# FIREBLIGHT CONTROL STRATEGIES USING THE BIOLOGICAL CONTROL *PSEUDOMONAS FLUORESCENS* STRAIN A506 (BLIGHT BAN A506)

(submitted to the Pear Pest Management Alliance)

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## ABSTRACT

Fireblight disease, caused by the bacterium *Erwinia amylovora,* has been shown to be partially controlled by the biological control agent *Pseudomonas fluorescens* Strain A506, currently sold as BlightBan A506® by Plant Health Technologies, Inc. Research has also shown that A506 is capable of colonizing blossom tissue at lower than current label rates as long as conditions for colonization are favorable. More recently, it has been observed in small scale trials that colonization of partially opened flower buds (1-5% bloom stage) could be enhanced by combining the A506 with a silicon based surfactant by facilitating penetration deep into bud tissue. A506 could then colonize buds before they became occupied by competing bacteria. This would also theoretically allow the user to apply A506 earlier in the season and eliminate concerns about its compatibility with scab fungicides. Finally, enhanced early colonization could eliminate later sprays.

A demonstration project was conducted in two Bartlett pear orchards in Yuba County to show that 1) A506 could successfully colonize pear trees at half the labeled rate, 2) the number of A506 sprays could be reduced with the use of a silicon-based penetrating surfactant, and 3) A506 could improve fireblight control versus an antibiotics alone program. Treatments were applied by commercial spray rig and consisted of 1) half rate of A506 applied at 20% bloom, full bloom, and rat-tail, with antibiotics, 2) half rate of A506 applied at 1-5% bloom with a silicon based surfactant, then again at rat-tail, with antibiotics, and 3) antibiotics alone. Measurements included monitoring of colonization using a flower rub technique and evaluation of fireblight strikes.

Colonization of A506 was unable to be directly measured because flower rub cultures became rapidly contaminated due to the loss of the antifungal agent cycloheximide for use in isolate petri plates. Fireblight strikes in one orchard, however, were reduced 38% in the A506 plus antibiotic plots and 11% in the A506 plus penetrating surfactant plots. This indirectly indicated successful colonization (very few strikes occurred in any treatment in the second orchard).

Using A506 three times at half rate added $34 per acre to the antibiotic program. The A506 plus penetrating surfactant treatment cost $31.00 but eliminated the full bloom A506 treatment. Program cost must take into consideration resistance to streptomycin, which was present in both orchards.

Plans in 2001 will be to continue to refine the use of A506 as a component of an integrated fireblight program, and to test new biological control methods such as *Bacillus subtillis* (Serenade®, Agra Quest).

## INTRODUCTION

Fireblight disease caused by *Erwinia amylovora* is the most severe disease of pear in California. Its incidence limits where pears can be grown, as well as requires great expense and vigilance to control. Control of the disease involves cutting out infected tissue and applying preventative antibiotic or copper treatments when infection is likely. Resistance to one of the two antibiotics, streptomycin, has reduced control options. Copper, while effective, causes fruit russeting, which reduces fresh market value.

Research by U. C. Plant Pathologist Dr. Steve Lindow has led to the commercial availability of a biological control agent, *Pseudomonas fluorescens* Strain A506, marketed as BlightBan A506®, by Plant Health Technologies. A506 works by colonizing flower tissue, thereby preventing colonization of flowers by the fireblight pathogen and other russet-inducing bacteria. Trials over the past decade have shown that fireblight and russet are reduced from 50 to 80% by A506 alone, and that it provides additive control when used in conjunction with streptomycin.

Commercial adoption of A506 has been hindered by several factors: 1) it is suppressed by the antibiotic terramycin and by copper and thus needs to be applied separately (it is totally resistant, however, to streptomycin); 2) there is evidence that it is suppressed by certain scab fungicides, particularly mancozeb (Dithane®) when tank mixed; and 3) it adds expense to an already costly fireblight control program.

Data from the past several years has shown that cost savings can be achieved without sacrificing efficacy by applying lower rates of A506. It was also shown that fewer applications of antibiotics were necessary in an A506 program, thus reducing both chances of resistance build up and program cost.

In 1999, a demonstration trial was established in a Bartlett pear orchard in Wheatland, Yuba County, to show growers that: 1) adequate colonization could be achieved by using a half-rate of A506; 2) the number of antibiotic applications could also be reduced; and 3) A506 would reduce fruit russet if russet conditions prevailed. It was confirmed that A506 is capable of successfully colonizing and spreading through the orchard when applied at half the labeled rate under conditions suitable for colonization (Elkins and Lindow 1999).

In 2000, based on the 1999 results, the half-rate of A506® was applied prior to bloom, then later in the spring to coincide with the onset of the early summer rat-tail bloom period. The pre-bloom timing was combined with a silicon-based surfactant to enhance A506 penetration into the buds, thereby establishing this competitive bacterium in flowers as they emerge. This would then displace other potential bud colonizing bacteria through the entire main bloom and petal fall period, and therefore eliminate additional A506 applications during this period. The later A506 application(s) could then target any potential infections well after the main bloom period ends.

## PROCEDURE

Two orchards in the Sacramento Valley were divided into multiple sections. Three treatments were applied: 1) half-rate of A506 (2.7 oz./acre) plus the silicon based surfactant Breakthru® (Plant Health Technologies, Boise, ID), (1 qt./acre) at 1-5% bloom, followed by half-rate of A506 at rat-tail; 2) half-rate of A506 at 20% bloom, full bloom, late rat-tail (grower discretion on exact timing); and 3) normal antibiotic program. Treatments 1 and 2 also received a normal antibiotic program at grower and PCA discretion. Treatments were applied to six replicates at the Wheatland location and three replicates at the Marysville location.

A506 and Breakthru® were applied at 100 gallons per acre. Antibiotics were applied at either 50 or 100 gallons per acre depending on timing. All treatments were applied using a commercial air blast sprayer. The A506 plus Breakthru® treatment was applied March 20-22; full-bloom A506 March 23 (Marysville) and April 3 (Wheatland), and rat-tail application April 19. There were 6 – 8 full antibiotic applications applied between March 23 and April 29 in the normal grower program.

Unopened buds were collected from the Wheatland orchard on March 7 to appraise the existence of any bacteria which could compete with A506. At weekly intervals from March 15 (pre-treatment) through April 27, newly opened blossoms were rubbed onto petri dishes containing agar allowing only growth of strain A506. Each dish was divided into nine sections, and 27 flowers were sampled per plot at each date. Dishes were brought to the laboratory and held for three days to allow the A506 to grow. The colonized sections were then recorded as no growth, some growth, or vigorous growth. A total of six samples were collected. For graphing and analysis, it was planned to convert sample data into ratings using weighted averages (1.0 = no growth to 3.0 = maximum growth), then perform an analysis of variance on ranked transformed data using the Kruskal-Wallis ANOVA for Ranks. This would reveal which effects were significant (i.e. level of A506 and level of antibiotic), as it had in the 1999 trial.

**Fireblight strike evaluation:** During the treatment period, the growers regularly observed incidence of fireblight in the plot areas. Extensive infection was observed in the Marysville plot in mid-April so fireblight strikes were counted at the Marysville orchard on April 18 and 28. Analysis of variance was performed on the combined number of strikes per tree the two dates. The Wheatland orchard had very few strikes in 2000 so no count was done in that orchard.

**Extension of information:** Results of the trial were reported at a field meeting held on June 7, 2000 at the Naumes C.E. Sullivan Ranch in Yuba City. This meeting was attended by over 80 apple and pear growers, as well as pest control advisers, media and others (attached agenda).

## RESULTS AND DISCUSSION

**Pattern of A506 colonization:** No significant numbers of competing bacteria were found in pre-treatment bud samples collected March 7. It was discovered soon after sampling began that fungal contamination of agar plates greatly hindered A506 colonization. The selective media used in the past had traditionally been treated with the antifungal agent cycloheximide. This

chemical became unavailable in the 2001 season, rendering the data unreliable and non-analyzable.

**Fireblight strike evaluation (Marysville):** There were 38% fewer strikes in the A506 plus antibiotic plot than in the plots receiving antibiotics alone. This corroborates previous data showing the additive effect of A506 when applied with antibiotics. Resistance of *Erwina amylovora* to streptomycin at this site was documented during the 2000 season, which may also explain the high incidence of strikes in the antibiotic only plots. The A506 plus Breakthru® plus antibiotic treatment had 11% fewer strikes than where antibiotics were used alone. Data was statistically different only at 0.15%, however, this was encouraging given the large plot size, small number of replications, and variable distribution of fireblight in the field (Table 1).

**Table 1:** Average number of fireblight strikes - April 18 and 28 combined
Marysville, California

| Treatment | No. Strikes/tree |
|---|---|
| A506 plus antibiotics | .55 a |
| A506 Breakthru® plus antibiotics | .78 a b |
| Antibiotics only | .88 b |

Significant at p = 0.15 (actual p value = 0.12), means separated by Fisher's protected LSD.

**Program cost:** Applying A506 three times at half-rate added a total of $34.00 per acre to the cost of antibiotic program consisting of six full treatments of streptomycin and terramycin. This must be viewed in the context of the higher number of strikes due to resistance to streptomycin. Previous data has shown that the number of antibiotic treatments can be successfully reduced in an A506 program (Lindow, McGourty, Elkins, 1996). Applying the half rate of A506 with Breakthru® at 1-5% bloom cost $31.00 per acre but eliminated the full bloom A506 application. Actual program cost will depend on number and severity of potential infection periods, streptomycin resistance status, and grower/PCA preference.

## CONCLUSIONS AND 2001 PLANS

The demonstration in 2000 failed to directly reconfirm 1999 data showing that *Pseudomonas fluorescens* Strain A506 (Blight Ban A506®) successfully colonized and spread through the orchard when applied at half the labeled rate under conditions suitable for colonization. This was strictly the result of contamination of media plates due to the unavailability of the antifungal chemical cycloheximide. A new anti-fungal compound has been located so that sampling for colonization status can be successfully resumed in 2001.

Fireblight infection in mid-April allowed the number of strikes to be evaluated for each treatment in the Marysville orchard. There were 38% fewer strikes per tree where antibiotics were supplemented with A506 at half the labeled rate. This indirectly indicates successful

colonization by A506 in the orchard. The A506 plus Breakthru® treatment also appears promising, but requires further study.

Plans in 2001 will be to continue to refine the use of A506 as a component of an integrated fireblight program. This is especially important where resistance to streptomycin is present, as it is in the Upper Sacramento Valley. 2001 treatments will also include the newly registered *Bacillus subtillis* biofungicide (Serenade®, Agra Quest) which has shown positive results in recent trials. The newly registered plant growth regulator prohexadione calcium, (Apogee®, BASF) will also be tried to reduce vigorous shoot growth which is associated with late-season infections of vegetative shoots.

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## REFERENCES

Lindow, E., G. McGourty, and R. Elkins. 1996. Interactions of antibiotics with *Pseudomonas fluorescens* strain A506 in the control of fireblight and frost injury to pear. *Phytopahtology* 86(8):841-848.

Elkins, R.B. and S. Lindow. 1999. Optimal use pattern of *Pseudomonas fluorescens* A506 (Blight Ban A506®) to control fireblight and russet in pears. 1999 California Pear Research Reports, p. 251-256.

**Table 1**
Severity of fruit russet and incidence of frost damage to pear fruit at harvest that was treated at bloom time with antagonistic bacteria and applied to trees previously treated with eradicant chemicals or treated before bloom with a single application of Blightban A506 with and without a penetrating surfactant

| Treatment | Russet Severity (% of surface) | Frost Damage (% of fruit) |
|---|---|---|
| Control | 2.85 bcd | 0.28 a |
| Kocide + 0.5% Breakthru (finger) + A506 weekly | 4.08 b | 0.00 a |
| Bleach + 0.5% Breakthru (finger) + A506 weekly | 3.91 b | 0.00 a |
| A506 weekly | 1.75 cd | 0.00 a |
| A506 + 0.05% Breakthru weekly | 5.99 a | 0.00 a |
| A506 + 0.5% Breakthru (popcorn) only | 2.65 bcd | 0.22 a |
| A506 + 0.1% Breakthru (popcorn) only | 3.03 bc | 0.25 a |
| A506 (popcorn) only | 1.59 cd | 0.00 a |
| A506 + 0.5% Breakthru (1$^{st}$ bloom) only | 2.09 cd | 0.23 a |
| A506 + 0.2% Breakthru (1$^{st}$ bloom) only | 1.59 cd | 0.24 a |
| A506 + 0.1% Breakthru (1$^{st}$ bloom) only | 1.28 d | 0.00 a |
| A506 (1$^{st}$ bloom) only | 1.48 cd | 0.00 a |
| Serenade WP 4lb/A (20%, 40%, 100%) bloom | 1.56 cd | 0.00 a |
| Serenade WP 2lb/A (20%, 40%, 100%) bloom | 1.59 cd | 0.00 a |
| Serenade WP 4lb/A (7 & 14 days prebloom) | 1.88 cd | 0.24 a |
| C9-1 Weekly | 1.49 cd | 0.00 a |
| Streptomycin + Terramycin weekly | 1.75 cd | 0.00 a |