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 have led to renewed interest in this tactic. Orchards were treated from 2010 to 2013 slightly before green tip to avoid potential russetting, with positive results in three of the four years. 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. Only in 2012, and in the Sacramento Delta district in all years, did populations of *E. amylovora* remain very low through the sampling period, with no statistical difference between treated and untreated plots. In these cases, long extended periods of cool and (in the case of 2012) wet weather between the copper application and infection period likely diminished the amount of copper on treated buds. There were also significantly fewer fire blight strikes in treated plots. There were no differences in russet or frost damage in any year. Four years of testing have confirmed the usefulness of the delayed dormant application strategy when the period between application and inoculum development is no more than about 40 days and there is a measurable amount of inoculum from mid-bloom through petal fall.

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 2004). The green tip timing, or just before bloom, was designated to ensure an adequate reservoir of intact copper when overwintering 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 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.

Conclusions from earlier studies about copper efficacy 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 et al. 1995) and more recently, loop-mediated amplification (LAMP) (Temple and Johnson, 2011; 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 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, and continued through 2013 to obtain robust verification of using this strategy to reduce initial *E. amylovora* inoculum. 2013 was the fourth and final year of this project.

2013 Continuing Objectives

1) Complete one final year to verify the efficacy and usefulness of (delayed) dormant copper treatments as a means to reduce inoculum and hence, delay and/or reduce fire blight infection;

2) Utilize the field-based technique (LAMP) to rapidly assess the amount of existing inoculum on blooms, in order to make timely treatment decisions; as previously mentioned, this technique is now being used commercially;

MATERIALS AND METHODS

8-acre sections of five orchards in Lake County, six orchards in Yuba County, and four orchards in Sacramento County with a history of fire blight were randomly divided into two 4-acre sections and either treated with formulated copper product 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 by cooperating growers at 125 gpa 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 (300 total) 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. A total of 30 100-cluster samples was collected from each treatment section in each of the sample 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 will indicate a positive LAMP reaction. Samples were then subjected 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 and count of fire blight strikes from late April through early June, as well as correlated with the Washington State University risk model, Cougarblight (Smith 2010) and the Zoller "California" risk model (Gubler et al. 2007, Zoller 1990). Fruit was collected from each treatment section just prior to harvest and rated for russet presence and severity at UC Berkeley.

RESULTS

2013 LAMP results – The seasonal total number of positive LAMP samples was significantly higher (p = 0.05) in oil alone versus copper plus oil (0.37 vs. 0.26 per 600 flowers) (Table 1). This pattern significance was similar to that of 2010 and 2011, but differed from 2012 (Table 4). Average log^{10} of colony forming units was very significantly higher for oil alone (1.88 vs. 1.06), the first year of significant differences in log values.

In contrast to previous years, differences were significant in mid-bloom rather than full bloom through rat tail, however, there was considerable overlap between sampling stages in 2013 due to rapid bloom and early fruit set weather (Table, 5, Figures 1-4). There were also location differences. The number of positive samples/600 flowers was numerically (0.29 treated vs. 0.41 untreated), but not statistically (p = 0.18) different in Lake County; log^{10} was also numerically 1.4 vs. 2.0 colony forming units) but not significantly (p = 0.20) different (Table 2). In contrast, Upper Sacramento Valley results were variable, but always numerically in favor of the copper treatment and Log^{10} highly significant in the case of one orchard and the two combined locations. Again, the earlier sampling period were the most significant (Table 3). As in previous years, very little or no inoculum was detected in samples from Sacramento County orchards (Table 6). There was only one positive LAMP sample from Sacramento County in 2013 and there were no significant differences between treatments in any respect.

2010-2013 cumulative results – Over the entire four year period (n=357 treated, 352 untreated), the number of positive LAMP samples very highly significantly higher (p = .0001) from untreated sites (0.26 vs. 016 per flower, a nearly 40% reduction). Log^{10} colony forming units in the treated samples (p = 0.10; 0.20 untreated, 0.13 treated, or 35% fewer colonies) (Table 5). As stated above, these results are possibly the cumulative effect of annual treatments to the same sites and demonstrates the concept of long-term inoculum reduction, analogous to mating disruption to reduce overwintering codling moth populations.

Fire blight strikes – The number of strikes was (close to) significantly reduced in all three years they were evaluated. There were more strikes in the copper-treated plots in 2012, but not significantly more (p = .07) (Table 7).

Fruit russeting and frost damage – There was no difference in russeting between treatments in Lake County in 2013. Fruit from the sampled Yuba County orchard decayed in storage before it could be rated. From 2010-2013, there has been no statistical difference between treatments using 6 lbs. per acre of copper ammonium hydroxide (Badge X2) and no observed frost damage (Table 8).

DISCUSSION

LAMP and dilution plate results from 2013 resembled those of 2010 and 2011, in that they showed that delayed dormant copper applications significantly reduced the amount of *E. amylovora* inoculum. In contrast, while the pattern of inoculum build up in 2012 was similar to 2010, 2011, and 2013, the overall level was negligible through the sampling period (Figure 5). Weather pattern is highly relevant to inoculum development. Versus the other three years, 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. This pattern contrasts with other years 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 (Figure 6). Thus, in 2012, though total spring rainfall from application to early bloom was no more than in other years, the amount of total active copper remaining at the time of bloom in 2012 was likely diminished due to the longer period between application and timing of flower opening and/or critical degree hour accumulation threshold (Table 4). This likely resulted 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 2013.

The Sacramento Delta has differed considerably from other locations in that over the course of the project, very little inoculum has been present in any of the LAMP samples, however, what little has shown up has been in petal fall samples, consistent with other districts (Tables 5-6). The lack of more robust inoculum levels might possibly be due to the earlier bloom and sampling regime prior to significant inoculum increase under Sacramento Delta risk conditions. Indeed, there is a perception of a greater amount of late season shoot blight in the Delta growing area. The fire blight season in the Sacramento Delta may be more consistently analogous to the 2012 season in which the interval between applying copper to incidence of positive LAMP samples may exceed the effective life of the treatment. It would be useful to vary application timing to learn if this would enhance the usefulness of the treatment at that location.

The number of recorded fire blight strikes can be related to LAMP results in that in 2012, there were actually more strikes in untreated blocks (Table 7). This is likely because the greatest benefit of the treatment was in orchards harboring more overwintering inoculum, and thus were more susceptible to in-season infection unchecked by the copper treatment. This may be seen in Figures 7a and 7b, which show that while 50% of total number of treated orchards benefitted from the copper treatment, those with high amounts of inoculum benefitted the most, particularly when the copper was applied closer to bloom.

Russet and frost evaluations performed at UC Berkeley revealed no significant difference between treated and untreated orchards in any year. 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 (Table 8).

SUMMARY AND CONCLUSIONS

Badge X2 cost was about $7.50 per lb. in 2013, or $45 per acre for the delayed-dormant spray ($45 in 2011). 2014 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. Most importantly, labor savings would be achieved by a reduced need to cut blight during and after the growing season. In summary, the delayed-dormant copper application could feasibly reduce the overall cost of a total fire blight IPM program, (including cutting) 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. Four years of results, 2010-2013 suggest that the treatment has been effective in three of four years, a probability of success greater than 50%.

As in past years, the 2013 outcome again demonstrated that LAMP results correlated will 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.

Based on results from 2010-2013 (Elkins, et al., 2012; Elkins et al., 2011), delayed dormant copper applications have been incorporated into the integrated fire blight management program being developed for organic growers faced with loss of antibiotics after the 2014 season, and can be a valuable tactic in conventional programs as well. Growers and PCAs now have access to a tool to utilize as part of their overall fire blight management program using a number of available registered products.

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REFERENCES

Anon. Revised 1972. Blight of pears, apples, and quinces. U.S. Department of Agriculture Leaflet No. 187.

Burr, T.J., H.S. Aldwinckle and S.V. Beer. 1980. Fire blight: Tree Fruit IPM Dis. Ident. Sheet No. 3. New York Agricultural Experiment Station.

Elkins, R., K. Johnson, S. Lindow and B. Zoller. 2012. Evaluation of delayed dormant copper as a component of a fire blight IPM program. 2011 California Pear Research Reports p. 78-90.

Elkins, R., K. Johnson, F. Niederholzer, S. Lindow and B. Zoller. 2011. Evaluation of delayed dormant copper as a component of a fireblight IPM program. 2010 California Pear Research Reports p. 88-93.

Ellis, M.A. 1980. Evaluation of a copper and oil dormant spray and streptomycin bloom sprays for control of fire blight in apple, 1980. Fungicide and Nematicide Tests 36:6.

Ellis, M.A. 1981. Evaluation of compounds for control of apple fire blight during bloom, 1981. Fungicide and Nematicide Tests 37:4.

Gubler, W.D, B.G. Zoller, R.A. Duncan, and S. Lindow. 2007. Diseases. In: Pear Production and Handling Manual, University of California Division of Agriculture and Natural Resources. Oakland, California, p. 145-156.

Johnson, K. and T.N. Temple. 2013. Evaluation of strategies for fire blight control in organic pome fruit without antibiotics. *Plant Disease* 97:(in press).

Johnson, K. and V. Stockwell. 2008. Development of a rapid detection protocol for the fire blight pathogen of pear and apple. CRIS No. 0214016.

Lindow, S., G. McGourty and R. Elkins. 1995. Control of fire blight, frost injury, and fruit russet using cultural, chemical and biological controls. 1994 Reports on Research Projects for California Bartlett Pears.

OptiGene. 2011. Genie® II field trial September 2011. http://www.optigene.co.uk/downloads/GenieR%20II%20Field%20trial%20September%20201.pdf. (accessed December 30, 2011).

Powell, D. 1965. Factors influencing the severity of fire blight infections in apple and pear. Proceedings 94th Annual Meeting, Michigan State Horticultural Society\ p.______.

Powell, D. and J.F. Reinhardt. 1955. The effect of copper sulfate as a dormant spray for fire blight control. Transactions of the Illinois State Horticultural Society p. 161-166.

Reimer, F.C. 1925. Value of Bordeaux mixture in blight control. Ann. Rept. Oregon Hort. Soc. p. 136-142.

van der Zwet, T. and S.V. Beer. 1999. Fire Blight - its Nature, Prevention, and Control. Bull. No. 631. USDA-ARS.

Smith, T. J. 2010 "Cougarblight 2010 EZ-F" Fire Blight Infection Risk Model. *http://www.ncw.wsu.edu/treefruit/documents/CougarBlight 2010EZver41F_001.xls* (accessed 12/29/2010).

Steiner, P.W. 2000. Integrated Orchard and Nursery Management for the control of fire blight. In: Fire Blight; The Disease and its Causative Agent, *Erwinia amylovora.* CABI International, Wallingford, Oxon, UK.

Temple, T.N. and K.B. Johnson. 2011. Evaluation of Loop-mediated isothermal amplification for rapid detection of *Erwinia amylovora* on pear and apple flowers. Plant Disease 95:423-430.

Temple, T.N. and K.B. Johnson. 2009. Rapid and early detection of *Erwinia amylovora* in pear and apple orchards using loop-mediated isothermal amplification (LAMP) (poster). Annual Meeting American Phytopathological Society.

Van der Zwet, T. and S.V. Beer. 1999. Fire Blight – its Nature, Prevention, and Control. Bull. No. 631. USDA-ARS.

Wilcox, W.F. 2004. Fire blight: Tree Fruit Crops Disease Identification Sheet No. D3 (rev.)

Table 1: Average number positive LAMP samples and average Log[10] of colony forming units for *E. amylovora* per 600 flowers detected in mid-bloom, full bloom, petal fall and rat tail samples collected from 12 orchards in Lake[2] and Yuba[3] Counties, California, 2013.

Treatment	Bloom Stage: Mid Bloom 3/25-4/2*		Full Bloom 3-26-4/8		Petal Fall 4/2-4/12*		Rat Tail 4/16-4/30		Total 3/25-4/30	
	No./600 flowers	Log[10]	No./600 flowers	Log[10]	No./600 flowers	Log[10]	No./600 flowers	Log[10]	No./600 flowers	Log[10]
Copper + oil	0.08	0.22	0.13	0.32	0.12	0.34	0.68	3.27	0.26	1.06
Oil alone	0.32	1.50	0.19	0.69	0.23	1.06	0.74	4.33	0.37	1.88
P-value[1]	0.03	0.01	0.50	0.27	0.21	0.08	0.58	0.19	0.05	0.01

Treated	n=25	n=25	n=31	n=31	n=34	n=34	n=31	n=31	n=121	n=121
Untreated	n=25	n=25	n=31	n=31	n=34	n=34	n=31	n=31	n=121	n=121

[1] Means analyzed using T-test, P<0.05. Data normalized with SQRT (value+1) transformation for P-value.
[2] Six plots
[3] Six plots
* Data includes on Bosc orchard in Lake County: Mid Bloom=4/12 and PF 4/22.

Table 2: Percent positive LAMP samples and average Log^{10} of colony forming units for *E. amylovora* detected in all bloom stage samples collected from six orchards in Lake County, California, 2013.

Treatment[1]	Bloom Stage: Mid Bloom 4/1-2*		Full Bloom 4/5-8		Petal Fall 4/10-12		Rat Tail 4/22-30		Total 4/1-30	
	No./600 flowers	Log^{10}	No./600 flowers	Log^{10}	No./600 flowers	Log^{10}	No./600 flowers	Log^{10}	No./600 flowers	Log^{10}
Copper + oil	0.12	0.34	0.31	0.75	0.12	0.41	0.69	4.58	0.29	1.40
Oil alone	0.25	0.95	0.31	1.05	0.31	1.28	0.77	5.14	0.41	2.00
P-value[2]	0.38	0.27	1.00	0.69	0.21	0.15	0.67	0.65	0.18	0.20

Treated	n=16	n=16	n=13	n=13	n=16	n=16	n=13	n=13	n=58	n=58
Untreated	n=16	n=16	n=13	n=13	n=16	n=16	n=13	n=13	n=58	n=58

[1] Additional positive LAMP samples (treated: mid-bloom=1, petal fall=2 and untreated: petal fall=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 for P-values.

[*] Data includes on Bose orchard: Mid-bloom = 4/12, PF = 4/22.

Table 3: Average number positive LAMP samples and average Log^{10} of colony forming units for *E. amylovora* per 600 flowers detected in all bloom stage samples collected from six orchards in Yuba County, California, 2013.

	Bloom Stage									
	Mid Bloom		Full Bloom		Petal Fall		Rat Tail		Total	
Treatment	No./600 flowers	Log^{10}	No./600 flowers	Log^{10}	No./600 flowers	Log^{10}	No./600 flowers	Log^{10}	No./600 flowers	Log^{10}
Dantoni	3/25		3/29		4/3		4/16			
Copper + oil	0.00	0.00	0.00	0.00	0.22	0.57	1.00	3.66	0.30	1.06
Oil alone	0.44	2.4	0.22	0.87	0.33	1.71	1.00	5.73	0.50	2.67
P-value[1]	0.02	0.02	0.15	0.15	0.62	0.31	-	<0.001	0.09	0.01
Dole	No data[2]		3/26		4/2		4/16			
Copper + oil	---	---	0.00	0.00	0.00	0.00	0.33	1.00	0.11	0.33
Oil alone	---	---	0.00	0.00	0.00	0.00	0.44	1.76	0.15	0.58
P-value[1]	~	~	~	~	~	~	0.65	0.45	0.69	0.51
Dantoni and Dole Combined										
Copper + oil	---	---	0.00	0.00	0.11	0.28	0.67	2.32	0.22	0.75
Oil alone	---	---	0.11	0.43	0.17	0.86	0.72	3.74	0.35	1.77
P-value[1]	~	~	0.15	0.15	0.64	0.34	0.73	0.14	0.12	0.01

~*No E amylovora*
[1]Means analyzed using T-test, $P<0.05$. Data normalized using (SQRT+1) transformation for P-values.
[2] No samples collected at mid-bloom in Dole orchard.

Dantoni: treated and untreated, n=9, Total data: treated, n=36, untreated, n=36. Dole: treated and untreated, n=9, Total data: n=27. Dantoni and Dole combined: n=18, Total data: treated, n=63, untreated, n=63.

Table 4: Average number positive LAMP samples and average Log^{10} of colony forming units for *E. amylovora* per flower (600 flowers in 2013) detected in all bloom stage samples collected in 12 orchards in Lake and Yuba Counties, California, 2010-2013.

	2010 Spray dates not available		2011 Treatment applied 36 (Yuba) to 37 (Lake) days prior to mid-bloom		2012 Treatment applied 28 (Yuba) to 59 (Lake) days prior to mid-bloom		2013 Treatment applied 25 (Yuba) to 35 (Lake) days prior to mid-bloom	
Treatment	No./flower	Log^{10}	No./flower	Log^{10}	No./flower	Log^{10}	No./ 600 flowers	Log^{10}
Copper + oil	1.0	1.6	0.10	0.22	0.11	0.11	0.26	1.06
Oil alone	1.1	1.7	0.23	0.32	0.11	0.14	0.37	1.88
P-value[1]	0.11	0.69	0.01	0.30	0.98	0.68	0.05	0.01

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

Table 5: Average number positive LAMP samples and average Log10 of colony forming units for *E. amylovora* per flower detected in all bloom stage samples collected from 12 orchards treated with and without delayed dormant copper in Lake and Yuba Counties, California, 2010-2013.

Treatment[1]	Bloom Stage										Average (all years)	
	Mid Bloom		Full Bloom		Petal Fall		Petal Fall II[2]		Rat Tail[3]			
	No. /flower	Log10	No. /flower	Log10	No. /flower	Log10	No. /flower	Log10	No. /flower	Log10	No. /flower	Log10
Copper + oil	0.04	<0.00	0.04	<0.01	0.21	0.36	0.60	1.54	0.47	0.16	0.16	0.13
Oil alone	0.07	<0.01	0.11	0.07	0.43	0.41	1.00	3.68	0.51	0.19	0.26	0.20
P-value[1]	0.22	0.18	0.05	0.08	0.003	0.60	0.14	0.03	0.72	0.80	0.001	0.10

Treated	n=111	n=111	n=100	n=100	n=80	n=80	n=5	n=5	n=61	n=61	n=357	n=357
Untreated	n=108	n=108	n=97	n=97	n=81	n=81	n=5	n=5	n=61	n=61	n=352	n=352

[1] Two sample comparison with means analyzed using T-test. Data for P-value (<0.05) normalized with (SQRT +1) transformation.
[2] Samples collected only in 2010.
[3] Samples collected only in 2012 and 2013.

Table 6 : Average number positive LAMP samples and average Log ^{10} of colony forming units for *E. amylovora* per flower detected in all bloom stage samples collected from four-six orchards treated with and without delayed dormant copper in Sacramento County, California, 2010-2013.

	Bloom Stage									
	Mid Bloom		Full Bloom		Petal Fall		Rat Tail[2]		Total	
Treatment[1]	No./flower	Log^{10}	No./flower	Log^{10}	No./flower	Log^{10}	No./flower	Log^{10}	No./flower	Log^{10}
Copper + oil	0.03	0.01	0.00	0.00	0.00	0.00	0.42	0.53	0.09	0.09
Oil alone	0.05	0.10	0.00	0.00	0.03	<0.00	0.46	0.26	0.10	0.07
P-value	0.56	0.30	~	~	0.32	0.32	0.58	0.31	0.84	0.59

Treated	n = 39	n = 39	n = 39	n = 39	n = 39	n = 39	n = 24	n = 24	n = 141	n = 141
Untreated	n = 39	n = 39	n = 39	n = 39	n = 39	n = 39	n = 24	n = 24	n = 141	n = 141

[1] Means analyzed using T-test, P<0.05. Data normalized with SQRT (value+1) transformation for P-value.
[2] Rat tail data for years 2012 and 2013 only.

Table 7: Number of fire blight infections[x] observed in 4 ha experimental replications located commercial orchards in Lake and Yuba Counties, California that had received a late dormant (green tip) treatment of horticultural oil plus copper on one half of the plot area and horticultural oil only[y] on the other half during the years of 2010-2013.

			Late dormant treatment		
Year	No. of replications in trial	No. of replications with fire blight	Copper and oil	Oil only	P-value
2010	5	0	-	-	-
2011	13	10	143.9	176.1	0.03
2012	12	7	410.0	281.7	0.07
2013	12	7	90	128.6	0.02

[x] In each replication, the number of fire blight strikes was determined by cutting and counting the number of individual infections on two to five dates between April 17 and June 10.
[y] Rates of fixed copper bactericide and horticultural oil were 5.6-6.7 kg and 37.4 liters, respectively, applied by air blast sprayer in 935 to 1169 L of water per hectare. Kocide 3000 (46% CuOH) was the copper product applied in 2010; Badge X2 (23% CuOH and 25% CuOC1) was applied in 2011 to 2013. The paired control plot received only horticultural oil.
[z] Means analyzed using paired T-test (P = 0.05). Prior to testing, data were normalized with (SQRT +1) transformation; non-transformed means are shown.

Table 8: Average fruit russeting severity and incidence of russet <3% and >7% for Bartlett pears[x] harvested from 4 ha experimental replications located within commercial orchards in Lake and Yuba Counties, California that had received a late dormant (green tip) treatment of horticultural oil plus copper on one half of the plot area and horticultural oil only[y] on the other half during the years of 2010 to 2013.

Year	No. of sections Sampled	Average Russet Severity (%)		Incidence of fruit with ≤3 % russet		Incidence of fruit with ≥7 % russet	
		Copper +oil	Oil Alone	Copper +oil	Oil Alone	Copper +oil	Oil Alone
2010	5	2.2	2.2	91	95	9	5
2011	12	2.7	2.7	76	76	10	10
2012	11	1.4	1.8	88	84	3	5
2013	5	1.0	1.3	99	99	1	1

[x] In each section, 75 to 100 fruit were sampled from both the horticultural oil plus copper and oil only plots; fruit russet ratings were done in a laboratory facility.

[y] Rates of fixed copper bactericide and horticultural oil were 5.6-6.7 kg and 37.4 liters, respectively, applied by air blast sprayer in 935 to 1169 L of water per hectare. Kocide 3000 (46% CuOH) was the copper product applied in 2010; Badge X2 (23% CuOH and 25% CuOC1) was applied in 2011 to 2013. The paired control plot received only horticultural oil.

[z] Within each season, the mean response in the horticultural oil plus copper plot was compared to the oil only plot with a paired T-test (P = 0.05). None of the T-tests were significant (NS); P=values ranged from 0.25 to 0.97. Prior to testing, data were normalized with (SQRT +1) transformation; non-transformed means are shown.

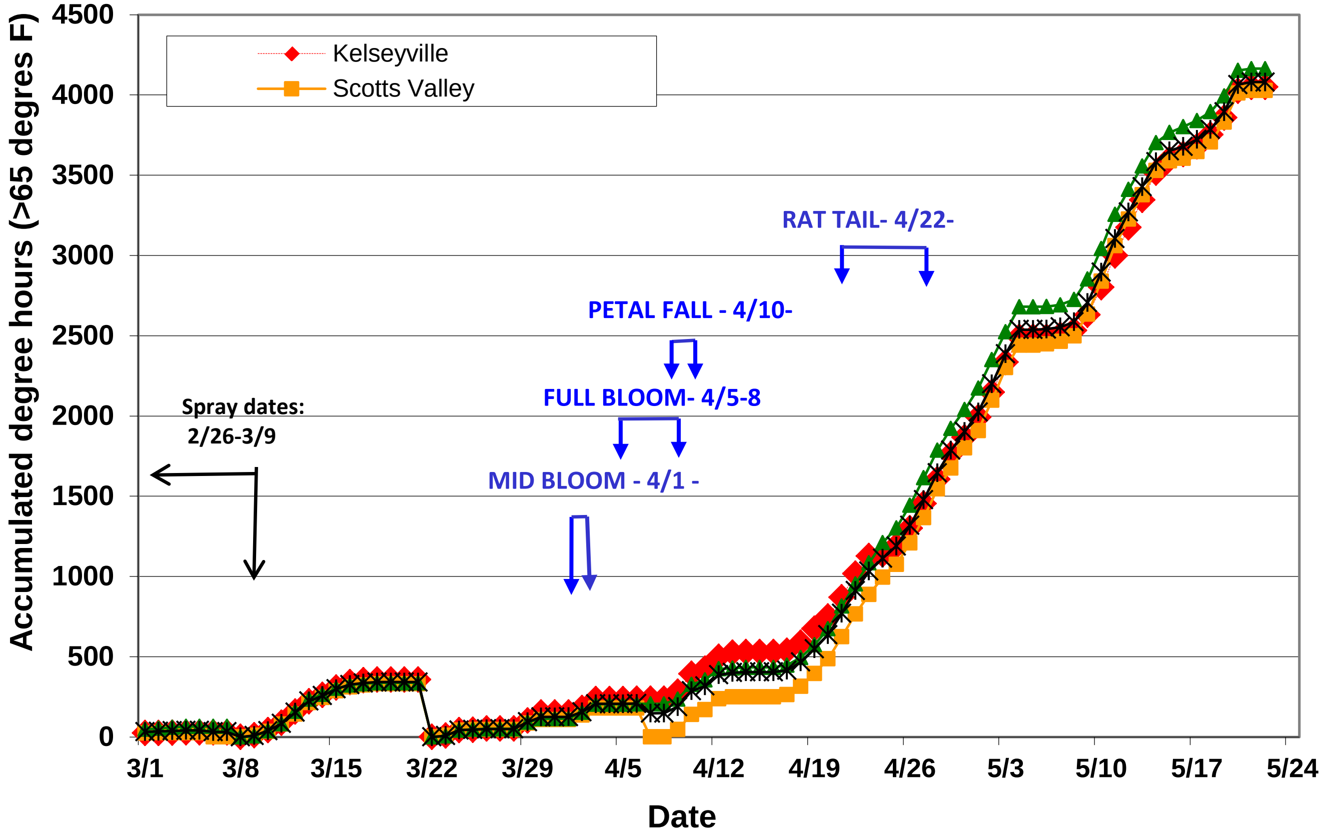

Figure 1: Relationship between accumulated degree hours (base $\geq$ 65 degrees F) for Kelseyville, Scotts Valley (Lakeport) and Upper Lake, Lake County, California, March 1 to May 23, 2013 and positive LAMP samples.

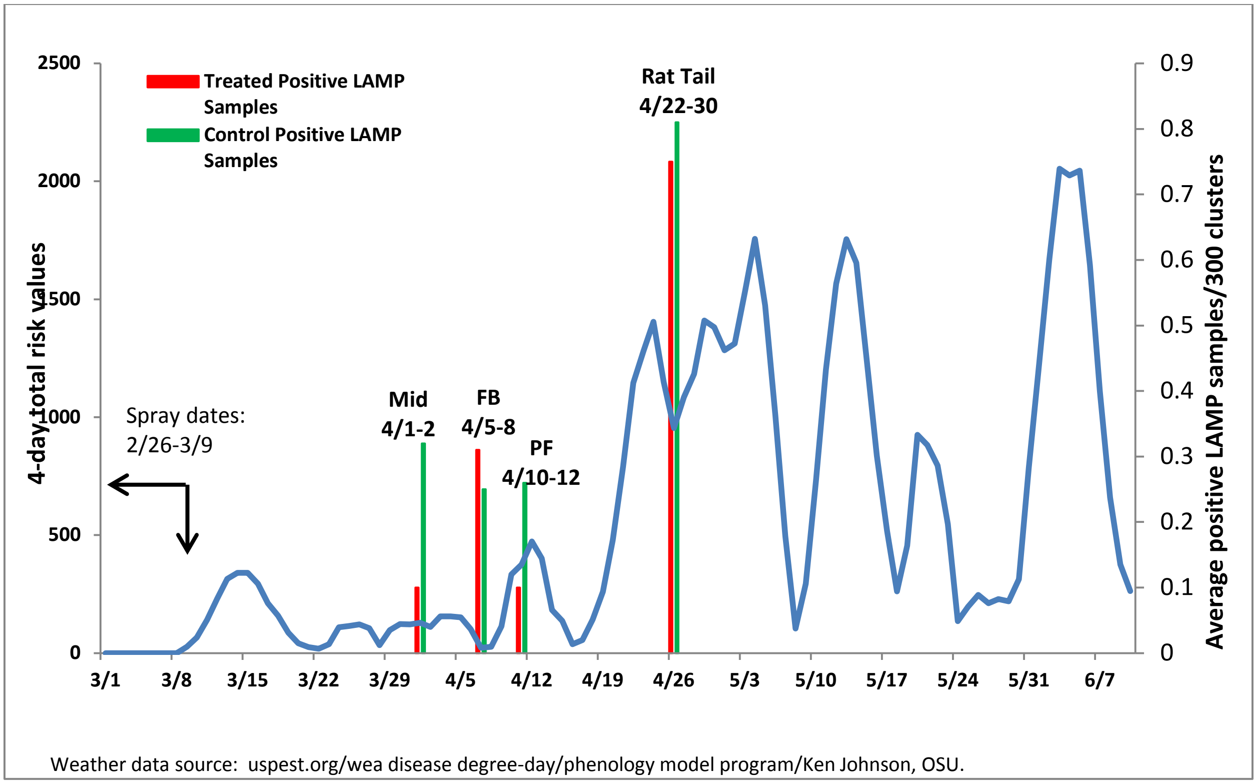

Figure 2: 4-day Cougarblight total temperature risk values and average positive LAMP samples per 600 flowers at varying bloom stages for 6 orchards, Lake County, CA, USA, 2013

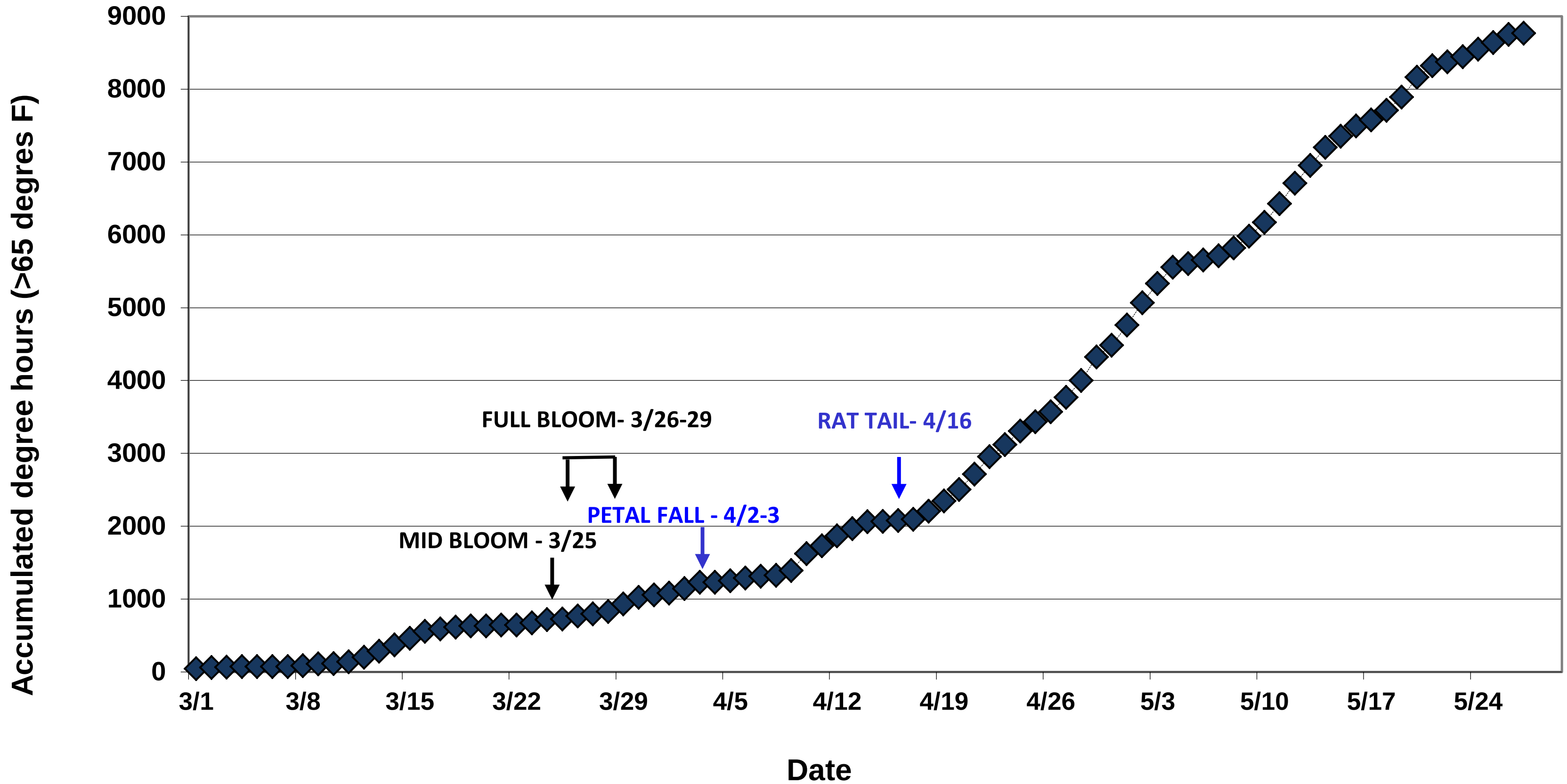

Figure 3: Relationship between accumulated degree hours (base ≥65°F) for Sutter/Yuba County, California, March 1 to May 28, 2013 and positive (shown in blue) and negative (shown in black) LAMP samples. Degree-hours calculated using data from Verona.A (CIMIS #235, Verona, Sutter Co.) and backup station in Woodland, Yolo County, California (Source: UCIPM).

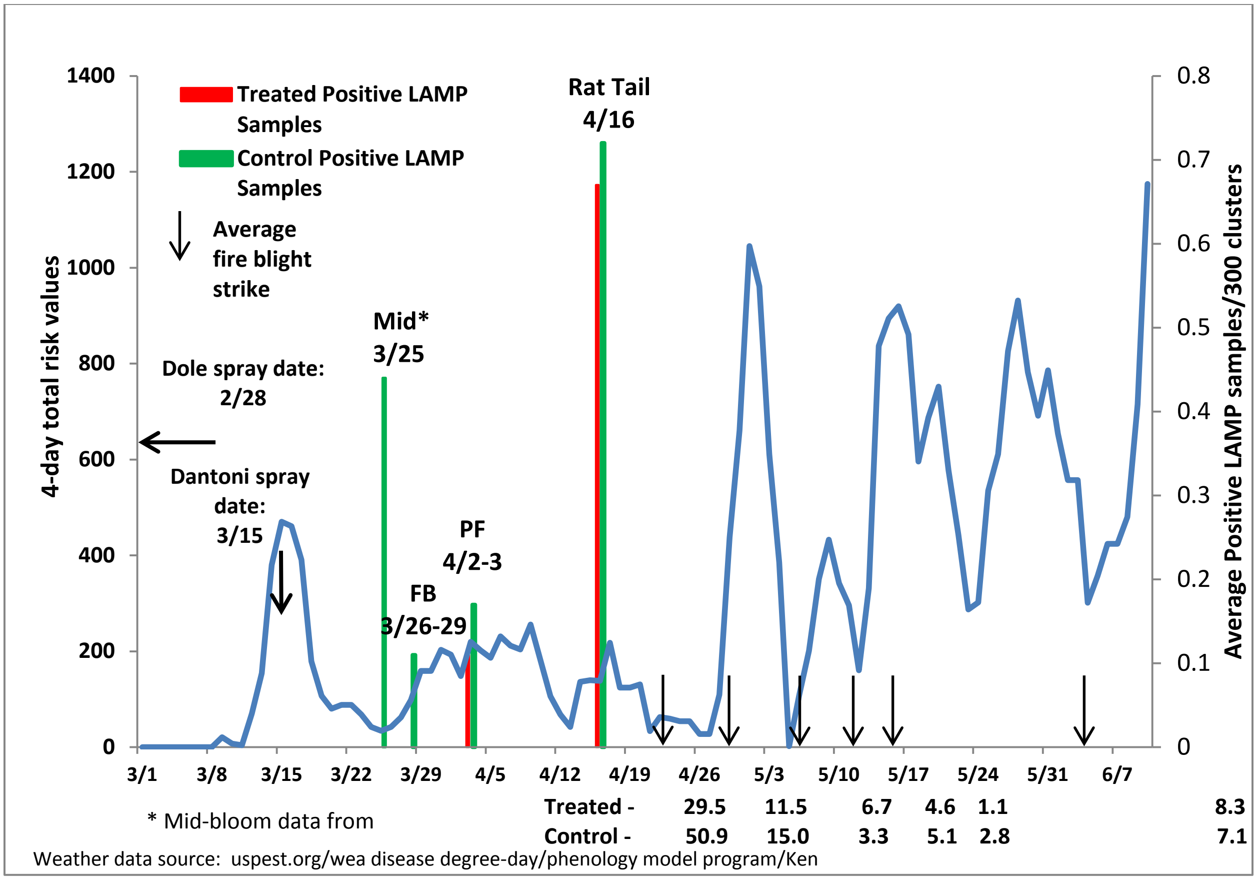

Figure 4: 4-day Cougarblight total temperature risk values and average positive LAMP samples per 600 flowers at varying bloom stages for 6 orchards, Yuba County, California, USA, 2013.

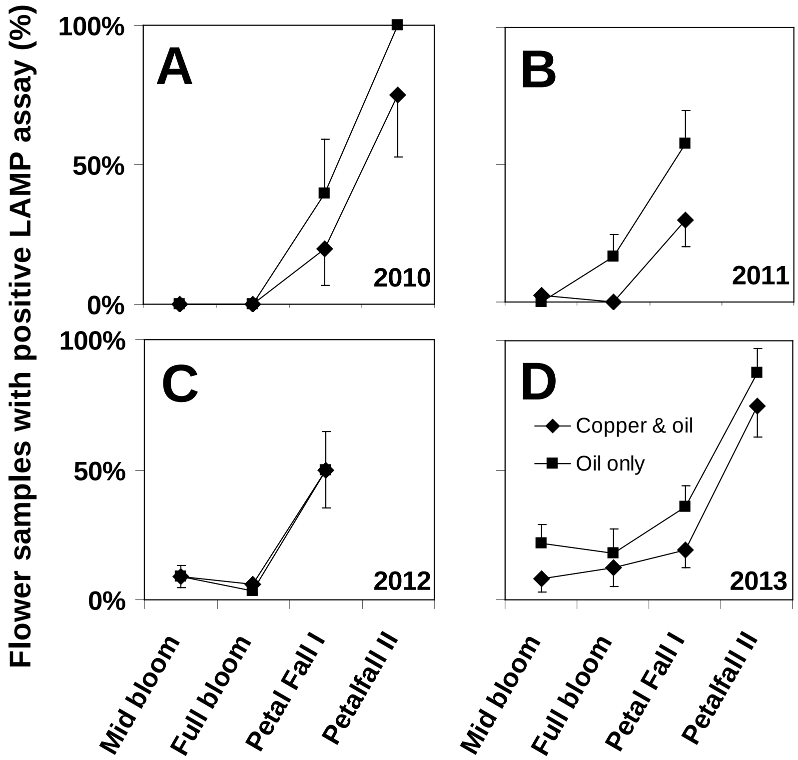

Figure 5: Percent positive loop mediated isothermal DNA amplification (LAMP) assays performed to detect *Erwinia amylovora* in 100-flower cluster samples collected at various bloom stages from commercial pear orchards in Lake and Yuba Counties of California during the bloom period for A-D) 2010 to 2013. Within orchards, five hectare sections were halved with a late dormant (green tip) timing of horticultural oil only applied to one half (solid square markers) and an oil plus copper treatment (solid diamond markers) applied to the other half. On each sampling date, three 100-flower cluster samples were made in each 2.5 ha section, with the washes of these samples subjected to LAMP assay and dilution plating. The number of 2.5-ha sections that comprised means represented by the markers were: 5, 13, 11, and 12 in 2010-2013, respectively. Bars associated with each point represent one standard error of the mean.

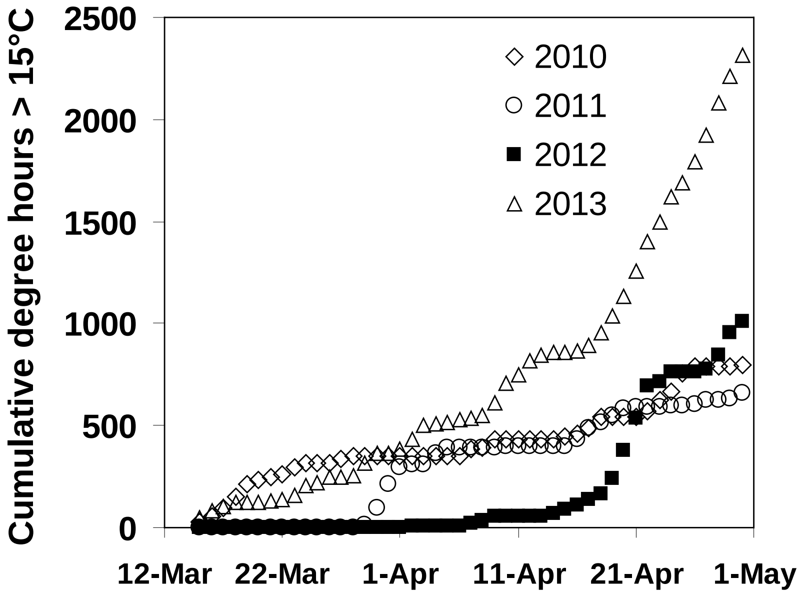

Figure 6: Cumulative degree hours >65 degrees F. for Lakeport, CA from March 15 to April 30 for the years 2010-2013.

Fig. 7a

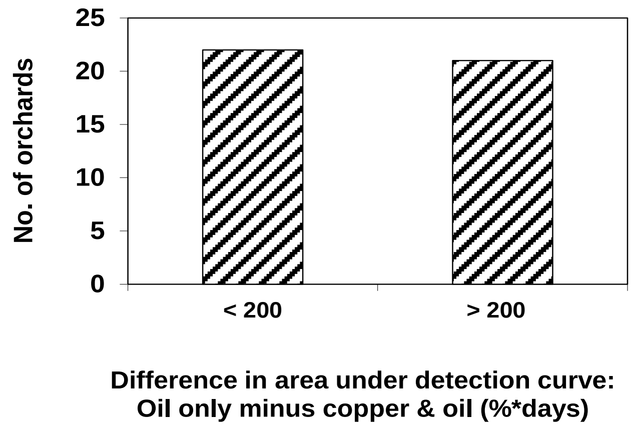

Fig. 7b

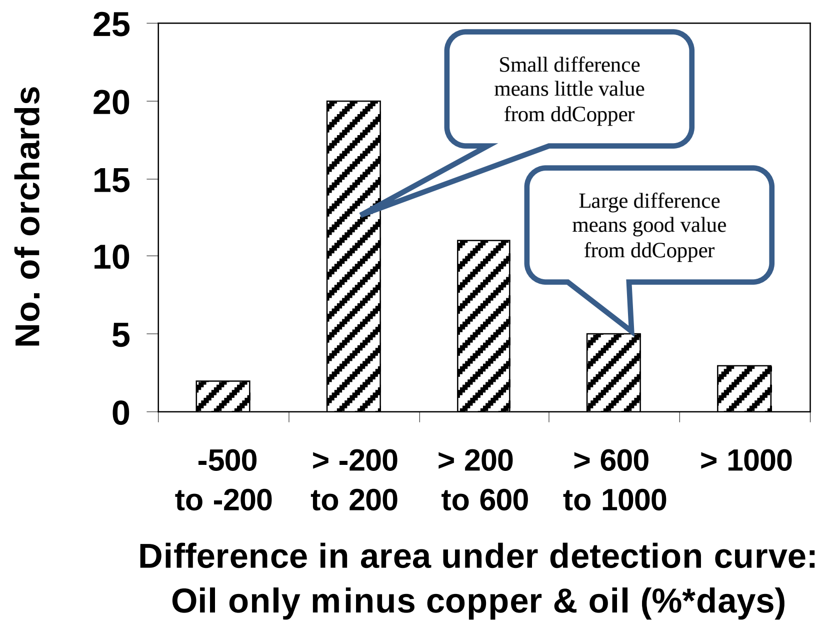

Fig. 7a and 7b. Histograms depicting the magnitude of the difference in area under the detection curve (AUDC) for *Erwinia amylovora* in 100-flower cluster samples collected from 2.5 ha sections of commercial pear orchards treated at a late dormant (green tip) timing with horticultural oil only or with oil plus copper. The orchards were located in Lake and Yuba Counties of California with 41 paired 2.5-ha sections treated and monitored during the years for 2010-2013. Each AUDC was comprised of three 100-flower cluster samples collected at each of several bloom stages: mid-bloom, full bloom, petal fall I, and petal fall II (2010 and 2013 only). From 2010-2013, approximately 50% of the treated orchards benefitted positively from the treatment. Figure 2b shows the range of benefit, from no (-500- -200) to excellent (>1000). Negative benefit (i.e. -500 - -200 was likely due to sampling “noise”. The greatest benefit was to those orchards with a high amount of overwintering inoculum, and those that are able to apply the copper as close to bloom as possible.