Management of Frost Injury, Fire blight, and Fruit Russeting of Pear Using Biological and Cultural Methods.

Principal Investigator: Steven E. Lindow
Department of Plant and Microbial Biology
University of California
Berkeley, CA 94720-3102.
email - icelab@berkeley.edu.
Telephone (510) 642-4174. Fax (510) 642-4995.

Cooperators: Rachel Elkins, Cooperative Extension, Lake County

Brent Holtz, Cooperative Extension, Madera County

ABSTRACT

The methods of application of antagonistic bacterium *Pseudomonas fluorescens* strain A506 (Blightban A506®) was tested in pear and apple in large replicated trials in commercial orchards subject to normal indigenous levels of the fire blight pathogen *Erwinia amylovora* as well as in smaller trials in which trees were inoculated with *E. amylovora* after treatment with Blightban A506 in different ways. The proportion of flowers that were colonized by strain A506 was generally low on pear in Lake country, irrespective of whether it was applied frequently in water alone or applied at first bloom with a penetrating surfactant; apparently the cold temperatures which occurred in the early spring in Lake county prevented multiplication of the bacterium after inoculation. In contrast, nearly all apple flowers had detectable A506 populations, irrespective of application methods in the warm conditions that prevailed in Madera and Fresno Counties. The incidence of fire blight to pear on trees inoculated with *E. amylovora* was reduced to a similar extent by application of Blightban A506 twice in water alone or a single time in 0.5% Breakthru. In contrast, the application of Blightban A506 in 0.5% Breakthru provided superior protection against first blight to both Fuji and Granny Smith apple in plots inoculated with *E. amylovora*. Serenade® provided poor control of fire blight in most trials. No enhanced russeting of fruit was observed in any plot in which surfactant was applied. These results suggest the number of applications of the bacterium needed for frost and disease control can be reduced by applying it early in the season with a penetrating surfactant. In addition, by applying the bacterium only once early in the early spring before applications of Dithane and Terramycin and other pesticides are subsequently made to trees, we can avoid potential problems with compatibilities of the bacterium with these other pesticides.

Comparison of spray methods to establishment of *P. fluorescens* strain A506 in flowers

Given that we had observed in previous years, that rapid colonization of flowers by indigenous bacteria could occur if flowers were inoculated with bacteria, we investigated

approaches by which *P. fluorescens* strain A506 could be introduced into pear or apple buds before bloom so that flowers would be exposed to this antagonistic bacterium as soon as they opened. We evaluated the potential of introducing bacteria into buds using relatively high rates of the penetrating surfactant Breakthru to ensure that it would be present in flowers as they opened. We hypothesized that suspensions of *P. fluorescens* strain A506 could be made to enter buds if applied with such surfactants. Organo-silicon surfactants such as Breakthru and related compounds have the unique ability to allow water solutions to penetrate into plant tissues through natural openings due to the low surface tension of such solutions. Normal sticker-spreaders do not have a sufficiently low surface tension to permit such penetration into plants. Laboratory tests had indicated that strain A506 was tolerant to over 3% Breakthru. Thus this bacterium was compatible with even high rates of surfactant.

In 2005 we compared the colonization of flowers by strain A506 when Blightban A506 was applied weekly 3 or more times in water alone starting at about 20% bloom with applications of Blightban A506 only once at "first bloom". We also compared weekly applications of Blightban A506 in water with applications of *Erwinia herbicola* C9-1, another antagonistic bacterium under development for fire blight control. We also evaluated a combination of these two bacteria for fire blight control. The control of fire blight by these bacterial treatments was compared with that conferred by application of a mixture of streptomycin and Terramycin as well as a copper formulation and Serenade® in some trials. The methods of application of antagonistic bacterium *Pseudomonas fluorescens* strain A506 (Blightban A506®) was tested in pear and apple in large replicated trials in commercial orchards in Lake and Madera Counties, respectively, that were subject to normal indigenous levels of the fire blight pathogen *Erwinia amylovora*. Treatments were also applied to trees in smaller trials which were inoculated with *E. amylovora* after treatment with Blightban A506 in different ways. Small scale testes on inoculated pear were performed at Berkeley and small scale tests on inoculated Granny Smith and Fuji Apple were performed at the Kearney Field Station in Fresno County.

Very cold and wet conditions were encountered in the large pear trial in Lake County which prevented natural occurrence of fire blight, and which prevented fruit set which made it impossible to rate frost injury to fruit or fruit russet at harvest. The proportion of flowers that were colonized by strain A506 was generally low on pear in Lake Country, irrespective of whether it was applied frequently in water alone or applied at first bloom with a penetrating surfactant (Figure 1); apparently the cold temperatures which occurred in the early spring in Lake county prevented multiplication of the bacterium after inoculation as has been observed on occasion in trials by Oregon State University researchers in trials in the Northwest. Thus, while strain A506 normally multiplies rapidly after inoculation onto flowers, even from very small numbers of cells, it might be inhibited under the very cold conditions which prevailed in the spring of 2005.

Under the much warmer conditions that prevailed in Madera County in the spring of 2005 nearly all Pink Lady apple flowers in our large scale plot had detectable A506 populations, irrespective of application methods (Figure 2). This trial, supported by the UC-IPM program, and done in cooperation with Brent Holtz of UC Cooperative Extension in Madera County was very similar in design to the large pear trial in Lake County described above. The presence of strain A506 was detected in flowers with a "flower rub" assay in which the pistils of the flowers are rubbed onto a selective medium for *P. fluorescens* strain A506. While this assay is very sensitive in detecting the presence of strain A506, it does not provide a measure of how many cells were present in a given flower. Thus our results

suggest that A506 had spread to a large percentage of flowers, irrespective of application method. Interestingly, there was also substantial spread to control flowers, apparently by bees or other flying insects that visit apple flowers. Because of the relatively warm temperatures at this site, and the fact that bees are attracted to apple flowers, movement of strain A506 to apple flowers is more pronounced than on pear flowers. There was very little fire blight in the apple plot area in 2005, apparently due to relatively low levels of inoculum and conditions unsuitable for the development of the disease.

The application of *E. amylovora* to trees ensured that fire blight infection would occur in small scale studies done on pear in Berkeley and on apple at the Kearney Field Station. In a small-scale plot on pear done at the Gill Tract research site Berkeley untreated control trees exhibited about 0.28 infections per flower cluster, thus allowing the effects of antagonistic bacteria and bactericides on infection to be measured, even on relatively small trees (Table 1). In this study, antagonistic bacteria applied in water alone as well as bactericides were applied both 10 days and 2 days before inoculation of trees with 10^5 cells/ml of *E. amylovora* to trees. Blightban A506 was also applied once, 10 days before application of *E. amylovora* with 0.5% Breakthru. While Serenade provided relatively little control of fire blight infection in this study, the antagonistic bacteria all provided about the same level of disease control (Table1). The disease control provided by the various antagonistic bacteria was similar to that provided by a mixture of streptomycin and Terramycin (Table 1). Antagonist *E. herbicola* C9-1 provided similar levels of disease control as Blightban A506, although there was no significant increase in efficacy in disease control provided by a mixture of thee two strains compared to either strain alone (Table 1). A single application of Blightban A506 with Breakthru provided similar disease control as two applications of this bacterium when applied in water alone (Table 1).

Antagonistic bacteria and bactericides were also tested for fire blight control on *E. amylovora*-treated apple trees in plots at the Kearney Field Station treated in a similar way as discussed above for pear. The application of Blightban A506 a single time in 0.5% Breakthru provided superior protection against first blight to both Fuji and Granny Smith apple in plots inoculated with *E. amylovora* compared to application of this bacterium twice in water alone (Tables 2 and 3). While Serenade provided inconsistent control of fire blight infection in this study, the antagonistic bacteria all provided about the same level of disease control when applied twice in water alone (Tables 2 and 3). The disease control provided by the various antagonistic bacteria was similar to that provided by a mixture of streptomycin and Terramycin (Tables 2 and 3). Antagonist *E. herbicola* C9-1 provided similar levels of disease control as Blightban A506, although there was no significant increase in efficacy in disease control provided by a mixture of thee two strains compared to either strain alone (Tables 2 and 3).

These results are encouraging in that they suggest that early season application of antagonistic bacteria may be a superior means of establishing these biological control organisms on trees. These results confirm that we should be able to greatly reduce the number of applications of the bacterium by applying it early in the presence of the penetrating surfactant. Apparently the stage of flower bud opening that allows flower colonization is critical to success of this strategy of inoculation of flowers. The best evidence is still that the best time to apply the single bacterial treatment with penetrating surfactants is after buds begin to open, but before many flowers appear (since open flowers appear to be at risk of phytotoxicity from the silicon surfactant).

While we usually see more colonization of emerging flowers when Blightban A506 is applied with 0.5% Breakthru compared to with 0.25% Breakthru, these differences are usually small, and probably do not justify the higher rate of surfactant. We thus expect that further tests will show that 0.25% Breakthru is sufficient to enable the colonization of flowers with strain A506 from early-season applications of Blightban A506 with this surfactant. In addition, by applying the bacterium only once early in the early spring before applications of Dithane and Terramycin and other pesticides are subsequently made to trees, we can avoid potential problems with compatibilities of the bacterium with these other pesticides. Since strain A506 can be established on trees before these other pesticides need to be applied, and since we have already shown that the bacterium is quite tolerant of other pesticides such as Dithane and Terramycin if it has established on trees before these pesticides are applied, we can greatly reduce any possibility that they will interfere with the performance of strain A506 in biological control of frost, fire blight and fruit russet. Such an application strategy should thus also help increase the adoption of biological control strategies for fire blight and fruit russet since they will make it easier to integrate into existing management strategies.

Table 1
Incidence of fire blight and severity of fruit russet on Bartlett pear treated with different biological and chemical control agents – 2005, Berkeley

Treatment	Fire Blight (# infections/cluster)	Fruit Russet (% of surface)
Control	0.28 a	15.0 a
Serenade	0.20 ab	11.1 ab
A506 + C9-1	0.19 ab	10.4 ab
A506 + Breakthru	0.14 bc	12.0 ab
Streptomycin + Terramycin	0.13 bc	12.0 ab
C9-1	0.12 bc	9.8 b
A506	0.07 c	11.2 ab

Table 2
Incidence of Fire Blight Strikes and Severity of Fruit Russet on Granny Smith Apple Treated at Bloom with Different Antagonistic Bacteria or Bactericides

Treatment rate/acre	Fire Blight Strikes # / tree	Fruit Russet (% of surface)
CuprofixA MZ, 5.5 lb	07.2 a	1.0 b
A506+Breakthru 0.5%	08.8 ab	1.8 a
Strep (100 ppm)+ Oxytet (200 ppm)	11.6 abc	0.8 cb
Serenade, 4lb/acre	14.0 abc	0.4 c
C9-1	14.3 bc	0.3 c
Blightban A506+C9-1 combination	16.0 bc	0.4 c
Blightban A506	19.3 cd	0.3 c
Untreated control	24.3 d	0.4 c

Table 3
Incidence of Fire Blight Infections and Severity of Fruit Russet at Harvest on Fuji Apple Treated at Bloom with Different Antagonistic Bacteria or Bactericides

Treatment rate/acre	Fire Blight Strikes (# / tree)	Fruit Russet (% of surface)
Blightban A506+Breakthru 0.5%	55.6 ab	5.3 a
Strep (100 ppm)+ Oxytet (200 ppm)	62.0 abc	2.7 cd
C9-1	66.2 abc	2.8 cd
Blightban A506+C9-1 combination	69.0 abc	2.7 cd
Blightban A506	90.8 abcd	3.0 cd
Serenade, 4lb/acre	102.2 bcd	3.9 bc
CuprofixA MZ, 5.5 lb	107.8 cd	3.8 bcd
Untreated control	132.8 d	2.9 cd

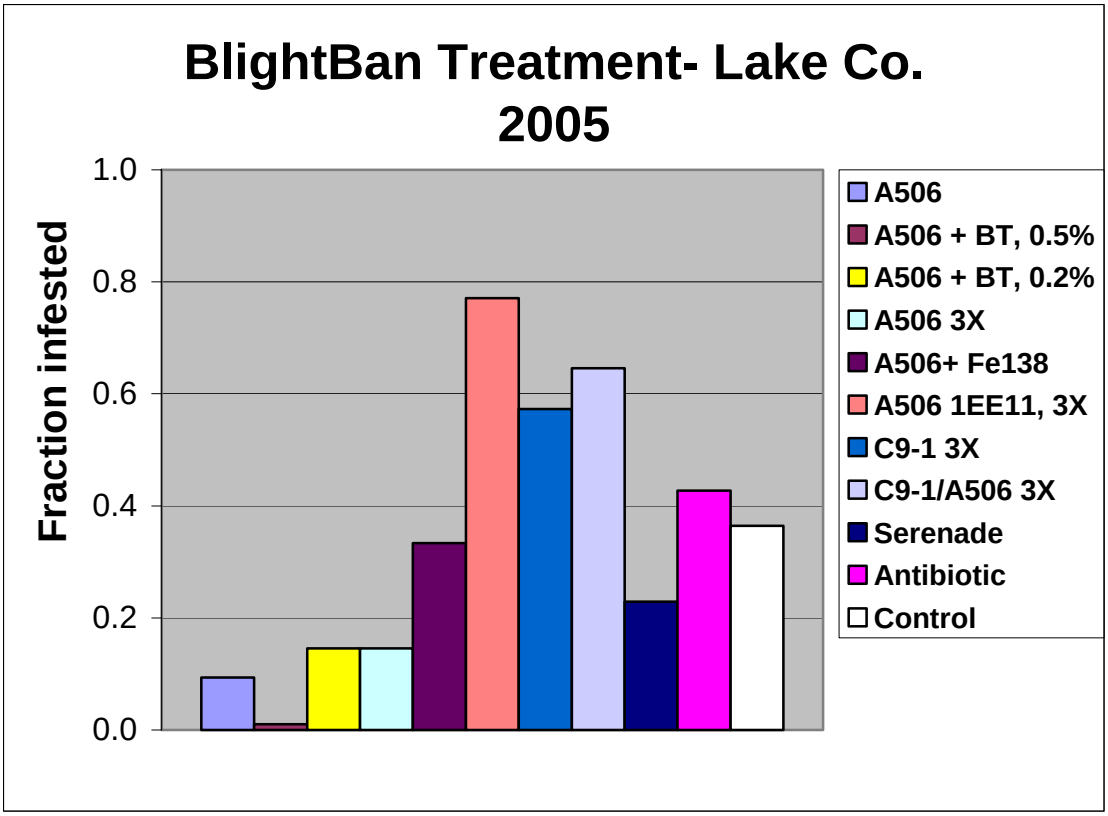

Figure 1. Fraction of flowers colonized with *Pseudomonas fluorescens* strain A506 or Erwinia herbicola C9-1 on Bartlett pear trees treated once only at the "first bloom" stage of growth [when only a few flowers were observed in an orchard] with a label rate of Blightban A506 in 0.5% Breakthru, compared with weekly applications of Blightban A506 in water E. herbicola C9-1, or weekly applications of Blightban and 1 lb/100 gal Sequestrene 138 or with bactericides alone in a Lake County plot.

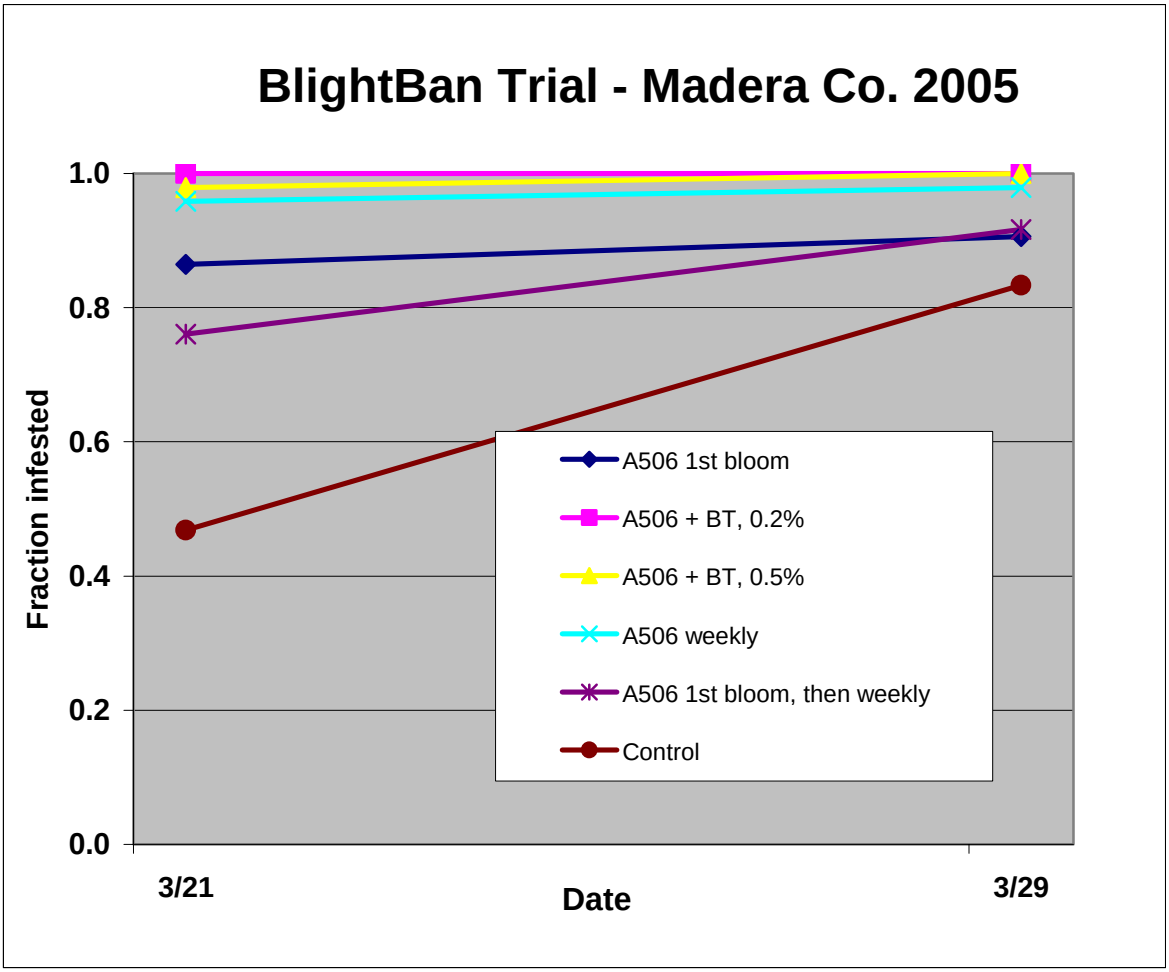

Figure 2. Fraction of flowers colonized with *Pseudomonas fluorescens* strain A506 on Pink Lady apple trees treated once only at the "first bloom" stage of growth [when only a few flowers were observed in an orchard] with a label rate of Blightban A506 in water alone or in 0.5% Breakthru, 0.2% Breakthru , compared with weekly applications of Blightban A506 in water alone, or weekly applications of Blightban and 1 lb/100 gal Sequestrene 138 or with antibiotics alone in a Madera County plot in 2004.