CONTROL OF FIRE BLIGHT DISEASE IN PEAR CAUSED BY *ERWINIA AMYLOVORA* USING BIOLOGICAL CONTROL AGENTS, COPPER, AND ANTIBIOTICS

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ABSTRACT

Several commercially-available biological control agents were applied in a replicated single tree trial in Upper Lake, Lake County, California. Treatments included the biological control agents *Pseudomonas fluorescens* A506, sold as Bight Ban A506® (Nufarm Americas, Burr Ridge, Illinois), Bloomtime Biological® FD Biopesticide "Strain E325" (Northwest Agricultural Projects, Pasco, Washington), biological yeast BCY-B, sold as Blossom Protect® (Westbridge, Vista, California), and (new in 2012) the plant extract of giant knot-weed *Reynoutia sachalinensis*, sold as Regalia® (Marrone Bio Innovations, Inc., Davis, California). Biologicals were compared to the grower standard tank mix of the antibiotics streptomycin and terramycin, and to the copper hydroxide plus copper oxychloride product, Badge X2 (Isagro-USA, Morrisville, North Carolina). Treatments were applied variously from April 14 to May 8 from 10% to "rattail" bloom. Fire blight infection potential reached treatable levels on April 19 and continued through June, well beyond the final treatment date. There were very few blight strikes (average 0.4 per week or 2.0 cumulative across treatments). Differences among treatments were insignificant, though there was a trend (p=.10). However, untreated controls harbored the lowest average weekly count (p=.4). thus block differences and treatment x block interactions (each p=.001) likely overwhelmed any treatment effects. Only A506 applied weekly at half rate resulted in greater than 5% of the fruit with noticeable russet (rating >7) perhaps due to diminished viability under warm conditions in late April and early May. BlossomProtect® at 20-30% bloom had the least russet while the 80-90% bloom and full season applications had some russet. There were no significant differences in frost damage.

INTRODUCTION

Until recently, commercial use of biological control of fire blight was largely limited to the formulation of *Pseudomonas fluorescens* A506 (sold as BlightBan A506, Nufarm Americas, Burr Ridge, Illinois). Two new products sold specifically for fire blight became available in 2011: Bloomtime Biological® FD BioPesticide "Strain E325 (Northwest Agricultural Products, Pasco, Washington), and the biological yeast BCY-B, sold as Blossom Protect® (Westbridge, Vista, California). These two products had shown promise in Northwest and California trials and offered another opportunity to expand the repertoire of biological control agents to supplement antibiotics and delay onset of resistance. In addition to the above agents, a more recent phenomenon is the

introduction of products to “boost” host plants’ natural “defense mechanisms” and increase their ability to “fend off” infection and avoid disease. One such product is an extract of giant knotweed, *Reynoutria sachalinensis*, sold as Regalia® (Marronne Bio Innovations, Davis, California). Finally, there has been renewed interest in using copper due to its relatively low cost and the availability of apparently effective fine particle formulations containing less actual metallis, for example, Kocide® 3000 (E.I. DuPont de Nemours and Co., Wilmington, Delaware) and Badge X2 (Isagro-USA, Morrisville, North Carolina).

Testing of these newer materials under North Coast conditions was initiated in 2010 to evaluate for efficacy (number of fire blight strikes) and propensity to reduce or exacerbate frost damage and russet, two conditions often influenced by these materials due to their modes of action. 2010 and 2011 results suggested that all biological control agents were able to colonize blossoms more or less successfully depending on weather conditions, and that the yeast could cause russeting under prolonged wetting conditions (Elkins and Lindow 2011). 2012 was the third year of this trial.

MATERIALS AND METHODS

Trial Design: Randomized complete block, five single-tree replicates.

Trial Location: Mature Bartlett pear orchard, Upper Lake, Lake County, California.
12’ x 24’ spacing, 151 trees per acre, 0.40 acre total.

Treatment Details: Applied at 100 gpa by handgun, 0.66 gal./tree = 2.5 liters/tree; at 10-30% (April 14-15), 40-70% (April 19-21), 90% - full bloom (April 21-24), petal fall (May 1), and rat-tail (May 8), depending on treatment (Table 1). Fire blight infection periods are shown below (Figure A).

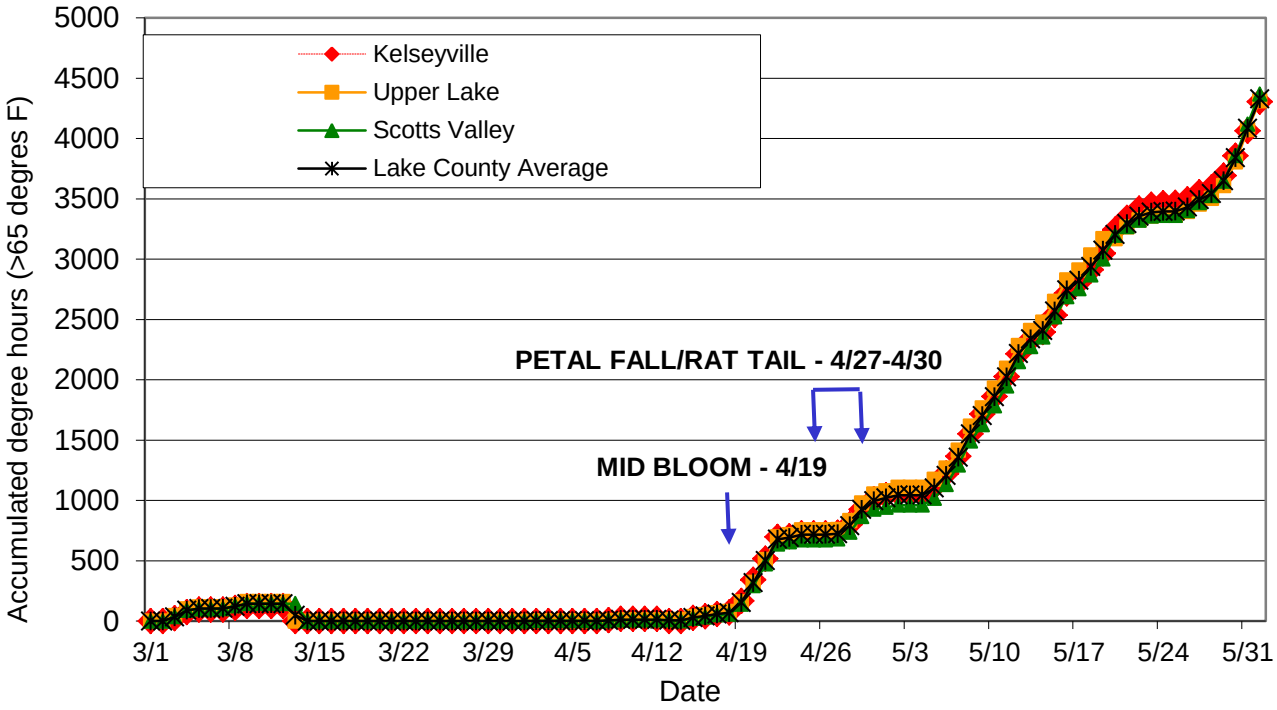

Figure A: Relationship between accumulated degree hours (base >65°F) for Kelseyville, Scotts Valley (Lakeport) and Upper Lake, Lake County, California, March 1 to June 1, 2012 and positive (shown in bold) and negative (shown in black) LAMP samples. Degree-hours calculated from using data from Kelseyville-0.1P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

Table 1. Combinations of biological control agents applied to control fire blight, Upper Lake, Lake County, California, 2012

No.	Treatment	Stage(s)	Application Dates[1]
1	Control (untreated)		
2	A506, 1/2 rate, weekly	weekly	4/19, 4/24, 5/1, 5/8
3	A506 @ 20% bloom, full bloom, rat-tail	2,6,8	4/14, 4/21, 5/8
4	A506 @ 20% bloom, E325 @ full bloom, A506 @ rat-tail	2,6,8	4/15, 4/19, 5/8
5	E325 @ 20% bloom, A506 @ full bloom, E325 @ rat-tail	2,6,8	4/15, 4/21, 5/8
6	E325 5x label @ 20-30% bloom	2	4/15
7	E325 5x label @ 80-90% bloom	6	4/19
8	Blossom Protect + Buffer A @ 20-30% bloom	2	4/14
9	Blossom Protect + Buffer A @ 80-90% bloom	6	4/21
10	Blossom Protect + Buffer A @ 10% bloom, full bloom, pre-petal fall	1,6,7	4/14, 4/21-24, 5/1
11	Regalia @ 20% bloom, full bloom, rat-tail	2,6,8	4/15, 4/19, 5/8
12	E325 @ 30% bloom, E325 + Regalia @ 70% bloom, Blossom Protect plus Regalia @ full bloom, Blossom Protect @ pre-petal fall	3,5,6,7	4/19, 4/21, 4/24, 5/1
13	E325 @ 80% and 90% bloom, Blossom Protect @ full bloom and pre-petal fall	3,5,6,7	4/19, 4/21, 4/24, 5/1
14	Badge X2 weekly	weekly	4/19, 4/24, 5/1, 5/8
15	Streptomycin/Terramycin tank mix, weekly	weekly	4/19, 4/24, 5/1, 5/8

[1]Dates were approximate with the following phenological stages and corresponding weather events which affected actual treatment timing due to rapidly changing conditions:

Stage	Phenology	Occurrence	Application Conditions
1	10%	4/14	
2-3	20-30%	4/14-15	wind 4/14; finished 4/15
4-5	40-70%	4/19-21	windy 4/19 then major bloom increase 4/20-21
6	90% - full bloom	4/21-24	max. 88ºF; finish 4/19 sprays (some stage 6 sprays applied 4/19)
7	petal fall	5/1	
8	rattail	5/8	

Following full bloom (April 20), 16 blossoms per treatment (4 per tree; one missing replicate) were collected on May 14, placed in individual polyethylene sample bags, and shipped to UC Berkeley to evaluate presence and population sizes of the biological control agents A506, E325, and BCY-B yeast. For those treatments in which more than one organism was applied, separate estimates of the population size of each strain was made. Population sizes were estimated by washing individual flowers in small volumes (2 ml) of sterile buffer and plating on appropriate selective medium. The plot was surveyed for fire blight strikes from May 16 through June 26 (5 sample dates) and fruit collected prior to harvest in late August to evaluate russet and frost damage.

Data was analyzed by ANOVA and means separated using Duncans Multiple Range Test ($p \geq .05$) (StatGraphics, Centurion XVI.1, StatPoint Technologies, Inc., Warrenton, Virginia).

RESULTS AND DISCUSSION

Infection conditions (Figure A). Major continuous fire blight infection conditions began April 20 (250 degree hours) and continued through June as indicated by a low level but continued appearance of strikes through June 26.

Bacterial populations - For all biological control agents, the population size of a given strain varied widely among the flowers sampled. In 2012, all treatments harbored some flowers with undetectably low numbers of a given biological control agent (less than about 100 cells per flower). Only a small number of untreated control trees harbored any of the biological control agents that had been applied on adjacent trees, as is normal (Figure 1). The frequency with which *Pseudomonas fluorescens* A506 was recovered from flowers in treated trees was much lower than those in previous years and substantially less than the other applied biological control agents (Figures 2 through 4). Only about half of the flowers sampled harbored any cells of *Pseudomonas fluorescens* A506, and the median population was around 10^4 cells per flower (Figures 2 through 4).

Several factors determine the population size of bacteria within an individual flower: 1) the number of cells deposited during a spray event, 2) whether the flower was open at the time the last application of the biological control agent was made, and 3) the growth and survival of the biological control agent after spray application. Based on past experimental observations, once applied, the biological control agents can then spread from one tree to the next either by rain splash after application, distribution by flying insects such as bees and flies, and from small amounts of spray drift during the time of application. It is unclear whether the unexpectedly low population sizes of strain A506 were due to poor survival of the bacterium on the flowers after application or whether the concentration of cells in the sprayed inoculum was lower than expected. The population size of *Pseudomonas fluorescens* A506 was similar in flowers in which it had been applied individually as compared to those in which it was applied in combination with *Pantoea agglomerans* E325 (Figures 4 and 5). In contrast, and in contrast to 2011, when A506 predominated on the blossoms, the population size of strain E325 was substantially higher in 2012, with a median population size of about 10^6 cells per flower (Figures 5 through 7). About 75% of the flowers on trees treated with strain E325 harbored at least some cells of this biological control agent. The population size of strain E325 on flowers that were also treated with Regalia were similar to those on flowers not also receiving this chemical treatment (compare Figures 11 and 12). The population size of strain E325 on flowers was largely independent of the application frequency (number of previous spray application before sampling) suggesting that the population sizes at sampling was of primary importance in determining the numbers of bacterial cells on a flower. Samples were collected on May 14, thus six days had

elapsed since the application of most treatments that were applied weekly, and 6-25 days since those applied at less frequent application schedules. The lower populations of strain A506 relative to strain E325 may thus be (at least) partially explained by the weather conditions prior to sampling. Maximum temperatures increased into the high 80's to low 90's the week prior to blossom sample collection. A506 was more competitive versus E325 in 2011 when temperatures were consistently cool through May. These contrasting results in 2011 and 2012 provide valuable information for a potential complementary use regime.

Surprisingly, all flowers on trees treated with Blossom Protect harbored this yeast (Figures 8 through 10). This corroborates 2011 results and similar results elsewhere (Johnson and Temple, 2013). The median population size of the yeast on trees treated with Blossom Protect was about 10^5 cells per flower. While the number of cells of the yeast on trees treated with Blossom Protect was somewhat smaller than those on trees treated with the bacterial biological control agents, it should be remembered that the large size of cells openly dictates that their numbers would always be lower than those of bacterial cells on a given flower.

Fire blight strikes (Table 2). Average weekly strikes numbered below 0.5 per tree except on May 23 (0.7 per tree). The average number was 0.4 across all weeks (cumulative = 2.0). There were no significant differences among treatments for any individual week and the untreated controls had the least number of strikes. Block differences, as well as interactions, were highly significant.

Fruit russeting and frost damage (Table 3). In contrast to 2011, there was very little russet in 2012 and block differences were greater than treatment differences. The untreated controls showed very little russet. Interestingly, there was a trend ($p=0.10$) toward higher russet on fruit treated weekly with a half rate of A506, while the full rate applied weekly had less russet (poor survival conditions may have accounted for some of the result). Unlike in 2011, when russet was observed on all fruit treated with Blossom Protect, 2012 results varied, with only the fruit treated three times during bloom showing notable russet. Conversely, a single treatment at 20-30% had the least russet of any treatment, and one treatment at 80-90% was intermediate. Copper (Badge X2) applied weekly had some russet but not different than the control.

There were no differences among treatments in frost damage.

REFERENCES

Elkins, R. and S. Lindow. 2011. Control of fire blight disease in pear caused by *Erwinia amylovora* using biological control agents, copper, and antibiotics, p. 91-103.

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).

ACKNOWLEDGEMENTS

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Table 2. Average number of fire blight strikes in Bartlett pear trees treated with varying combinations of biological control agents in Upper Lake, Lake County, California, 2012.

Treatment[1]	Average Number of Weekly Strikes					Average No. Weekly Strikes	Average Cumulative Strikes
	5/16/12	5/23/12	5/30/12	6/6/12	6/26/12		
Control (Untreated)	0.0	0.2	0.0	0.0	0.0	0.04 c	0.2
A506 alone 1/2 rate, weekly	0.6	0.8	0.0	0.2	1.0	0.52 abc	2.6
A506 alone 3X @ 20% bloom, full bloom, rattail	0.0	0.0	0.0	0.2	0.0	0.19 c	0.2
A506 @ 20% bloom, E325 @ full bloom, A506 @ rattail	0.0	1.0	0.0	0.2	0.4	0.32 bc	1.6
E325 @ 20% bloom, A506 @ full bloom, E325 @ rattail	0.0	0.4	0.4	0.4	0.2	0.28 bc	1.4
E325 alone 5x label @ 20%-30% bloom	0.6	1.2	0.6	0.6	0.6	0.72 ab	3.6
E325 alone 5x label @ 80%-90% bloom	0.4	1.6	0.6	0.4	0.6	0.72 ab	3.6
Blossom Protect + Buffer A @ 20%-30% bloom	0.6	1.2	0.6	0.2	0.2	0.56 abc	2.8
Blossom Protect + Buffer A @ 80%-90% bloom	0.2	0.2	0.0	0.2	0.0	0.12 bc	0.6
Blossom Protect + Buffer A @ 10% bloom, full bloom, pre-petal fall	0.2	0.8	0.4	0.2	0.2	0.36 bc	1.8
Regalia alone @ 20% bloom, full bloom, rattail	0.6	0.6	0.2	0.2	0.8	0.48 abc	2.4
E325 @ 30% bloom, E325 + Regalia @ 70% bloom, Blossom Protect + Buffer A + Regalia @ full bloom, Blossom Protect + Buffer A @ pre-petal fall	1.4	2.0	0.2	0.8	0.6	1.00 a	5.0
E325 @ 30% bloom, 70% bloom, Blossom Protect+ Buffer A @ full bloom, pre-petal fall	0.0	0.0	0.4	0.0	0.0	0.08 bc	0.4
Badge X2 weekly	0.0	0.6	0.2	0.2	0.6	0.32 bc	1.6
Streptomycin/Terramycin tank mix, weekly	0.0	0.2	0.0	1.4	0.6	0.44 abc	2.2
Average	**0.3**	**0.7**	**0.2**	**0.3**	**0.4**	**0.4**	**2.0**
ANOVA[2]							
Treatment (P-value)	NS (0.41)	NS (0.58)	NS (0.22)	NS (0.86)	NS (0.58)	** (0.01)	NS (0.23)
Block (P-value)	*(0.02)	** (0.01)	*** (<0.001)	NS (0.32)	*(0.03)	*** (<0.001)	*** (0.001)
Date (P-value)	----	----	----	----	----	* (0.02)	----
Treatment x Block (P-value)	----	----	----	----	----	*** (<0.001)	----

[1] Within columns, treatment means significantly different (Duncan $P \leq 0.05$).

[2] *, **, *** Indicates significance at $P \leq 0.05$, 0.01 and 0.001 respectively. NS indicates not significant $P > 0.05$. Data normalized using (SQRT +1) transformation.

Table 3. Average fruit russeting, percent russet severity and percent frost damage in Bartlett pears treated with varying combinations of biological control agents and harvested in Upper Lake, Lake County, California, 2012.

Treatment[1]	Average Russeting	Russet Severity (greater than 7%)	Russet Severity (less than 3%)	Frost Damage (%)
Control (untreated)	0.4 bc	0.7 b	99.4 ab	16.7
A506 alone 1/2 rate, weekly	1.4 a	5.4 a	90.7 c	12.1
A506 alone 3X @ 20%, full bloom, rattail	0.7 bc	1.3 b	98.0 ab	12.7
A506 @ 20% bloom, E325 @ full bloom, A506 @ rattail	0.5 bc	0.0 b	98.7 ab	19.4
E325 @ 20% bloom, A506 @ full bloom, E325 @ rattail	0.7 abc	0.0 b	98.6 ab	18.2
E325 alone 5X label @ 20%-30% bloom	0.5 bc	0.7 b	99.3 ab	20.0
E325 alone 5X label @ 80%-90% bloom	0.5 bc	0.0 b	99.4 ab	14.6
Blossom Protect + Buffer A @ 20%-30% bloom	0.3 c	0.0 b	100.0 a	17.1
Blossom Protect + Buffer A @ 80%-90% bloom	0.6 bc	0.0 b	98.7 ab	17.4
Blossom Protect + Buffer A @ 10%, full bloom, pre-petal fall	1.1 ab	1.5 b	93.3 bc	12.6
Regalia alone @ 20% bloom, full bloom, rattail	0.8 abc	1.3 b	96.0 abc	12.1
E325 @ 30% bloom, E325 + Regalia @70% bloom, Blossom Protect + Buffer A + Regalia @ full bloom, Blossom Protect + Buffer A @ pre petal fall	0.8 abc	0.7 b	95.8 abc	18.8
E325 @ 30% bloom, 70% bloom, Blossom Protect + Buffer A @ full bloom, pre-petal fall	0.8 abc	1.3 b	96.6 abc	14.6
Badge X2 weekly	1.0 abc	1.3 b	95.3 abc	14.1
Streptomycin/Terramycin tank mix, weekly	0.6 bc	0.7 b	97.8 ab	8.4
Average	0.7	1.0	97.2	15.3
ANOVA[2]				
Treatment (P-value)	NS (0.10)	NS (0.18)	NS (0.08)	NS (0.87)
Block (P=value)	NS (0.06)	* (0.02)	NS (0.10)	NS (0.49)
Treatment x Block (P-value)				

[1] Within columns, rootstock treatment means significantly different (Duncan, $P \leq 0.05$).

[2] * Indicates significance at $P \leq 0.05$. NS indicates not significant $P > 0.05$.

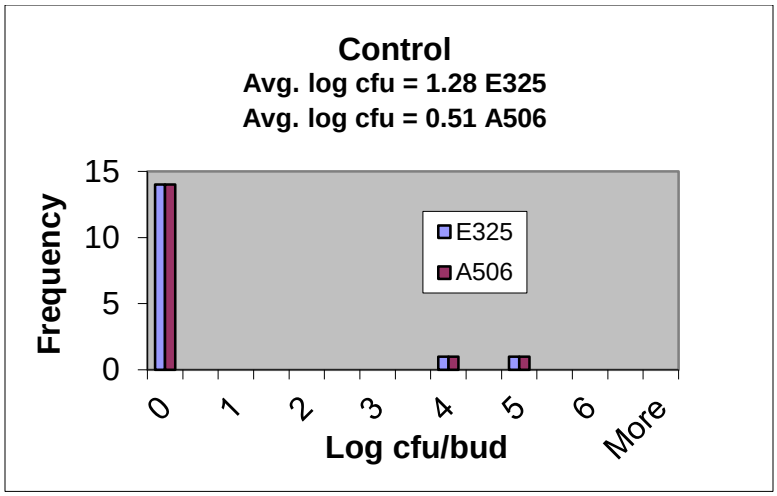


Figure 1

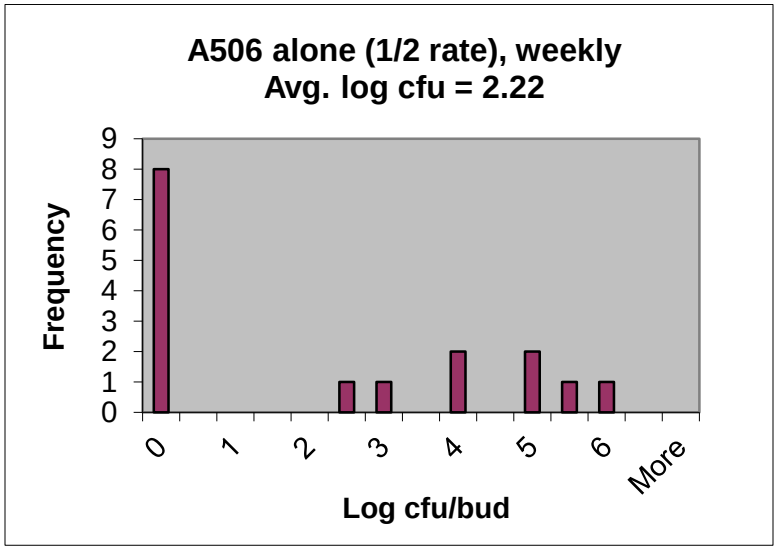


Figure 2

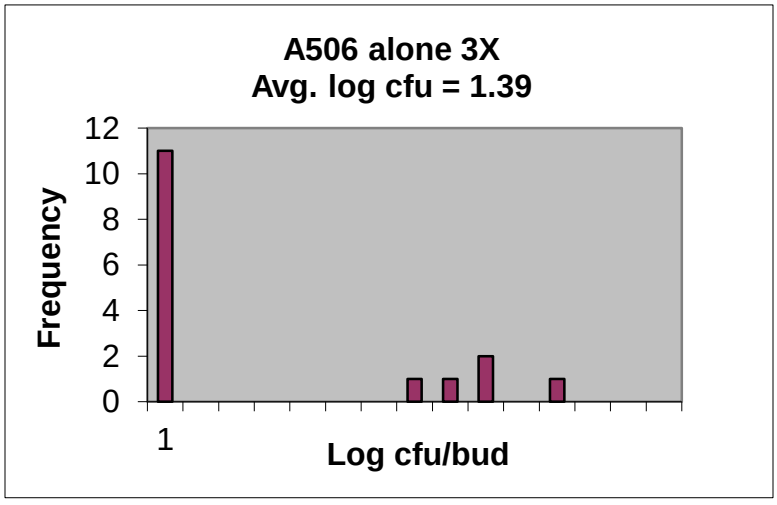
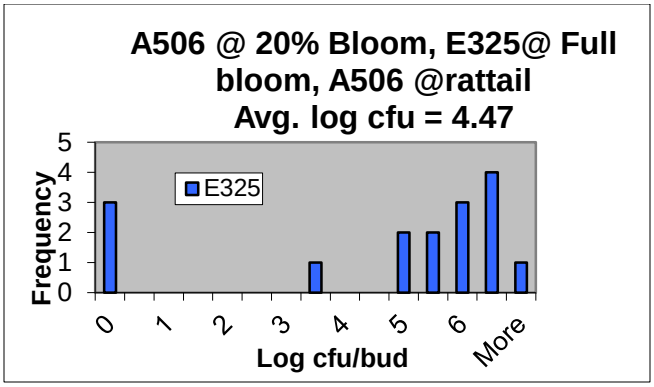


Figure 3

Figures 1-3. Distribution of the number of flowers having a log-transformed population size of *Pseudomonas fluorescens* strain A506 and *Pantoea agglomerans* strain E325 collected May 14 from blossoms of untreated Bartlett pear trees (Treatment 1, Figure 1); trees treated weekly with strain A506 at 50% the full label rate (Treatment 2, Figure 2); trees treated with strain A506 at 20% full bloom and rattail (Treatment 3, Figure 3).



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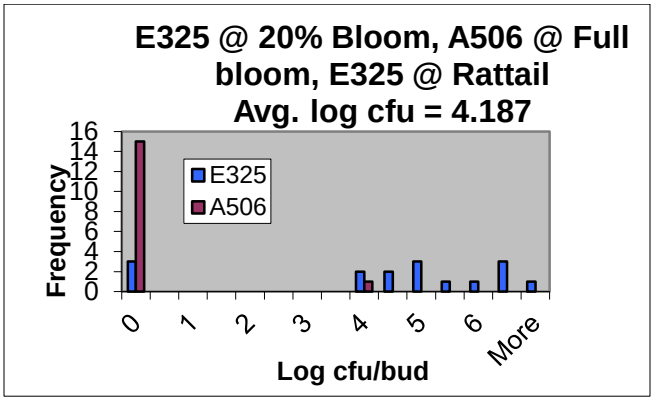

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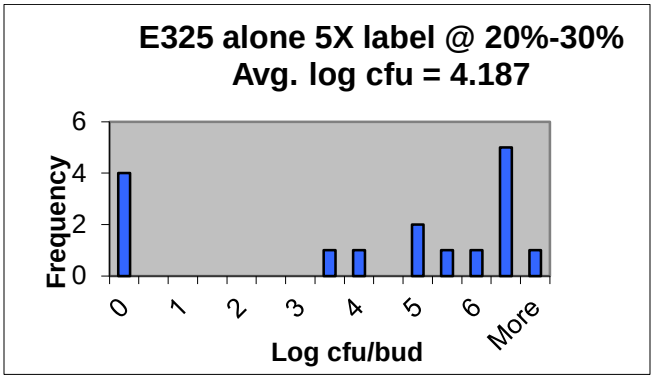

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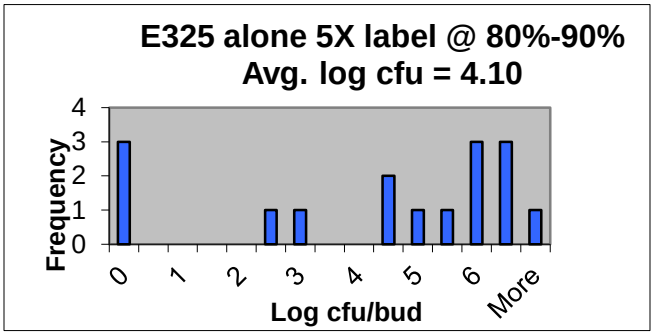


Figure 7

Figures 4-7. Distribution of the number of flowers having a log-transformed population size of *Pseudomonas fluorescens* strain A506 and *Pantoea agglomerans* strain E325 on blossoms collected May 14 from Bartlett pear trees treated with strain A506 and strain E325 on blossoms collected May 14 from Bartlett pear trees treated with strain A506 at 20% bloom, strain E325 at full bloom, and strain A506 at rattail (Treatment 4, panel 4); strain E325 at 20% bloom, strain A506 at full bloom and strain E325 at rattail (Treatment 5, Figure 5); strain E325 at 5x label rate at 20-30% bloom (Treatment 6, Figure 6); and strain E325 at 5x label rate at 80-90% bloom (Treatment 7, Figure 7).

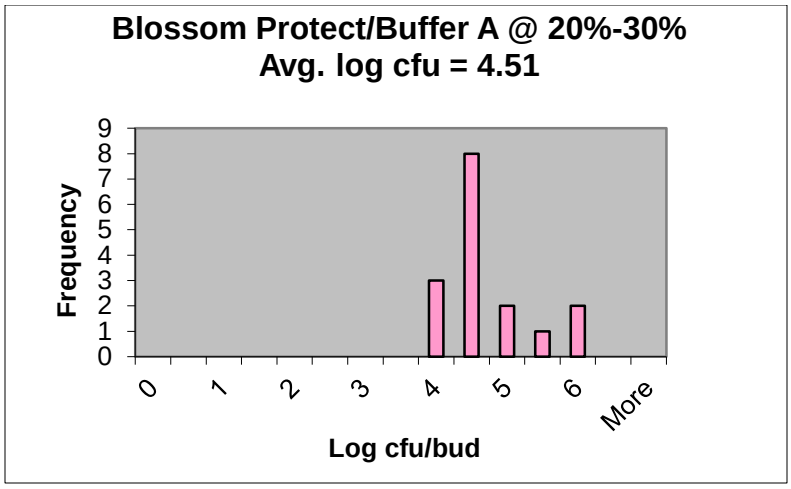


Figure 8

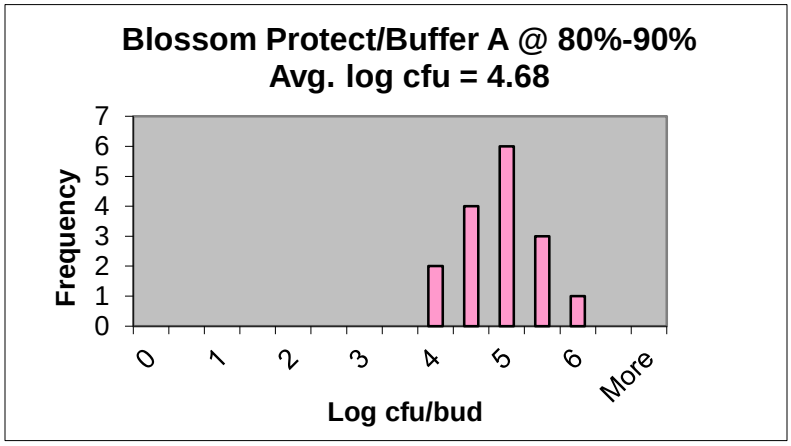
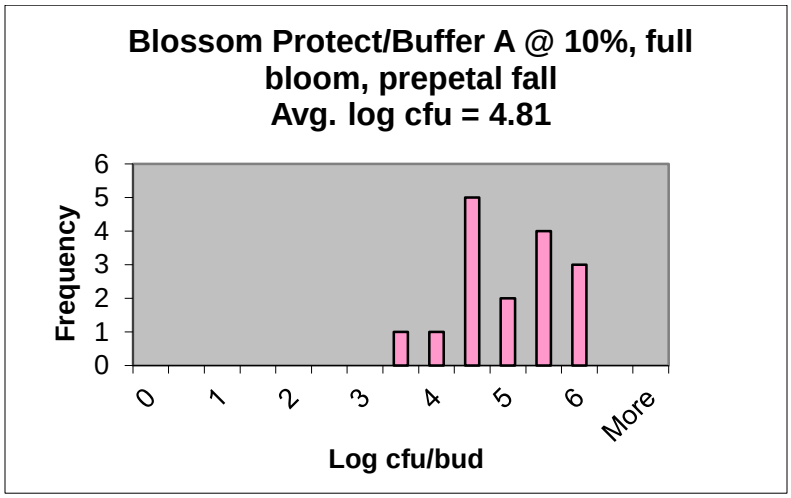


Figure 9

Figures 8-10. Distribution of the number of flowers having a log-transformed population size of the yeast Blossom Protect® BC4-B on blossoms collected May 14 from Bartlett pear trees treated with the label rate (and Buffer A) of this yeast at 20-30% bloom (Treatment 8, Figure 8); 80-90% bloom (Treatment 9, Figure 9); and 10% full bloom and pre-petal fall (Treatment 10, Figure 10).



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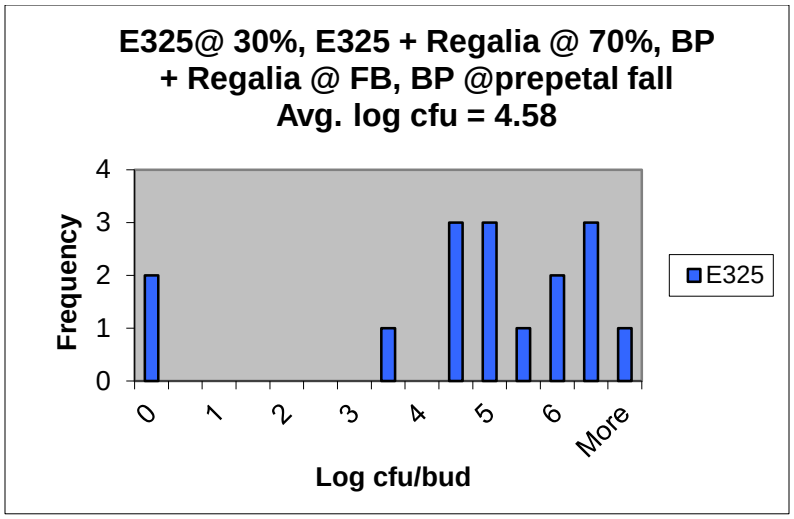

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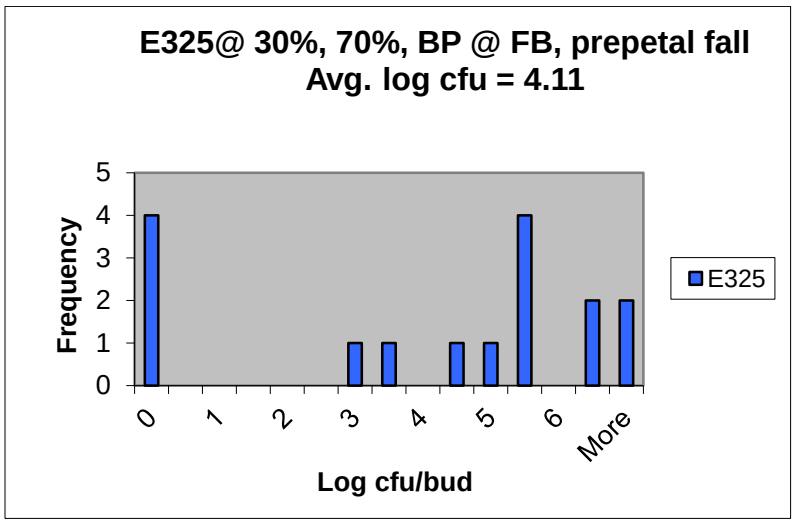

Figure 12

Figures 11-12. Distribution of the number of flowers having a log-transformed population size of the antagonistic bacteria *Pantoea agglomerans* strain E325 on blossoms collected May 14 from Bartlett pear trees treated with strain E325 at 30% bloom, followed by strain E325 plus Regalia at 70% bloom, Blossom Protect (plus Buffer A) plus Regalia at full bloom and Blossom Protect (plus Buffer A) at pre-petal fall (Treatment 11, Figure 11), and strain E325 at 30% and 70% bloom, followed by Blossom Protect (plus Buffer A) at full bloom and pre-petal fall (Treatment 12, Figure 12).