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 bacteria (*Erwinia amylovora*) associated with overwintering ("holdover") cankers is a component of some fire blight management programs, but is not currently utilized in California. Resistance, increasing costs and regulatory pressures has led to renewed interest in this practice. The second year trialing in replicated plots was carried out in 2011 to continue to assess the 1) efficacy and phytotoxicity of using copper at bud swell (just prior to green tip) and 2) the ability of the detection assay method loop-mediated amplification (LAMP) to rapidly detect the presence of *E. amylovora* on blossoms from copper-treated versus untreated orchards. Results from LAMP sampling in eight orchards in Lake County and two orchards in Sutter County continue to suggest that delayed dormant copper does reduce inoculum and that no observable phytotoxicity occurs. Observations of fire blight shoot strikes also suggest that inoculum reductions due to the delayed dormant applications can result in a reduced number of strikes, although further verification is needed. Effort will continue in 2012, re-treating current plots to continue suppressing inoculum and adding new ones in the Sacramento Delta.

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) 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). 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 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.

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 overplayed 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, continued in 2011, and will be continued in 2012 to obtain robust verification of using this strategy to reduce initial *E. amylovora* inoculum. Project objectives, as well as methodologies, were established in year 1 and remain the same. Special attention in 2012 will be given to repeating delayed dormant copper treatments those orchards treated in previous years to confirm whether inoculum levels can be further suppressed and fire blight shoot strikes further reduced.

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 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. Treatments were applied at 125

gallons per acre by cooperating growers using commercial air blast sprayers. Badge X_2 replaced Kocide 3000® in 2011 due to its less expensive cost, necessary to reduce potential cost of this added treatment tactic to the existing fire blight IPM program. 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.

Two (Sutter County) and eight (Lake County) samples of 100 flower clusters each (3300 to 4500 clusters total per sample timing per treatment) 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 to coincide with periods of building fire blight risk (Fig. 1). A total of 37-41 100-cluster samples were collected from each treatment section in each of the eight sample orchards in mid- to full bloom and 28-29 at petal fall, for a total of 104-108 samples in Lake and Yuba 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 *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 late June and early July, as well as correlated with the Washington State University risk model, Cougarblight (Smith, 2010) and 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 at UC Berkeley.

RESULTS

LAMP results (Tables 1 to 5) – 2011 results resembled those from 2009 and 2010. There was no inoculum detected in the mid-bloom sample in the northern Sacramento Valley and only a very small, insignificant amount in Lake County. Levels increased slightly, but again insignificantly, at full bloom in Yuba County; however, levels in the copper-treated plots were significantly lower versus oil-alone plots in Lake County at full bloom, both in number of *E. amylovora* positive samples and average log 10 per flower. The number of positive LAMP samples, but not the average log 10 was significantly greater at petal fall in Lake County only. Total number of positive LAMP samples over all sampling dates and locations was very significantly greater in the oil-alone plots (trend in Yuba County), but the average log 10 was equal in both treatments.

Fire blight strikes (Tables 6 and 7) – Data was analyzed using Spearman Rank Correlation Coefficient. There was no difference between treatments in Lake County; however, there were significantly fewer strikes in Yuba County and in both counties combined.

Fruit russeting (Tables 8 to 12) – As in 2010, there was no difference in russeting between treatments. However, there was a trend (0.09) toward increased average russeting at one Yuba County location.

CONCLUSIONS AND 2012 PLANS

LAMP and dilution plate results from this second year of testing continues to 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, with corresponding average reduction in average Log (CFU) per flower at mid-bloom and full-bloom. There were no differences between treatments in Lake County; however, there were significantly fewer strikes in Yuba County and in both counties combined. Results suggest that the use of delayed-dormant copper should continue to be explored as a new tool in fire blight IPM programs to reduce initial inoculum. Russet evaluations performed at UC Berkeley (Tables 8 to 12) revealed no significant difference between copper and untreated fruit. However, there was a trend (0.09) toward increased average russeting at one Yuba County location. In general, it appears that delayed-dormant applications prior to green tip are safe for pears destined for fresh market.

Badge X_2 cost was about $7 per lb. in 2011, less expensive than Kocide 3000 used in 2010.. 2012 retail price for one every row (two every other row) application 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 $12. If two antibiotic applications are eliminated, net savings for material alone would be $18. 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 can feasibly reduce the overall cost of a fire blight IPM program at the current price of Badge 2_X or similarly priced copper. In any case orchards with severe fire blight history, indicated antibiotic resistance, as well as organic orchard not marketing under IFOAM (which disallows copper) will benefit the most from this strategy.

LAMP results again correlated well with risk model output (Table , Figure) The LAMP assay again 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.

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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, and petal fall in Lake and Yuba Counties, CA, 2011.

	Bloom Stage							
	Mid Bloom		Full Bloom		Petal Fall		Total	
Treatment[1]	3/31-4/19/2011		4/8-22/2011		4/26-5/26/2011			
	No.	Log10	No.	Log10	No.	Log10	No.	Log10
Copper + oil	0.02	<0.01	0.00	<0.01	0.32	0.81	0.09	0.21
Oil alone	0.00	<0.01	0.13	1.80	0.62	0.85	0.22	0.30
P-value[2]	0.34	0.34	0.02	0.06	0.02	0.84	0.01	0.30
Treated	n=41	n=41	n=39	n=39	n=28	n=28	n=108	n=108
Untreated	n=38	n=38	n=37	n=37	n=29	n=29	n=104	n=104

[1] Additional positive LAMP samples (treated: Mid=1, PF=2 and untreated: Mid=4, FB=2, PF=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, and petal fall in Lake County, CA, 2011.

	Bloom Stage							
	Mid Bloom		Full Bloom		Petal Fall		Total	
Treatment[1]	3/31-4/19/2011		4/12-22/2011		5/12-26/2011			
	No.	Log10	No.	Log10	No.	Log10	No.	Log10
Copper + oil	0.04	<0.01	0.00	0.00	0.53	1.44	0.14	0.33
Oil alone	0.00	0.00	0.17	0.59	0.94	1.39	0.33	0.62
P-value[2]	0.37	0.37	0.03	0.06	0.01	0.99	0.01	0.16
Treated	n=24	n=24	n=24	n=24	n=15	n=15	n=63	n=63
Untreated	n=20	n=20	n=23	n=23	n=17	n=17	n=60	n=60

[1] Additional positive LAMP samples (treated: Mid=1, PF=2 and untreated: Mid=4, FB=1, PF=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 3. Average number positive LAMP samples per 300 flower clusters and average Log 10 *E. amylovora* per flower at mid-bloom, full bloom, and petal fall in combined Dole and Dantoni orchards, Sutter County, CA, 2011

	Bloom Stage							
	Mid Bloom		Full Bloom		Petal Fall		Total	
Treatment	3/31/2011		4/8-12/2011		4/26/2011			
	No.	Log10	No.	Log10	No.	Log10	No.	Log10
Copper + oil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Oil alone[1]	0.00	0.00	0.07	0.01	0.17	0.08	0.07	0.02
P-value[2]	~	~	0.28	0.28	0.15	0.15	0.08	0.12

~ No *E. amylovora* detected.

Treated	n=15	n=15	n=18	n=18	n=12	n=12	n=45	n=45
Untreated	n=18	n=18	n=15	n=15	n=12	n=12	n=45	n=45

[1] One positive LAMP sample (Dantoni, untreated-FB) 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 4. Average number positive LAMP samples per 300 flower clusters and average Log 10 *E. amylovora* per flower at mid-bloom, full bloom, and petal fall in Dantoni orchard, Yuba County, CA, 2011.

	Bloom Stage							
	Mid Bloom		Full Bloom		Petal Fall		Total	
Treatment	3/31/2011		4/8-12/2011		4/26/2011			
	No.	Log10	No.	Log10	No.	Log10	No.	Log10
Copper + oil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Oil alone[1]	0.00	0.00	0.11	0.01	0.17	0.08	0.08	0.03
P-value[2]	~	~	0.33	0.33	0.34	0.34	0.15	0.24

~ No *E. amylovora* detected.

Treated	n=9	n=9	n=9	n=9	n=6	n=6	n=24	n=24
Untreated	n=9	n=9	n=9	n=9	n=6	n=6	n=24	n=24

[1] One positive LAMP sample (Dantoni, untreated-FB) 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, and petal fall in Dole orchard, Yuba County, CA, 2011.

	Bloom Stage							
	Mid Bloom		Full Bloom		Petal Fall		Total	
Treatment	3/31/2011		4/8-12/2011		4/26/2011			
	No.	Log10	No.	Log10	No.	Log10	No.	Log10
Copper + oil	0.0	0.0	0.0	0.0	0.00	0.00	0.00	0.00
Oil alone	0.0	0.0	0.0	0.0	0.17	0.08	0.05	0.02
P-value[1]	~	~	~	~	0.34	0.34	0.32	0.32
Treated	n=9	n=9	n=6	n=6	n=6	n=6	n=21	n=21
Untreated	n=9	n=9	n=6	n=6	n=6	n=6	n=21	n=21

~ No *E. amylovora* detected.

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

Table 6. Average number of fire blight strikes in Dole and Dantoni orchard, Lake and Yuba Counties, 2011

Orchard	Lake County (5/24-6/8)	Yuba County (5/27-7/8)	Combined Lake and Yuba Counties
Treated	26.3	89.6	68.5
Control	49.3	97.1	81.1
P-value[1]	NS (0.60)	* (0.03)	* (0.04)
Sample size (complete cases)	n = 7	n = 14	n = 21

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

Table 7. Average number of fire blight strikes in Dole and Dantoni orchard, Yuba County, CA, 2011.

Orchard	Dole (5/27-6/10)	Dantoni (6/6-7/8)	Combined Dole and Dantoni
Treated	91.8	85.6	89.6
Control	108.3	76.8	97.1
P-value[1]	* (0.04)	NS (0.20)	* (0.03)
Sample size (complete cases)	n = 9	n = 5	n = 14

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

Table 8. Comparison of average fruit russeting, percent russet severity and percent frost damage in Bartlett pears[3] harvested in Lake and Yuba Counties, CA, 2011.

			Russet Severity					
	Average Russeting		(greater than 7%)		(less than 3%)		Frost Damage (%)	
Treatment[2]	Copper + oil	Oil alone	Copper + oil	Oil alone	Copper + oil	Oil alone	Copper + oil	Oil alone
Lake County	2.5	2.8	9.8	12.9	76.5	75.0	8.8	5.7
Yuba/Sutter Counties	2.9	2.5	11.1	7.4	75.3	77.3	0.2	0.2
P-value[1]	0.31	0.72	0.8	0.48	0.90	0.84	0.01	0.08

[1] Means analyzed using T-test, P< 0.05.

[2] Treated and control: n=6.

[3] Samples rated August 12, 2011

Table 9. Average fruit russeting, percent russet severity and percent frost damage in Bartlett pears[3] harvested in Lake County, CA, 2011.

Treatment[2]	Average Russeting	Russet Severity (greater than 7%)	Russet Severity (less than 3%)	Frost Damage (%)
Copper + oil	2.5	9.8	76.6	8.8
Oil alone	2.8	12.9	75.0	5.7
P-value[1]	0.76	0.71	0.91	0.45

[1] Means analyzed using T-test, P< 0.05.
[2] Treated and control: n=12.
[3] Samples rated August 12, 2011

Table 10. Average fruit russeting, percent russet severity and percent frost damage in Bartlett pears[3] harvested in Dole and Dantoni orchards in Yuba County, CA, 2011.

Treatment[2]	Average Russeting	Russet Severity (greater than 7%)	Russet Severity (less than 3%)	Frost Damage (%)
Copper + oil	2.9	11.1	75.3	0.2
Oil alone	2.5	7.4	77.3	0.2
P-value[1]	0.46	0.30	0.77	1.00

[1] Means analyzed using T-test, P< 0.05.
[2] Treated and control: n=6.
[3] Samples rated August 12, 2011

Table 11. Average fruit russeting, percent russet severity and percent frost damage in Bartlett pears[3] harvested in Dantoni orchard in Yuba County, CA, 2011.

Treatment[2]	Average Russeting	Russet Severity (greater than 7%)	Russet Severity (less than 3%)	Frost Damage (%)
Copper + oil	3.7	15.9	65.1	(No damage)
Oil alone	3.0	10.3	72.7	(No damage)
P-value[1]	0.09	0.18	0.22	

[1] Means analyzed using T-test, P< 0.05.
[2] Treated and control: n=3.
[3] Samples rated August 12, 2011

Table 12. Average fruit russeting, percent russet severity and percent frost damage in Bartlett pears[3] harvested in Dole orchard in Yuba County, CA, 2011.

Treatment[2]	Average Russeting	Russet Severity (greater than 7%)	Russet Severity (less than 3%)	Frost Damage (%)
Copper + oil	2.1	6.5	85.6	0.5
Oil alone	2.1	4.5	81.8	0.5
P-value[1]	0.96	0.64	0.65	1.00

[1] Means analyzed using T-test, P< 0.05.
[2] Treated and control: n=3.
[3] Samples rated August 12, 2011

Table 13. Accumulated Zoller 'California' Model degree-hours (base < 65F) on (sampling dates) in Yuba County versus Lake County, 2011

Location	Mid Bloom	Full Bloom	Petal Fall
Yuba County	188 (3/31)	392 - 12[1]* (4/8-4/12)	361[1] (4/26)
Lake County	229 - 96[1]* (3/31-4/18)	0 - 98[1] (4/12-4/22)	897 - 282[1]* (5/12-5/26)

[1] LAMP detection of *E. amylovora*. Note that the detection threshold of this model in Yuba County is 150 degree-hours versus detection level of 250 degree-hours in Lake County. (Source: Broc Zoller, personal communication).

[2] Degree-hour data taken form Yuba City, Sutter County.

* Four-day crash occurred; see graph for crash dates.

Figure 1.

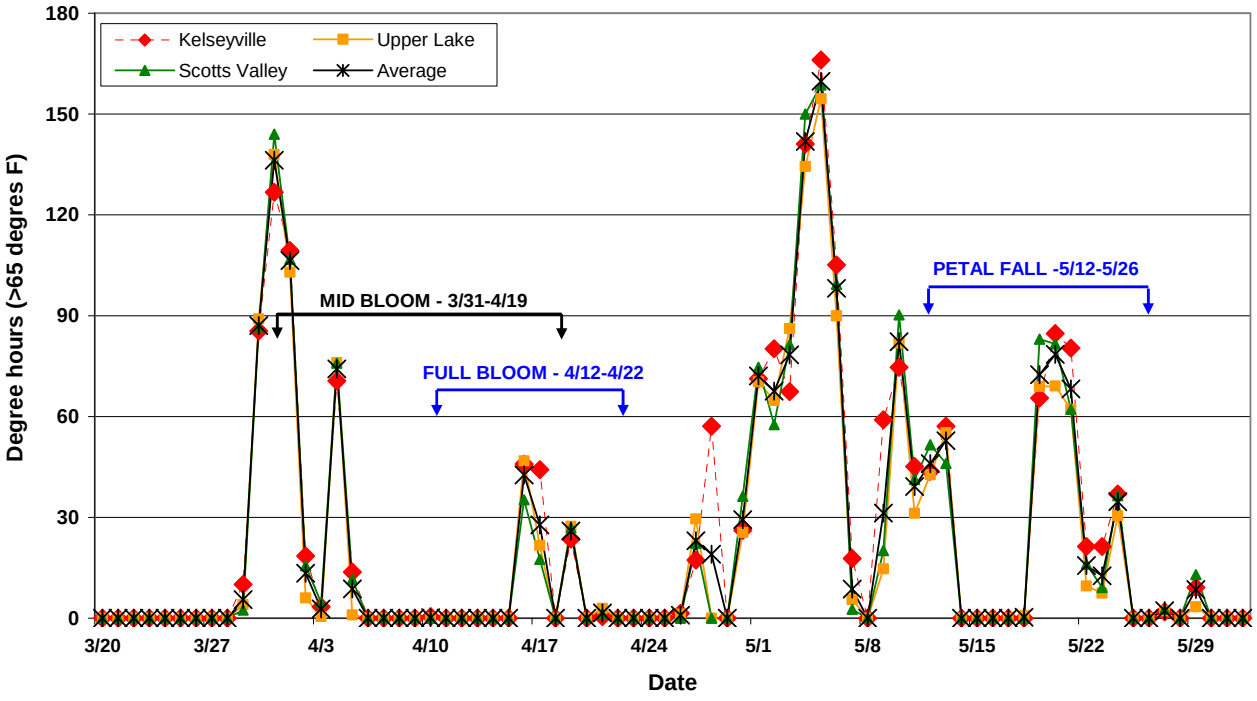

Figure 1: Relationship between degree hour (base >65°F) for Kelseyville, Scotts Valley (Lakeport) and Upper Lake, Lake County, California, March 20 to June 1, 2011 and positive (shown in blue) and negative (shown in black) LAMP samples.

Figure 2.

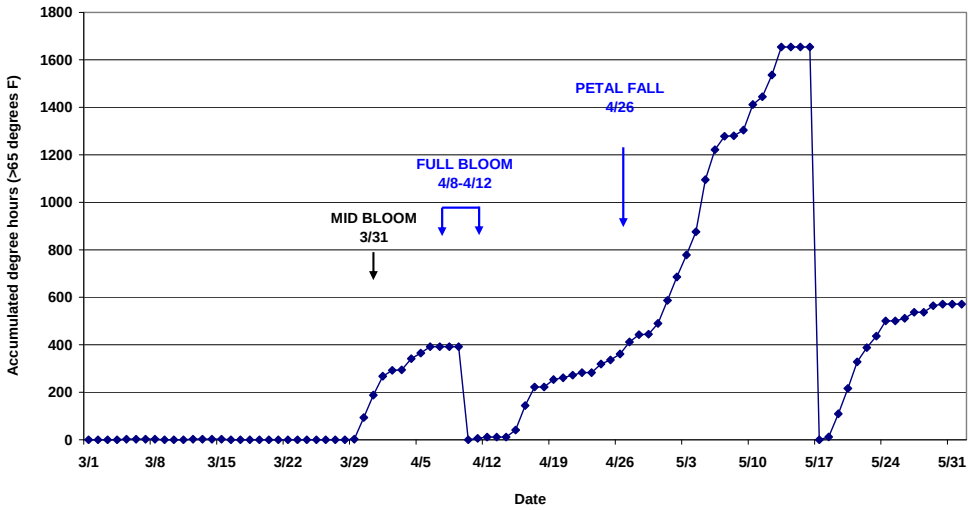

Figure 2: Relationship between accumulated degree hours (base >65°F) for Yuba County, California, March 1 to June 1, 2011 and positive LAMP samples (shown in blue). (Taken from Yuba City station, Sutter County)