartlett pears harvested in Dantoni orchard in Yuba County, California, 2012

Treatment[1]	Average Russeting	Russet Severity (7% or greater)	(3% or less)	Frost Damage (%)
Copper + oil	1.7	4.2	88.4	0.0
Oil alone	2.5	6.7	79.1	0.0
P-value[2]	*(0.04)	0.38	0.07	~

[1] Treated and control: n=3.
[2] Means analyzed using T-test; indicates significance at P≤ 0.05.
~ All values equal.

Table 13. Average fruit russeting, percent russet severity and percent frost damage in Bartlett pears harvested in Dole orchard in Yuba County, California, 2012.

Treatment[1]	Average Russeting	Russet Severity (7% or greater)	(3% or less)	Frost Damage (%)
Copper + oil	1.5	0.77	90.6	0.0
Oil alone	1.4	2.08	89.2	0.0
P-value[2]	0.61	0.05	0.67	~

[1] Treated and control: n=3.
[2] Means analyzed using T-test, $P \leq 0.05$.
~ All values equal.

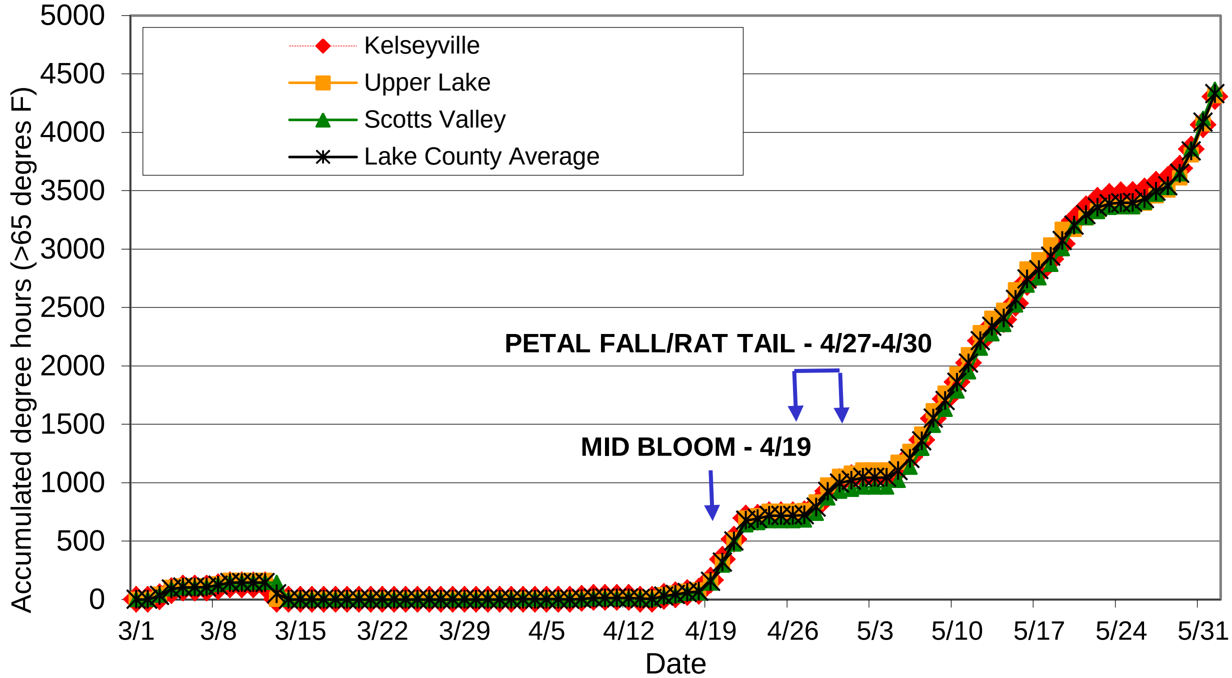

Figure 1: Relationship between accumulated degree hours (base >65°F) for Kelseyville, Scotts Valley (Lakeport) and Upper Lake, Lake County, California, March 1 to June 1, 2012 and positive (shown in bold) and negative (shown in black) LAMP samples. Degree-hours calculated from using data from Kelseyville-0.1P (Kel), Scotts_Valley-0.2 P (SVL), and Upper_Lake-0.1 P (UPL) (Source: UCIPM).

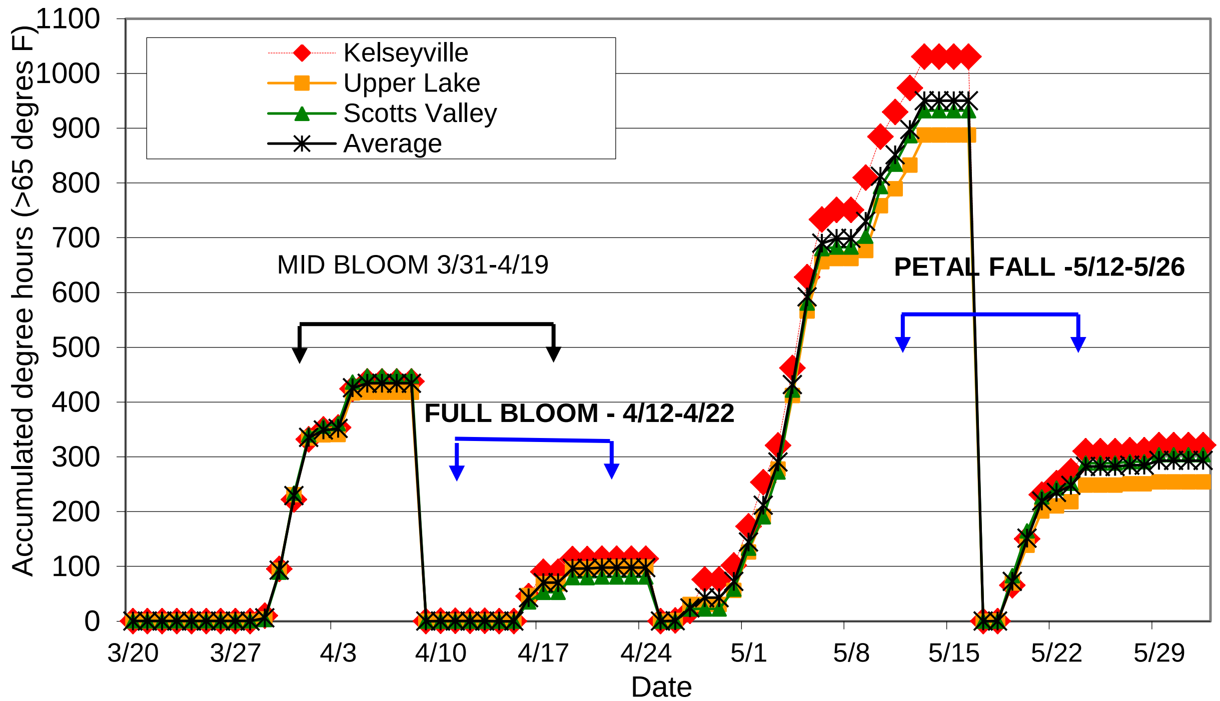

Figure 2: Relationship between accumulated degree hour (base >65°F) for Kelseyville, Scotts Valley (Lakeport) and Upper Lake, Lake County, California, March 20 to June 1, 2011 and positive (shown in bold) and negative (shown in black) LAMP samples. Degree-hours calculated from using data from Kelseyville-0.1P (Kel), Scotts_Valley-0.2 P (SVL), and Upper_Lake-0.1 P (UPL) (Source: UCIPM).

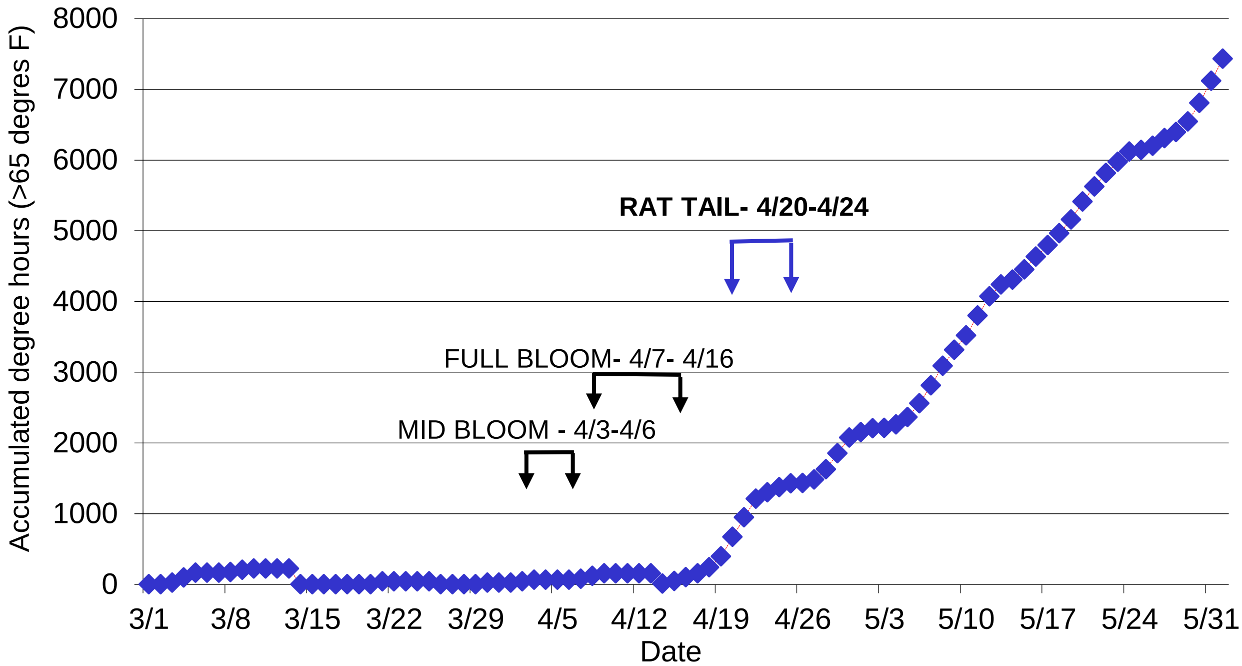

Figure 3: Relationship between accumulated degree hours (base >65°F) for Sutter/Yuba County, California, March 1 to June 1, 2012 and positive (shown in bold) and negative (shown in black) LAMP samples. Degree-hours calculated using data from CIMIS #235, Verona A, Sutter County. (Source: UCIPM)

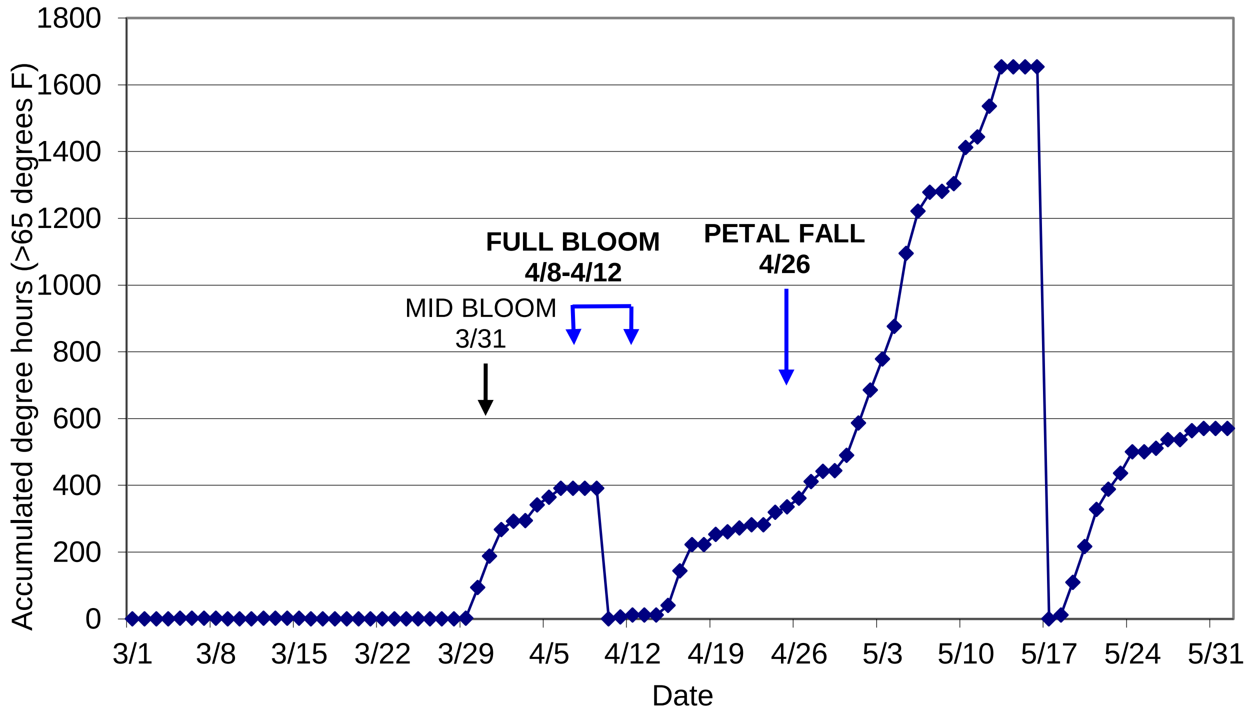

Figure 4: Relationship between accumulated degree hours (base >65°F) for Yuba County, California, March 1 to June 1, 2011 and positive (shown in bold) and negative (shown in black) LAMP samples. (Degree hours calculated using data from Touchtone #24, Yuba City, Sutter County) (Source: UCIPM).