Control of Fire blight Disease in Pear caused by *Erwinia amylovora* Using Biological Control Agents, Copper and Antibiotics

Rachel Elkins, UC Cooperative Extension, Lake and Mendocino Counties
Steven Lindow, Department of Microbial Biology, University of California, Berkeley

INTRODUCTION

Commercial use of biological control of fire blight control has largely been limited to the formulation of *Pseudomonas fluorescens A506* sold as Blight Ban® A506 (Nufarm Americas, Burr Ridge, Illinois). New biological agents have recently become available but had yet to be tested in Lake and Mendocino Counties in the North Coast of California. Of particular interest were Bloomtime Biological® FD BioPesticide "Strain E325" (Northwest Agricultural Products, Pasco, Washington), which had looked promising in previous trials in the Northwest and Sacramento Valley and the biological yeast BCY-B, now named Blossom Protect® (Westbridge, Vista, California), which had also successfully controlled fire blight in previous tests. These materials offered another opportunity to expand the repertoire of biological control agents to supplement antibiotics and delay onset of resistance. Finally, renewed interest in copper due to its relatively low cost and the availability of apparently effective fine particle formulations containing less actual metallic deemed it reasonable to test a relatively new copper formula Kocide® 3000 (E.I. dePont de Nemours and Co., Wilmington, Delaware), particularly to evaluate potential russeting. A replicated trial was established in spring 2010 to obtain preliminary data on using these materials. This work continued in 2011, replacing Kocide 3000® with the less expensive Badge 2_X (Isagro-USA, Morrisville, North Carolina) and adding the biological fungicide Actinovate® (*Streptomyces lydicus*) (Natural Industries, Inc., Houston, Texas). Blossom Protect® was also applied with and without Buffer A combined with Blight Ban® A506 to gauge sensitivity of strain A506 to the buffer versus the yeast alone. Work will continue in 2012 with the addition of alternative copper materials and focus on the better-appearing treatment and timing combinations from 2010 and 2011.

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/acre, 0.40 acre total.

Treatment Details: Applied at 100 gpa by handgun, 0.66 gal./tree = 2.5 liters/tree; at 20% bloom (March 27), full bloom (April 3) and petal fall ("rattail") (April 23), or weekly, depending on treatment.

	Treatment	Spray dates
1	Control (Untreated)	
2	A506 alone 1/2 rate, weekly	4/3,4/12,4/19,4/26,5/3,5/10
3	A506 alone 3X @ 20% bloom, full bloom, rattail (PF)	4/3,4/12,5/13
4	A506 @ 20% bloom, E325 @ full bloom, A506 @ rattail (PF)	4/3,4/12,5/13
5	A506 alternated with Blossom Protect/Buffer A, weekly	4/4,4/12,4/19,4/27,5/3,5/10
6	E325 alone 1/2 rate, weekly	4/4,4/12,4/19,4/27,5/3,5/10
7	E325 alone 3X @ 20% bloom, full bloom, rattail (PF)	4/4,4/12,5/13
8	E325 @ 20% bloom, A506 @ full bloom, E325 @rattail (PF)	4/4,4/12,5/13
9	A506+E325 tank mix @ 20% bloom, full bloom, rattail (PF)	4/4,4/12,5/13
10	Blossom Protect/Buffer A @ 10% bloom, 40% bloom, 70% bloom, 90% bloom	4/2,4/4,4/12,
11	A506+Blossom Protect/Buffer A @ 20%, full bloom, rattail (PF)	4/4,4/12,5/13
12	A506+Blossom Protect @ 20%, full bloom, rattail (PF)	4/4,4/12,5/13
13	Actinovate, weekly starting at 10%	4/3,4/14,4/22,4/28,5/5
14	Streptomycin/Terramycin tank mix, weekly	4/4,4/14,4/22,4/28,5/5
15	Badge X2, weekly	4/4,4/14,4/22,4/28,5/5

RESULTS AND DISCUSSION

Bacterial populations (Figures 5-15) - The population sizes of the different biological control agents were quantified on 20 individual flowers collected from trees on April 20, 2011. The population size of the biological control agents was evaluated from all treatments, and on those treatments in which more than one organism was applied, separate estimates of the population size of each strain was made.

For all treatments, the population size of a given strain varied widely among the flowers sampled. For example, in all treatments, at least some flowers had undetectably low population sizes of a given biological control agent, while others had in excess of 10^6 cells per flower. Given that samples were collected on April 20, 16-17 days had elapsed since the first application of most treatments that were applied weekly and as much as 18 days since the first application of biological control agents applied at particular stages of growth such as those in treatments 3, 4, 7, and 8-12. As is commonly observed, the great potential of biological control agents such as *Pseudomonas fluorescens* strain A506 to spread from flower to flower via insects and rain splash led to the contamination of flowers from untreated control to treated trees. While the population sizes of biological control agents were generally less than 1000 cells per flower on untreated control trees, the majority of the flowers harbored at least some cells.

The population size of strain A506 was similarly high on trees treated with this strain at any frequency alone, or in combination with other biological control agents. Surprisingly, the population size of strain A506 was somewhat lower on average and a lower proportion of flowers were colonized on trees treated with this strain at one half the normal dose on a weekly basis compared with a full dose at three times during the season. The population size of strain

A506 was similarly high on flowers in which it was alternated with *Pantoea agglomerans* E325 or with the yeast in Blossom Protect.

The population size of strain E325 was generally lower than that of strain A506 when applied individually or when applied in combination with strain A506. This was particularly obvious when these two strains were alternated in their application to the trees, and was evident by the presence of flowers with much higher population sizes of A506 and those that harbored strain E325. Given that the spring of 2011 was relatively cold and wet, strain A506 apparently preferred such conditions compared to strain E325. The relative efficacy of strain E325 under warm and dry conditions compared to strain A506 have been noted before. Likewise, strain A506 had previously appeared superior under cold conditions.

The population size of the yeast in Blossom Protect was generally substantially lower than the population size of either strain A506 or strain E325. No flowers treated with the yeast in Blossom protect harbored large population sizes ($>10^4$ cells per flower). The larger cell size of the yeast may account for the lower population size that it achieves on flowers.

Fire blight strikes (Table 1) – The average number of strikes was low, ranging from 0.0 to 1.7. There was a trend (0.10) toward more strikes in the weekly applications of strain A506 alternating with Blossom Protect/Buffer A.

Fruit russeting and frost damage - (Table 2, Figure 1-4) – There were significant differences. Blossom Protect/Buffer A alone and tank mixed with strain A506 had the most russet, although strain A506 alternated with Blossom Protect /Buffer A had low russet. Russet was reduced when Buffer A was omitted from the strain A506 plus Blossom Protect tank mix, suggesting a negative effect of the buffer on strain A506 when directly tank mixed (however, this is not in agreement with measured bacterial populations, per above). Fruit russeting was equal on control and copper-treated fruit. There were no differences in frost damage.

CONCLUSIONS

California pear growers will have several alternative biological control selections available in 2012. Blossom Protect® (BP) will be available as of March, 2012; strains A506 and E325 are already registered. Strain A506 continues to perform well under the cool, wet conditions of the North Coast, and significantly reduced russet under all treatment strategies except when applied at ½ rate weekly and when combined with PB/Buffer A. It complements strain E325, which performs best under warmer conditions later in the season. It should be noted that in 2011 strain A506 populations were lower under the weekly ½ rate regime than when the full rate was applied three times. This contrasts with past results in which the weekly ½ rate colonized equally as well. It thus appears that populations this year were less able to “pre-colonize” the blossom stigmas ahead of the russet-causing bacteria and may have affect russet results. This difference will be tested again in 2012. The BP yeast apparently colonizes the blossom at the base of the stigma (ovary) versus strain A506 which colonizes the stigma. BP has performed well as a fire blight control agent in previous test in the Northwest. In contrast to dry climates such as central Washington, which is unaffected the by the problem, the primary concern with it in wetter

growing areas is fruit russeting. Russet severity was noticeably higher in several BP treatments, however, plot variability precluded absolute statistical differences. Interestingly, russet was reduced when combined with strain A506 *without* Buffer A and also when strain A506 was *alternated* with BP/Buffer A. It may thus be that the two might be incompatible as a tank mix, but alternating is quite safe for the bacteria. Russet was also somewhat increased by Actinovate (*Streptomyces lydicus*), as well as by the ½ rate of strain E325 and strain A506 alternated with E325. However, more testing is needed to confirm these results. In any case Actinovate may show promise as a biological control based on these preliminary results and should be re-tested in 2012. In summary, both conventional and organic pear growers should be heartened by the availability of new biological additions to the fire blight "IPM toolbox". Optimal treatment strategies will continue to be developed in 2012.

ACKNOWLEDGEMENTS

The authors wish to thank Sarah Johnson, Renee Koutsoukis, Carolyn Shaffer and Lawrence Stutsman for their assistance in establishing the trial and collecting and summarizing data.

We also wish to thank the manufacturers of the materials tested in this trial for their interest and support of this work.

Table 1. Average number of fire blight strikes in Bartlett pears, Seely Orchard, Lake County, CA, 2011.

Treatment[1]	Number Strikes by Date			Average No. Strikes
	6/2/2011	6/9/2011	6/16/2011	
Untreated	0	2	1	1.0 ab
A506 alone 1/2 rate, weekly	0	2	0	0.7 ab
A506 alone 3X @ 20% bloom, full bloom, rattail (PF)	0	2	0	0.7 ab
A506 @ 20% bloom, E325 @ full bloom, A506 @ rattail (PF)	0	1	0	0.3 b
A506 alternated with Blossom Protect/Buffer A, weekly	1	2	2	1.7 a
E325 alone 1/2 rate, weekly	0	0	0	0.0 b
E325 alone 3X @ 20% bloom, full bloom, rattail (PF)	0	1	0	0.3 b
E325 @ 20% bloom, A506 @ full bloom, E325 @rattail (PF)	0	0	0	0.0 b
A506+E325 tank mix @ 20% bloom, full bloom, rattail (PF)	0	1	1	0.7 ab
Blossom Protect/Buffer A @ 10% bloom, 40% bloom, 70% bloom, 90% bloom	0	0	0	0.0 b
A506+Blossom Protect/Buffer A @ 20%, full bloom, rattail (PF)	0	1	0	0.3 b
A506+Blossom Protect @ 20%, full bloom, rattail (PF)	1	0	0	0.3 b
Actinovate, weekly starting at 10%	0	0	0	0.0 b
Streptomycin/Terramycin tank mix, weekly	0	2	0	0.7 ab
Badge X2, weekly	2	1	0	1.0 ab
ANOVA[2]				
Treatment (P-value)				NS (0.10)
Date (P-value)				* * (0.003)

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

[2] ** Indicates significance at P$\leq$ 0.01 . NS indicates not significant P>0.05

Table 2. Average fruit russeting, percent russet severity and percent frost damage in Bartlett pears harvested in Seely Orchard, Lake County, CA. 2011

	Average Russeting	Russet Severity		Frost Damage
		(greater than 7%)	(less than 3%)	(%)
Treatment [1]				
Control	4.6 abc	22.9 ab	55.3 abc	5.4
A506 Alone 1/2 rate, weekly	3.7 abc	16.8 ab	63.8 abc	9.7
A506 Alone 3X @ 20%, Full, rattail	3.0 c	8.4 b	76.8 ab	7.2
A506 @ 20%, E325 @ Full, then A506 @ rattail	3.7 abc	15.8 ab	68.2 abc	7.9
A506 alternated with Blossom Protect/Buff A, weekly	3.3 bc	9.5 b	70.5 abc	9.0
E325 alone 1/2 rate, weekly	4.2 abc	21.0 ab	64.3 abc	7.6
E325 alone 3X @ 20%, Full, rattail	3.0 c	10.5 b	75.9 ab	8.4
E325 @ 20%, A506 @ Full, then E325 @ rattail	2.6 c	8.1 b	77.5 ab	7.6
A506 + E325 Tank mix @ 20%, Full, rattail	2.7 c	8.6 b	79.5 a	6.2
Blossom Protect/Buff A @ 10%, 40%, 70%, 90%	5.9 a	36.4 a	44.7 c	5.5
A506+Blossom Protect/Buff A @ 20%, Full, rattail	5.7 ab	36.2 a	47.3 bc	4.2
A506+Blossom Protect @ 20%, Full, rattail	3.9 abc	15.8 ab	64.7 abc	11.9
Actinovate, weekly starting @ 10%	4.0 abc	19.5 ab	62.0 abc	7.5
Strep/Terra Tank Mix, weekly	3.0 c	9.4 b	75.7 ab	6.6
Badge X2, weekly	3.9 abc	16.1 ab	65.8 abc	7.3
ANOVA [2]				
Treatment (P-value)	NS (0.09)	* (0.05)	NS (0.18)	NS (0.74)
Block	NS (0.69)	NS (0.57)	NS (0.42)	* (0.04)

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

[2] * Indicates significance at P ≤0.05. NS indicates not significant P>0.05.

[3] Samples rated August 12, 2011.

Figure 1.

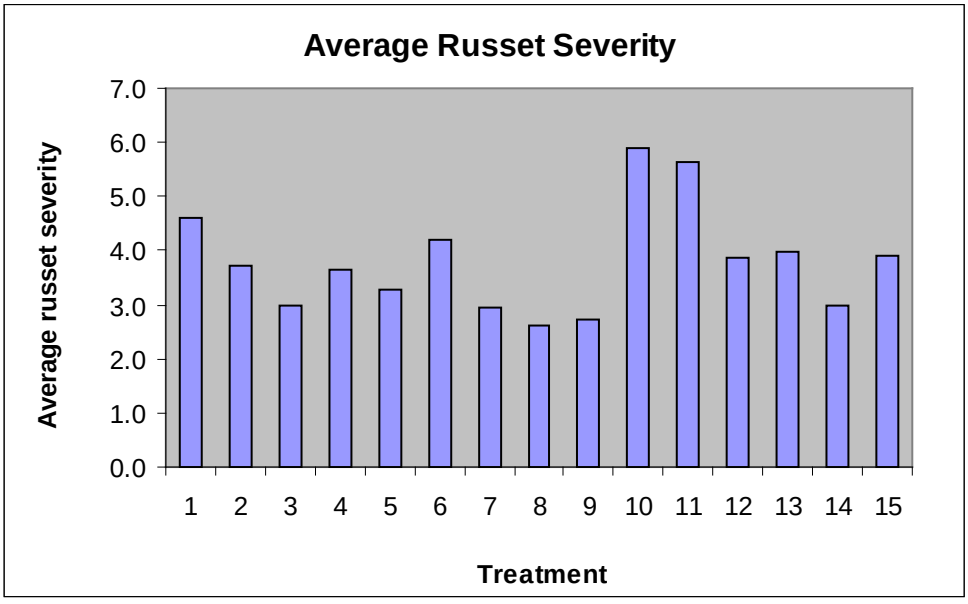


Figure 2.

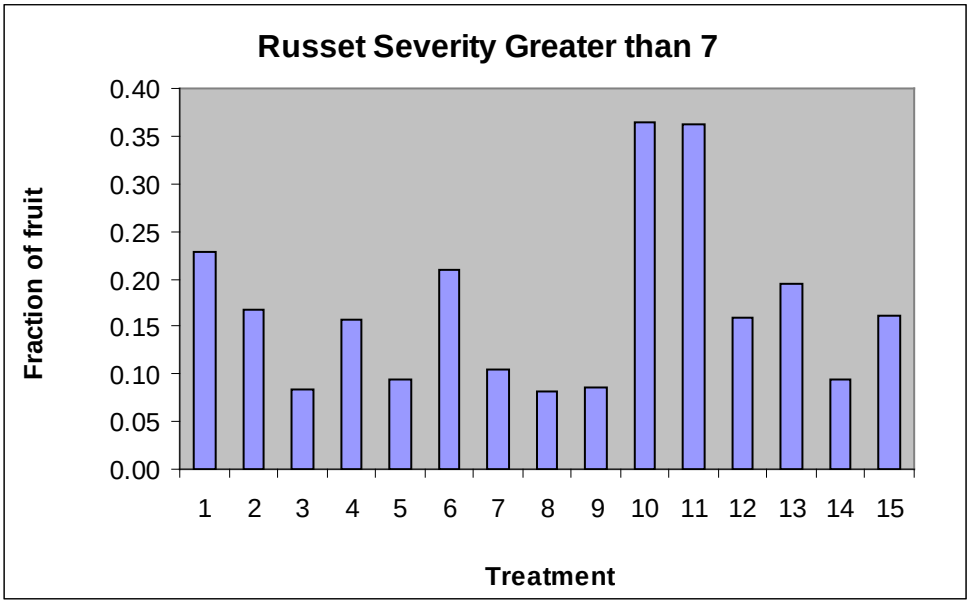


Figure 3.

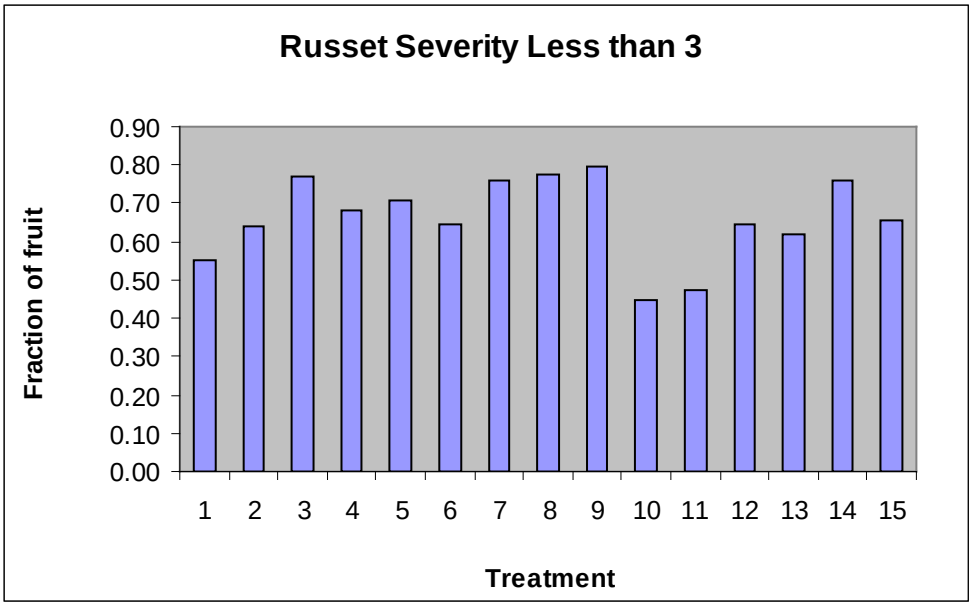


Figure 4.

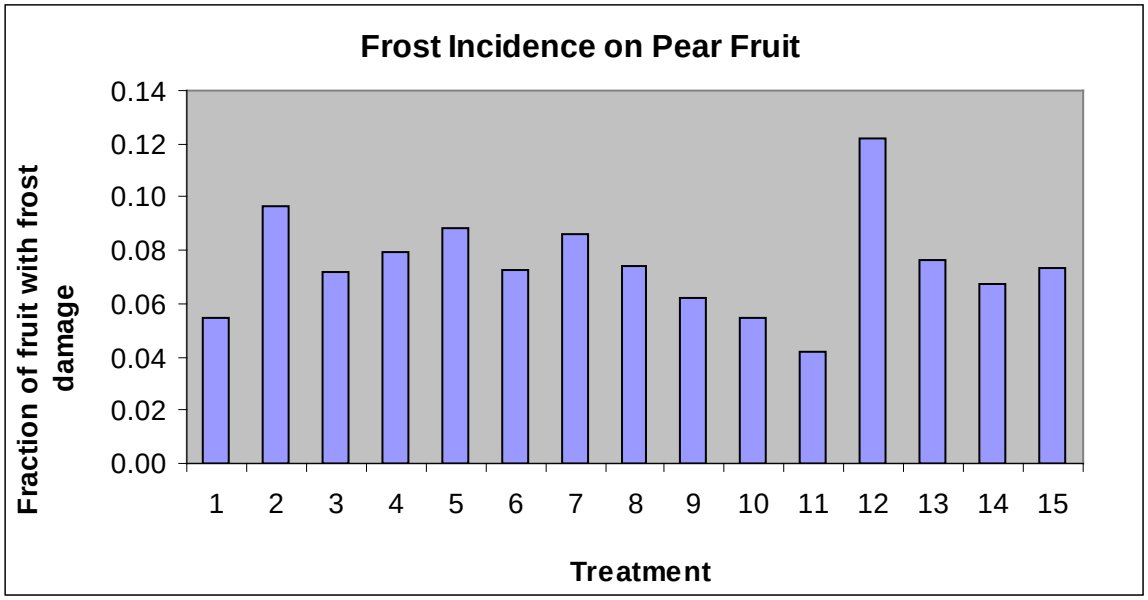


Figure 5.

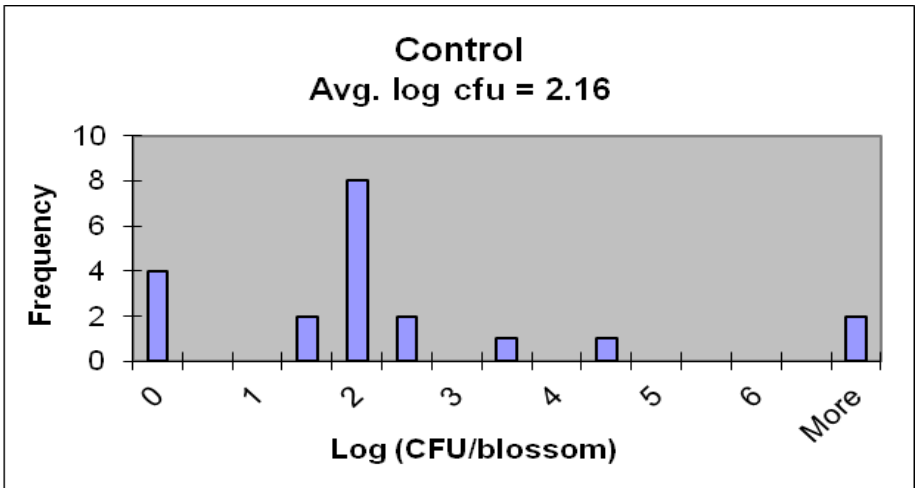


Figure 6.

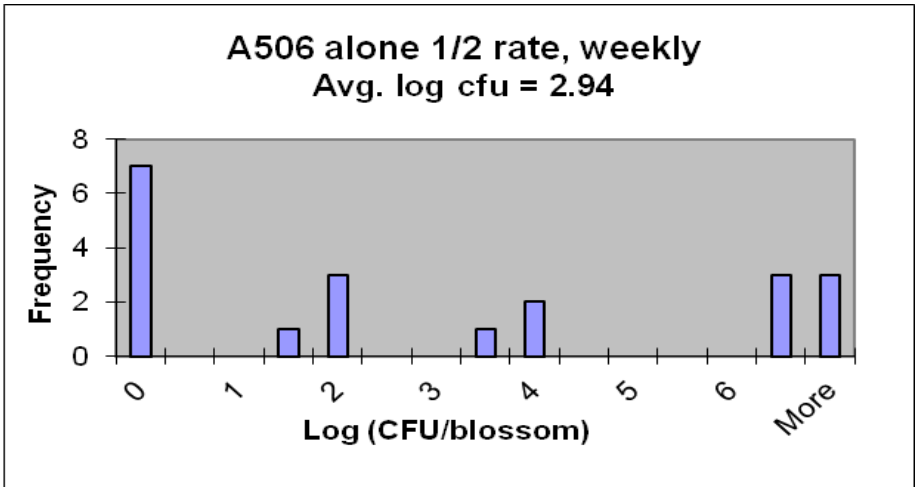


Figure 7.

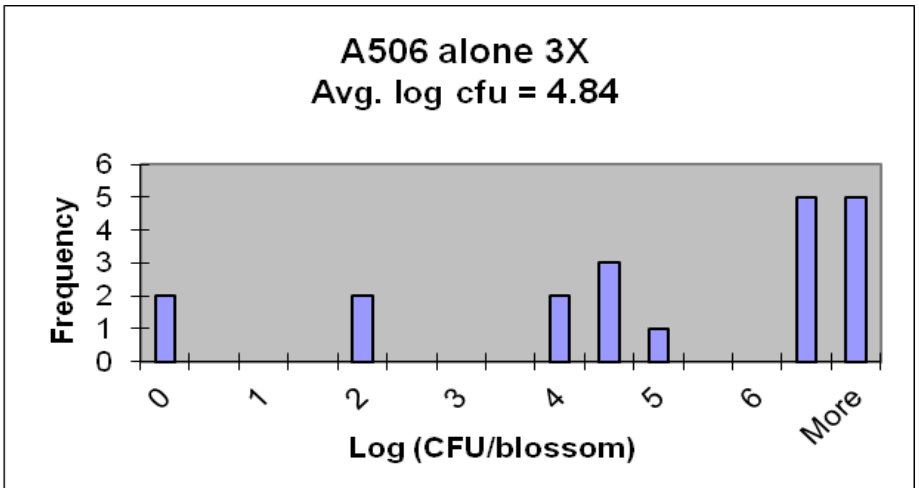


Figure 8.

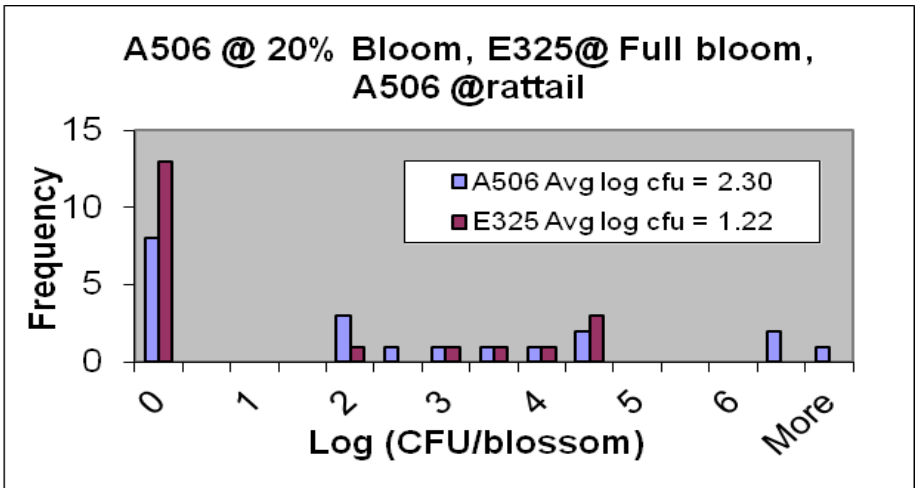


Figure 9.

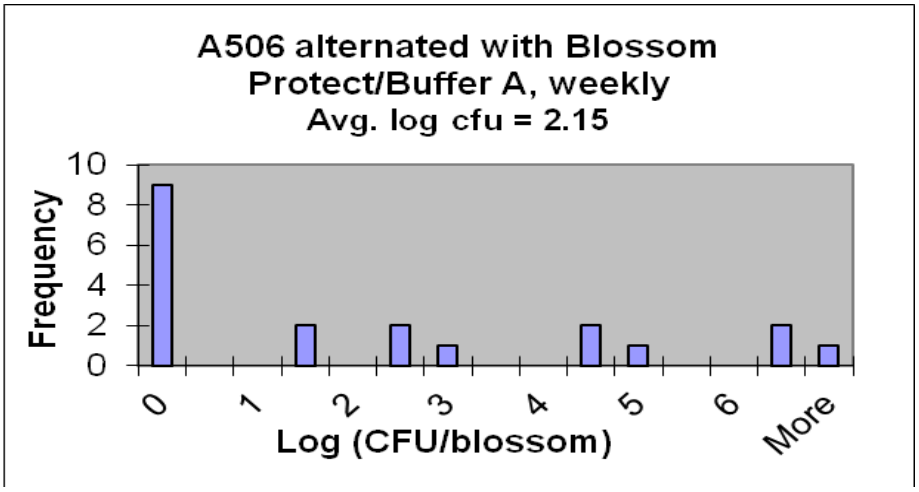


Figure 10.

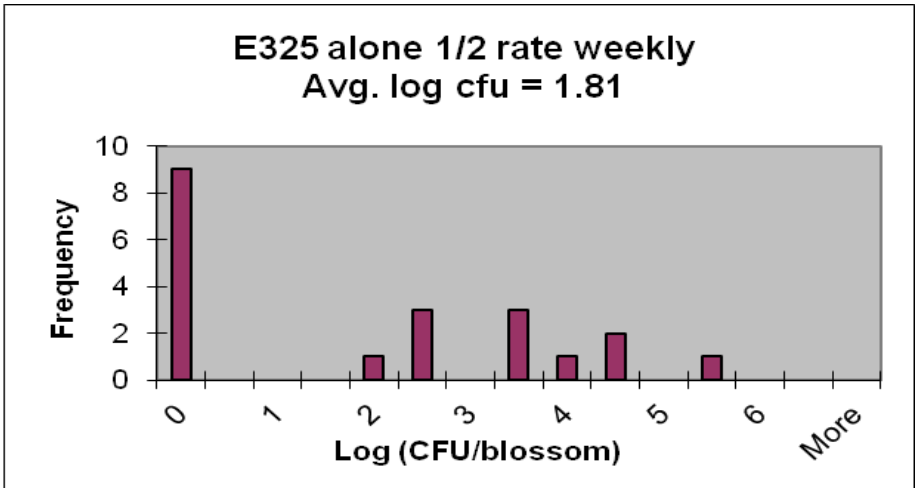


Figure 11.

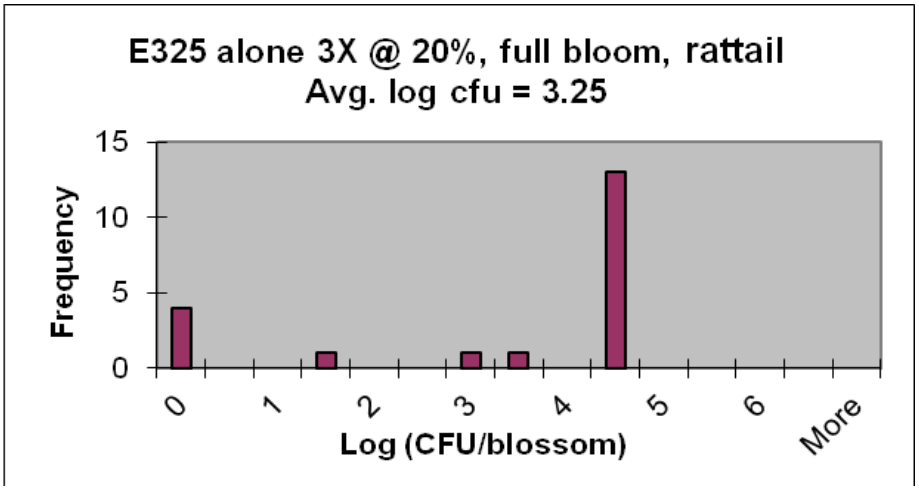


Figure 12.

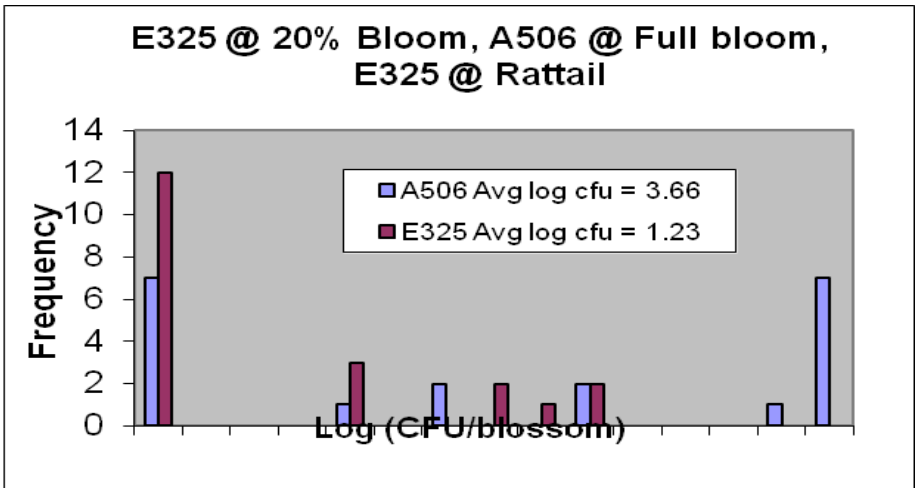


Figure 13.

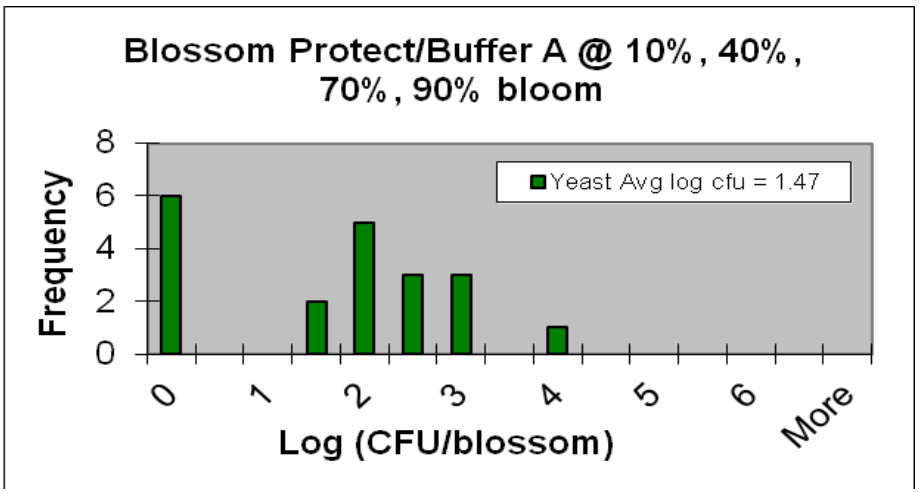


Figure 14.

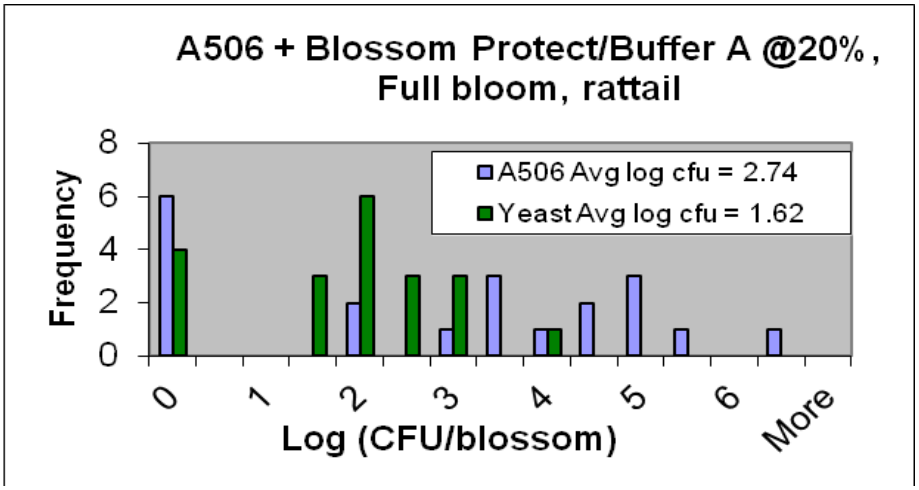
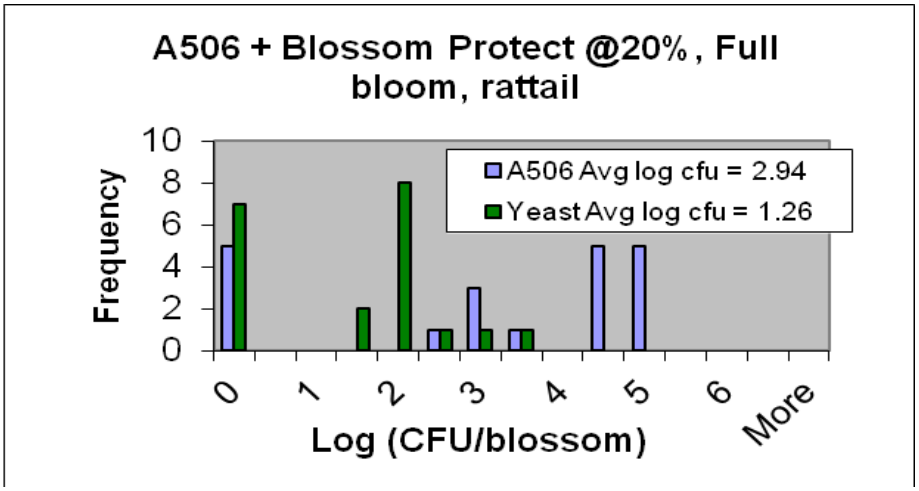


Figure 15.



Figures 5 to 15. Distribution of population sizes of *Pseudomonas fluorescens* strain A506 (blue bars), *Pantoea agglomerans* strain E325 (red bars) and the yeast in Blossom Protect (green bars) collected from trees treated with these strains and sampled on April 20, 2011. Shown are the number of individual flowers harboring a given population size shown on the abscissa. Treatments are indicated in each figure.