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

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ABSTRACT

The process of colonization of pear buds and flowers by indigenous bacteria of all kinds was monitored in 2 commercial pear orchards. The total number of indigenous bacteria in buds as well as the proportion of those bacteria that were "internal" to pear tissue varied substantially between the orchards. Total indigenous bacterial populations associated with pear in the early spring in 2004 were generally somewhat higher in number compared to that observed in these same sites previous years, with mean populations in individual buds ranging from about 10^4 to 10^6. Generally, a majority of the bacteria associated with buds were external. The fraction of the total bacterial population associated with pear buds that was "internal" was generally less than 10% in both orchards indicating that they are superficial colonists and probably not systemic in the trees. Application of Blightban A506 in 3 large replicated trials in commercial pear and apple orchards with the organo-silicon surfactant Breakthru resulted in a higher proportion of colonized flowers, especially early in the growing season in two of the trials than when the bacterium was applied in water alone. The proportion of flowers colonized with strain A506 throughout the spring generally increased with increasing concentrations of surfactant in which the bacteria were applied in a single application at the time of first bloom. Importantly, most flowers emerging from trees that were inoculated with strain A506 only a single time at "first bloom" with Blightban A506 in 0.5% Breakthru were as high as or higher throughout the main bloom, and into delayed bloom than that on trees that received weekly applications of the same amount of Blightban A506 without surfactant. No 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.

Colonization of pear buds and flowers with indigenous bacteria in the early spring

Work during 2004 addressed important issues in the microbial ecology of pear trees that relate to the management of fruit russet, frost injury, and fire blight disease of pear. One major objective was to monitor populations of indigenous bacteria in pear buds and emerging florets during winter/early spring and relate them to weather parameters such as spring rainfall. An earlier analysis of data in which we had measured indigenous bacterial populations on the flowers of pear at the beginning of bloom revealed large variations in population size from year to year. In some years such as 1995 and 1998 there were large populations of bacteria of all types in flowers shortly after they open in the spring, while in other years flowers emerged nearly sterile and become colonized by bacteria that apparently immigrated to the open flower via the air or insect vectoring from nearby plant sources such as orchard cover crop and weed species. We presumed that winter rains allow colonization of the buds and/or the emerging florets as the buds begin to open. Importantly, we had found that the incidence of early season fire blight infection is generally inversely proportional to the population size of the indigenous bacteria in the emerging flowers on control trees (eg. there is less disease in years when there are a lot of indigenous bacteria - "natural biological control" of fire blight seems to be operating). We also noted that the colonization of flowers by antagonistic bacteria such as *Pseudomonas fluorescens* strain A506 is less efficient in years when there are large indigenous bacterial populations. Fortunately, the presence of large numbers of indigenous bacteria can apparently confer some degree of "natural biological control" in those years when A506 itself is inhibited in its growth on pear by the presence of indigenous bacteria. The presence of large numbers of indigenous bacteria on emerging pear flowers is also associated with a relatively high incidence of frost injury during mild frost events compared to orchards and years when indigenous bacteria populations were low. We therefore undertook a detailed temporal analysis of the processes that allow bacteria to develop in emerging pear flowers to better understand how to manage biological control agents of fire blight disease as well as frost injury and fruit russet and to better predict when indigenous bacteria will be sufficiently numerous to present a high hazard of fruit russet and frost injury. Beginning about 4 weeks before bud break in 2004 we monitored the process of colonization of pear buds on a frequent basis to determine how rapidly bacterial populations changed and what weather factors were associated with the development of bacterial populations in buds and flowers. Bacterial populations were monitored on buds and flowers in a commercial pear orchard in both Lake County and Sacramento County. In addition to measuring the total bacterial population on buds and flowers we also measured the "internal" populations. At each sampling time the 40 bud or flower samples for each orchard were divided into two separate pools of 20 samples each. Total bacterial populations were determined by macerating the bud or flower samples from one pool individually in a small amount of buffer and plating appropriate dilutions onto non-selective media. In contrast, "internal" populations were determined as before on the other 20 samples in a given pool after the buds or flowers were surface sterilized by treatment with 0.5% sodium hypochlorite.

Both the total number of indigenous bacteria in buds as well as the proportion of those bacteria that were "internal" to pear tissue varied somewhat between orchards (Figs. 1-2). Total indigenous bacterial populations associated with pear in the early spring in 2004 was higher than in the past several years, reflecting the somewhat more normal winter rainfall. Average total bacterial populations per bud ranged from about 10^4 to 10^6 cells in

the various orchards. The numbers of bacteria on buds in these orchards did not change appreciably during the spring, and actually decreased with time in both orchards, being highest early in the spring (Figs. 1-2). The fraction of the total bacterial population associated with pear samples that were "internal" was generally about 10% or less in all of the orchards before bud break (Figs. 1-2). Thus it seems that most bacteria on buds are not "inside" the buds and thus might be expected to be influenced strongly by both winter pesticide applications, as well as weather conditions. The results of 2004 suggest that bacterial population development in pear occurs rapidly only after flowers emerge, and is not associated with large internal populations that developed during winter. Thus it appears that weather conditions at the time of flowering are more important in determining the populations of bacteria that will develop on newly forming flowers and fruit than weather conditions before buds open. This study was designed to be a relatively long-term study in which we will examine colonization of pear buds and flowers under a variety of weather conditions over a number of years so that the influence of weather conditions on bacterial populations can be better predicted. We will now be correlating the bud populations that have been monitored in these past several years with winter/spring weather conditions to obtain insight into the conditions that contribute to contamination of the buds by various bacteria. Predictions of indigenous bacterial populations before bloom will be useful in future predictions of the severity of fruit russet and of frost injury that are associated with these indigenous bacteria.

Establishment of *P. fluorescens* strain A506 in pear flowers by single early season applications with surfactants

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 tissues 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 pear tissues 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 pear tissues 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.

Blightban A506 was applied with high rates of surfactant in 2 large replicated trials in commercial pear orchards in 2004. We inoculated pear trees at the time of the first bloom with Blightban A506 with different concentrations of Breakthru and then measured the colonization of flowers by strain A506 after they emerged. The colonization of emerging flowers from early-season applications of bacteria and/or surfactant differed substantially in the different commercial orchards sprayed with airblast sprayers. At the Lake County orchard the fraction of flowers that were colonized by the biological control organism, strain A506, were nearly as high on flowers treated a single time with Blightban A506 with 0.5% Breakthru as on trees treated weekly with Blightban A506 in water alone throughout the

spring (Fig. 3). It is noteworthy that a substantially lower fraction of flowers were colonized in the early part of the bloom period when Blightban A506 was applied with 0.2% Breakthru than with 0.5% Breakthru a single time at first bloom (Fig. 3). Likewise, application of Blightban A506 at first bloom without any penetrating surfactant did not result in significant flower colonization. It thus appears that a penetrating surfactant is required for early season applications of Blightban A506 to successfully colonize the un-opened pear flowers. In this trial we also evaluated the inclusion of iron chelate (1 lb/100 gal of Sequestrene 138) with the repeated Blightban applications (Fig. 3). The addition of iron was evaluated since studies from Oregon researchers had indicated that the efficacy of Blightban A506 for fire blight control could be increased in the presence of added iron by stimulating the bacterium to produce an antibiotic inhibitory to *Erwinia amylovora*. The addition of iron did not increase nor inhibit the colonization of flowers with strain A506. Irrespective of the treatments compared in this trial, the incidence of flower colonization decreased in the later stages of flowering (Fig. 3). The bloom was very compact and accelerated in 2004 due to the extensive chilling and the warm weather at the time of bloom. Thus there were very few flowers available to colonie by early April. Our previous wok had indicated that the efficincy of movement of the biological control agent from treated flowers (flowers open when the spray was applied) to flowers that opened after the spray treatment decreased decreasing the number of flowers, presumably since there were fewer "source" flowers from which inoculum could be spread by flower-visiting insects. This seems to most easily explain the relative poor colonization of those few flowers that emerged in mid to late April, 2004.

There was relatively little fire blight in this plot in Lake Countyin 2004. Most of the fire blight occurred after mid-May, long after spray applications of strain A506 had ceased, and on flowers where there was little A506 colonization. There were, none-the-less, detectable differences in the incidence of fire blight on trees treated with Blightban in different ways (Table 1). The lowest incidence of fire blight strikes was on trees treated 3 times (20% bloom, Full, Bloom, and Petal Fall) with a mixture of Blightban A506 and Sequestrene 138 (Table 1). The possibilities if enhancing fire blight control with iron additions is promising and we will continue to evaluate it in trials in 2005. The severity of fruit russet was low and not significantly different between treatments in this plot (Table 2). The application of high rates of Breakthru in combination with Blightban A50 did not increase the severity of fruit russeting significantly compared to trees treated with antibiotics alone (Table 2).

Since the fireblight pathogen *Erwinia amylovora* multiplies primarily on the pistil of flowers, and that is the site where interaction with biological control agents must occur to achieve control of fireblight disease, we investigated the location of *P. fluorescens* strain A506 on flowers that have been treated with Blightban A506 to ensure that the occurrence of the antagonist measured with the flower-rub assays as noted above was because of its colonization of the pistil. On flowers from trees treated weekly with either Blightban A506 or treated only once at first bloom with Blightban A506 in 0.5% Breakthru, the majority of cells of strain A506 was associated with the pistil (Figs. 4 and 5). To determine the location of bacteria in flowers, they were dissected to remove the pistil from the remainder of the flower. Similar numbers of cells of strain A506 were seen on the pistil of flowers from these treatments (Fig. 4). Likewise, similar numbers of cells of strain A506 were recovered form the remainder of the flowers form these two treatments (Fig. 5). Generally about 90% of all of the cells of strain A506 on a given flower were on the pistil (Figs. 4 and 5). Since

the mass of the pistil is much less than the mass of the rest of the flower it is clear that the cells are highly concentrated on the pistil. The population sizes of strain A506 on pistils were also very high, generally nearly 10^5 cells/pistil during the main bloom period. This is generally considered to be close to the so-called carrying capacity for bacteria on the pistilate surface. We thus conclude that the pistil was colonized nearly to its fullest extent by the antagonist *P. fluorescens* strain A506 and that biological control of fireblight disease should have been maximum under these conditions. We also can be confident that estimates of the incidence of colonization of flowers made using the flower rub assay provide evidence that the pistil itself is colonized since nearly all of the bacteria in the flower were on the pistil.

The colonization of flowers by strain A506 in an orchard in Sacramento County that was treated with Blightban at different times was generally much less than that in the Lake County trial. While most pear flowers on trees that had been treated weekly with Blightban A506 alone or with Seqestrene 138 were highly colonized with strain A506, at least during the main bloom period, a much lower fractio of flowers from trees treated a single time with Blightban A506 + 0.25% Breakthru at first bloom that were sampled during the main bloom were colonized by strain A506 (Fig. 6). As in the Lake County trial, the fraction of flowers that emerged later in the growing season that were colonized decreased (Fig. 6). We do not know what to attribute the lesser colonization of flowers from the single application of Blightban + Breakthru compared to the trial in this trial. Unlike in other tests at this site, the Blightban A506 was applied with Breakthru at about 10 % bloom, a timing that has led to a high incidence of colonization in other trials such as in Lake County in 2004 (Fig. 3). The reasons for this poor colonization of flowers from the early Blightban applicaiotn will be further evaluated, such as to investigate whether the use of surfactants with the bacteria might have liberated inhibitory pesticide residues in the spray tank.

The large majority of bacteria in flowers treated with Blightban A506 in the Sacramento County trial were located on the pistil, as in the Lake Country trial. An average of about 10^5 cells/pistil were recovered from flowers from trees treated weekly with Blightban A506 alone or with Sequestrene 138 (Fig. 7). The pistilate populations on these trees represented on average about 90% of all of the bacteria recovered from these flowers (Figs. 7 and 8). While the populations of bacteria on the pistils of flowers treated a single time at first bloom with Blightban A506 and 0.25% Breakthru were much lower than on trees treated weekly with Blightban A506 (Fig. 7), the pistilate populations of strain A506 also represented about 90% of all of the bacteria recovered from dissected flowers from trees receiving this treatment (Figs. 7 and 8). These are important findings since it shows that while the bacterium was applied to closed flower buds, cells of the biological control agent were able to penetrate to the interior of the flower where it provided the inoculum for the colonization of the pistil as the flower opened.

The severity of fruit russet at harvest on trees from the Sacramento County trial (like that of the Lake County trial) was similar irrespective of the treatment to which trees had been given at the time of bloom (Table 2). There was no evidence of phytoxicity to fruit due to applicaiotns of 0.25% Breakthru. There was very little fire blight in this plot area in 2004, and hence no significant effects of the treatments on disease were observed.

The results of large-scale field tests of early season applications of Blightban A506 in 2004 continue to support the idea that the timing of such a treatment is very important to maximize the effectiveness of biological fireblight control. It is possible that if sprays are applied too early reduced colonization can result. Our work in 2000 had indicated that

colonization of flowers from single applications to “fingers” was much less effective than to buds at “first bloom”. 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).

An additional large-scale field trial to test the efficacy of early-season application of Blightban A506 done on Pink Lady apple supported the findings on flower colonization reported above for pear. 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 two large pear trials described above. The results of this study of colonization of apple after application of Blightban A506 were very similar to that obtained in the Lake Country pear trial. The percentage of flowers that were colonized by strain A506 on trees treated a single time at first bloom with Blightban A506 containing either 0.2% or 0.5% Breakthru were as high as or higher than that on trees treated weekly with Blightban A506 in water alone at all sampling times (Fig. 9). The proportion of flowers colonized by strain A506 on flowers treated once with the Blightban A506 + 0.5% Breakthru mixture were always much higher at a given date than that on flowers treated once with Blightban without surfactant. (Fig. 9). This points out the importance of the penetrating surfactant in making this strategy of biological control possible. The proportion of flowers on trees treated with Blightban A506 + 0.5% Breakthru were generally higher than that on trees treated with Blightban A506 + only 0.2% Breakthru (Fig. 11). Thus the higher concentration of Breakthru substantially enhanced colonization of apple flowers by strain A506 as it had in the Lake County pear trial. The addition of Sequestrene 138 to weekly applications of Blightban A506 did not appreciably affect the proportion of flowers that became colonized by strain A506 (Fig. 9).

Dissection of apple flowers revealed that application of Blightban A506 by a variety of methods always resulted in high relative populations of the *P. fluorescens* strain A506 cells in a flower to be on the pistil of flowers compared to the rest of the flower (Figs. 10 and 11). Average population sizes of strain A506 on the pistils of flowers sampled later in the main bloom period from trees treated weekly with Blightban A506 alone averaged about 10^4 cells, which represented about 90% of all bacteria found on that flower (Figs 10 and 11). Likewise, about 10^5 cellsof strain A506 per pistil were found on flowers early in the main bloom on trees treated a single time at first bloom with Blightban A506 with 0.5% Breakthru (Fig. 10). These populations represented the majority of the bacteria on flowers at a given sampling time (compare Figs 10 and 11). Thus it is clear that application of Blightban A506 even before flowers open can provide inoculum of strain A506 that reaches the pistil of flowers.

Very little fire blight occurred in the apple plot in 2004 and thus we could not determine the effects of treatments on disease control. The severity of fruit russet was very low on the Pink Lady apples at harvest. There was slightly more fruit russet on fruit treated with Breakthru at first bloom compared to other treatments (Table 2), but the degree of fruit russet was very low, even in these treatments.

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. 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. We will further test this approach in 2005 with the hope that we will encounter sufficient fire blight in our test plots to demonstrate that alternative application strategies of Blightban A506 can yield satisfactory disease control.

Table 1. Incidence of fire blight infections on Bartlett pear trees treated at various times with Blightban A506 with and without a penetrating surfactant – Kelseyville, 2004.

Treatment	Strikes/tree
Blightban A506 1X 1% bloom + 0.5% Breakthru	0.22 b
Blightban A506 1X 1% bloom + 0.2% Breakthru	0.15 ab
Blightban A506 1X 1% bloom – no Breakthru	0.20 b
Blightban A506 20% bloom + FB + petal fall +Sequestrene 138	0.09 a
Blightban A506 20% bloom + FB + petal fall	0.13 ab
Antibiotics only	0.18 ab

Table 2
Severity of pear and apple fruit russeting at harvest from trees treated with Blightban A506 in different ways before and during bloom -- 2004

Treatment	Fruit Russet (% of surface)		
	Pear		Apple
	Lake Co.	Sacramento Co.	Madera
Antibiotics only	0.71 a	0.44 a	0.80 c
Blightban A506 weekly	0.43 a	0.47 a	0.88 bc
Blightban A506 1st bloom + 0.5% Breakthru	0.74 a		1.69 a
Blightban A506 1st bloom + 0.2% Breakthru	0.84 a	0.72 a	1.47 ab
Blightban A506 weekly + Sequestrene 138	0.48 a	0.62 a	0.68 c
Blightban A506 1st bloom – no surfactant	0.60 a		0.80 c

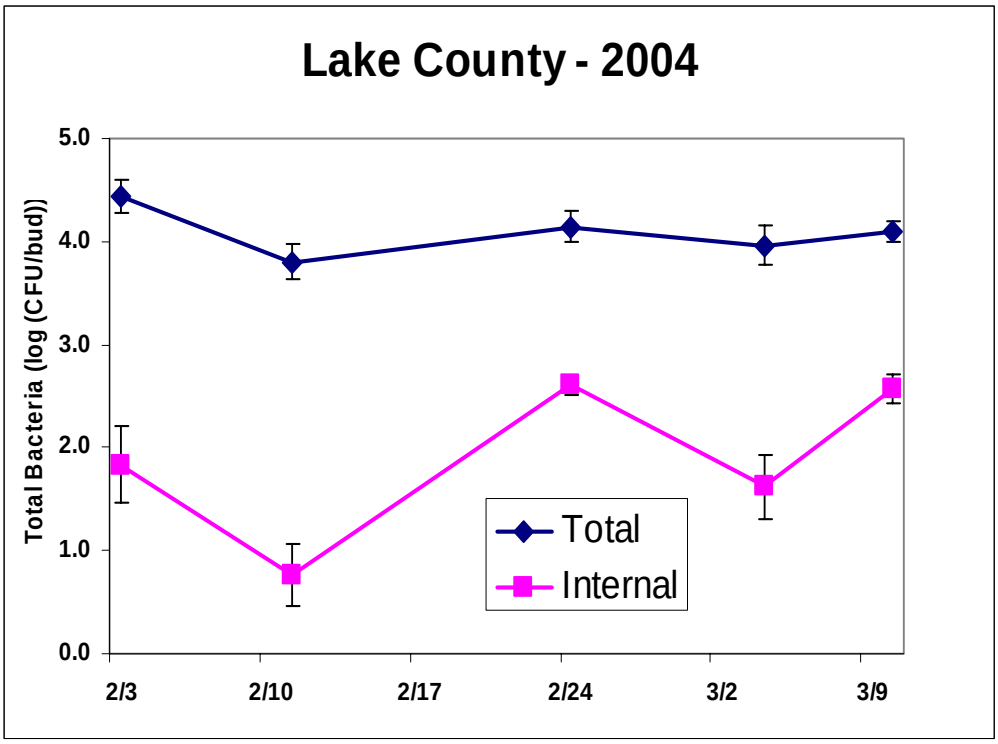

Figure 1. Total bacterial populations (diamonds) and "internal" bacterial populations remaining in buds and clusters of pear that were surface sterilized with bleach (squares) that were collected from a commercial Bartlett pear orchard in Lake County in the spring of 2004.

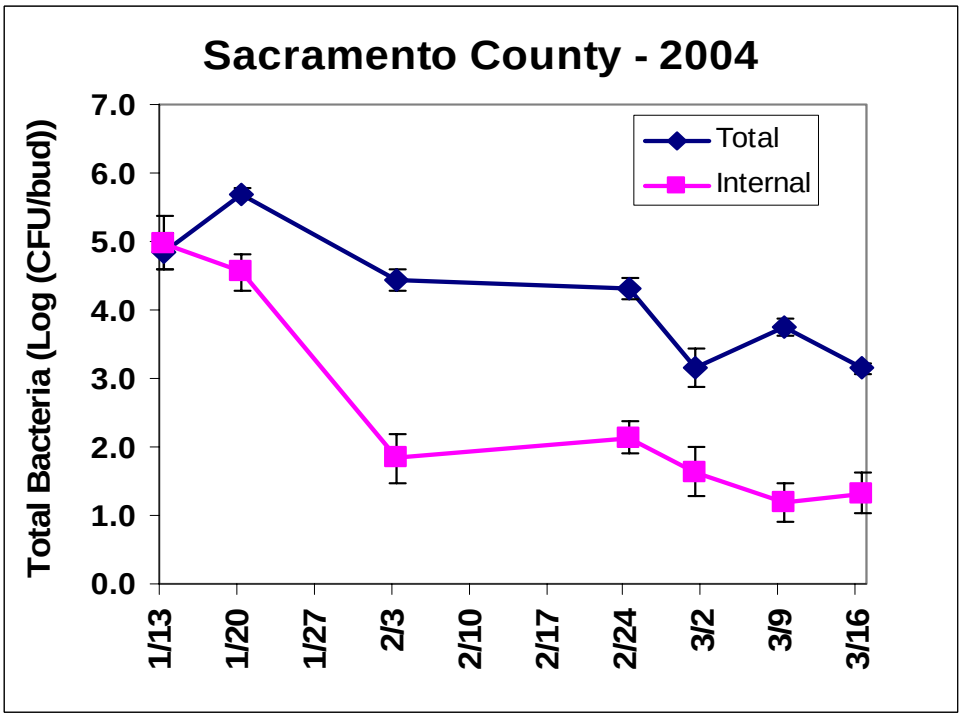

Figure 2. Total bacterial populations (diamonds) and "internal" bacterial populations remaining in buds and clusters of pear that were surface sterilized with bleach (squares) that were collected from a commercial Bartlett pear orchard in Sacramento County in the spring of 2004.

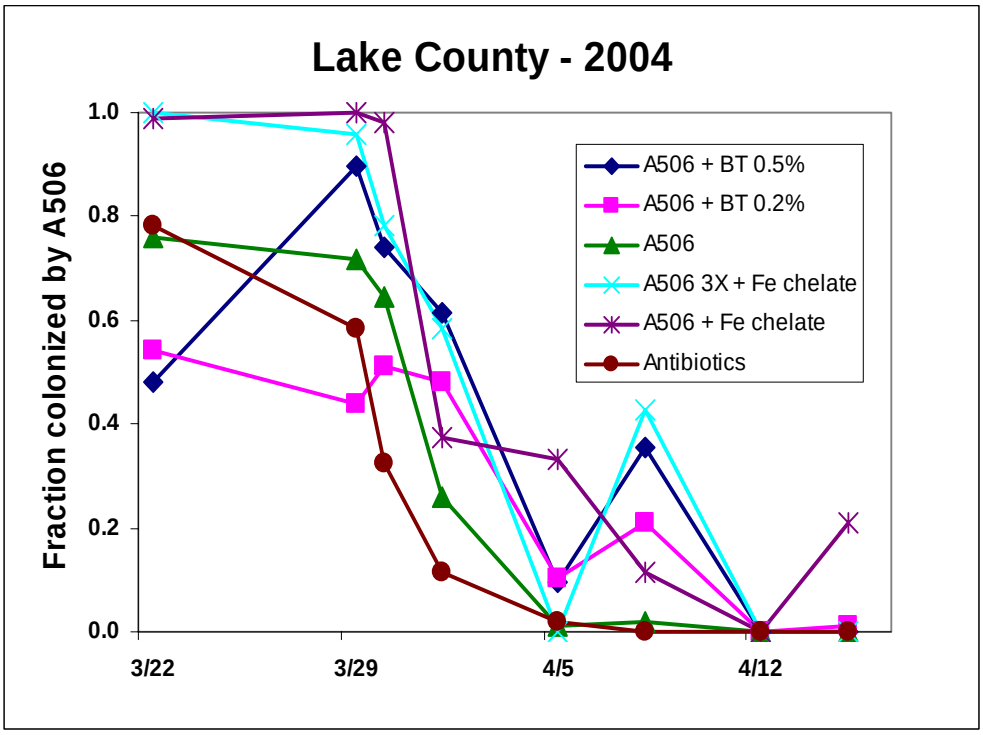

Figure 3. Fraction of flowers colonized with *Pseudomonas fluorescens* strain A506 on Bartlett pear trees treated once only at the "first bloom" stage of growth [when only a few flowers were observed in an orchard] with a label rate of Blightban A506 in water alone (triangles) or in 0.5% Breakthru (diamonds) or 0.2% Breakthru (squares) compared with weekly applications of Blightban A506 in water (stars) or weekly applications of Blightban and 1 lb/100 gal Sequestrene 138 (x's) or with antibiotics alone (circles) in a Lake County plot at Kelseyville in 2004.

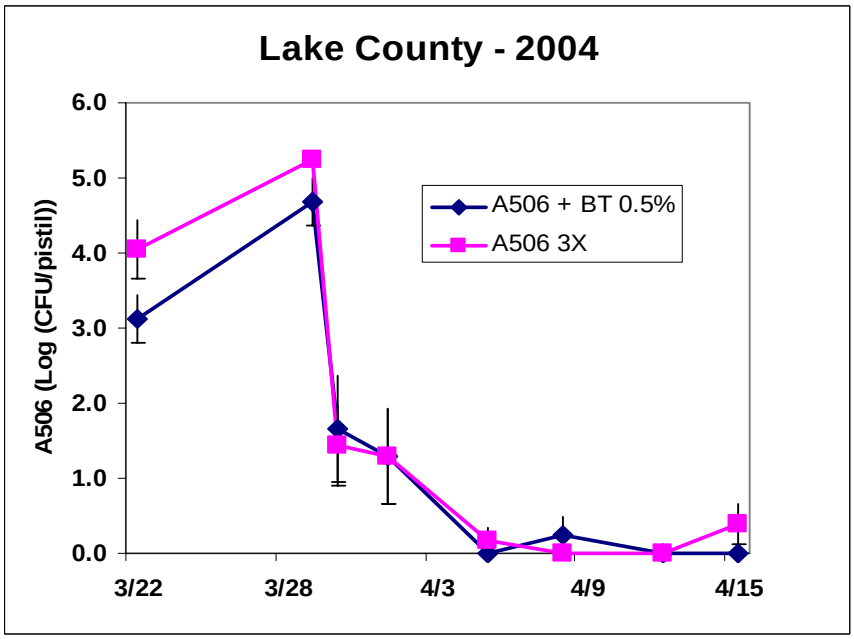

Figure 4. Population size of *Pseudomonas fluorescens* strain A506 on the pistils of flowers of Bartlett pear trees treated weekly with a label rate of Blightban A506 3 times (20 % bloom, full bloom, and petal fall) in water alone (squares) or once at 1% bloom in 0.5% Breakthru (diamonds) in a Lake Country trial in Kelseyville in 2004.

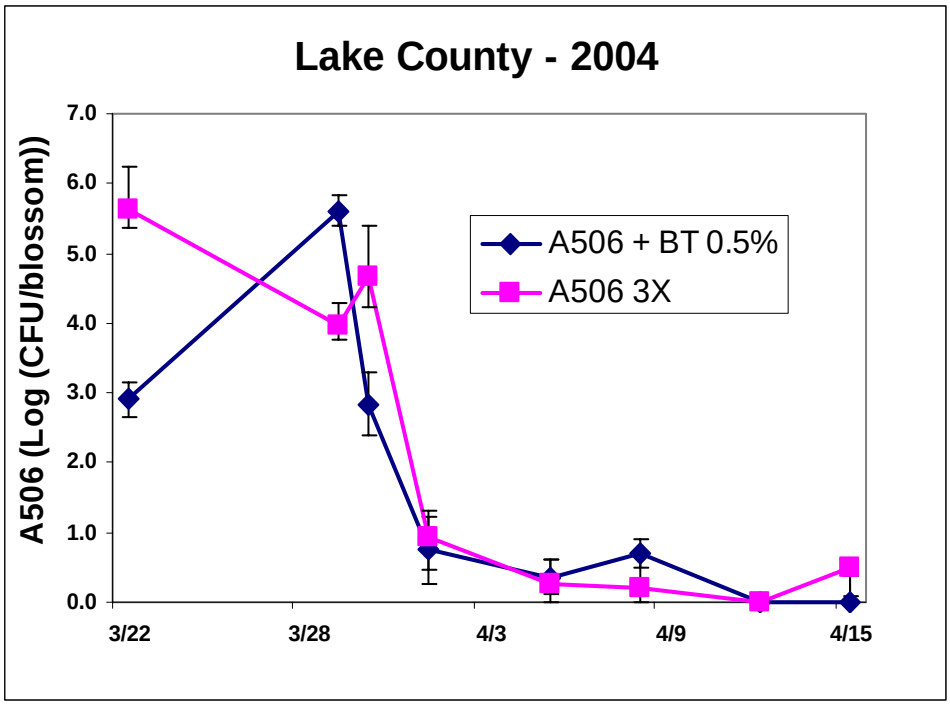

Figure 5. Population size of *Pseudomonas fluorescens* strain A506 on the remainder of the flower (after removal of the pistil) of flowers of Bartlett pear trees treated weekly with a label rate of Blightban A506 3 times (20 % bloom, full bloom, and petal fall) in water alone (squares) or once at 1% bloom in 0.5% Breakthru (diamonds) in a Lake Country trial in Kelseyville in 2004.

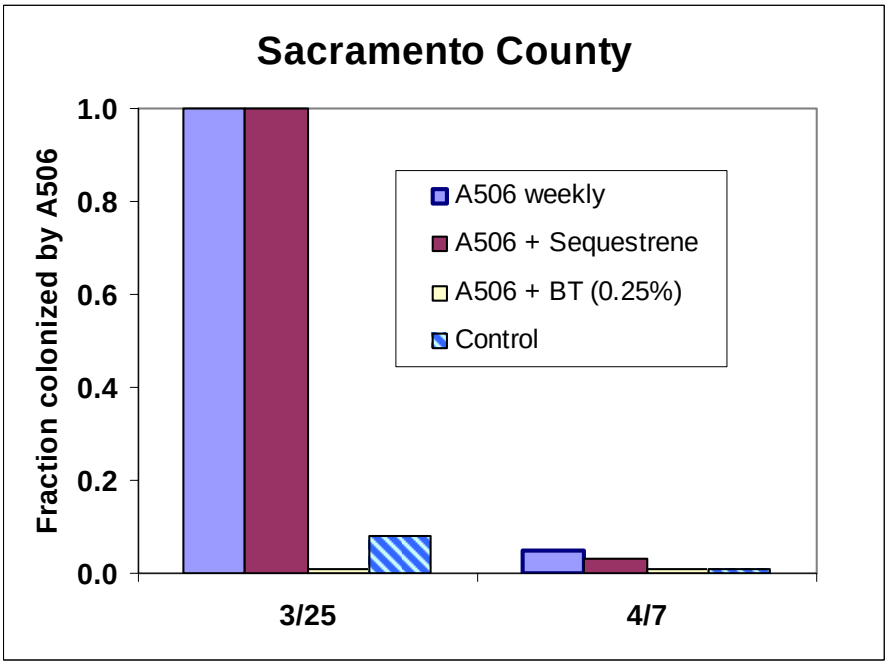

Figure 6. Fraction of flowers colonized with *Pseudomonas fluorescens* strain A506 on Bartlett pear trees treated once only at the "first bloom" stage of growth [when only a few flowers were observed in an orchard] with a label rate of Blightban A506 in 0.5% Breakthru, compared with weekly applications of Blightban A506 in water or weekly applications of Blightban and 1 lb/100 gal Sequestrene 138 or with antibiotics alone in a Sacramento County plot in 2004.

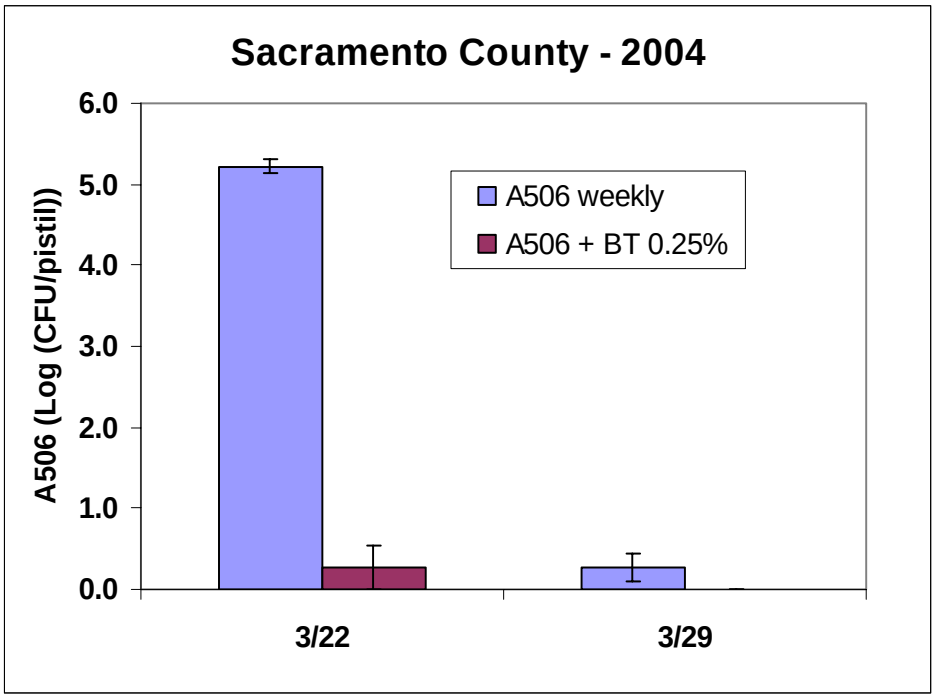

Figure 7. Population size of *Pseudomonas fluorescens* strain A506 on the pistils of Bartlett pear flowers on trees treated weekly with a label rate of Blightban A506 in water alone or treated a single time at 10% bloom with Blightban A506 in 0.25% Breakthru in a Sacramento County trial in 2004.

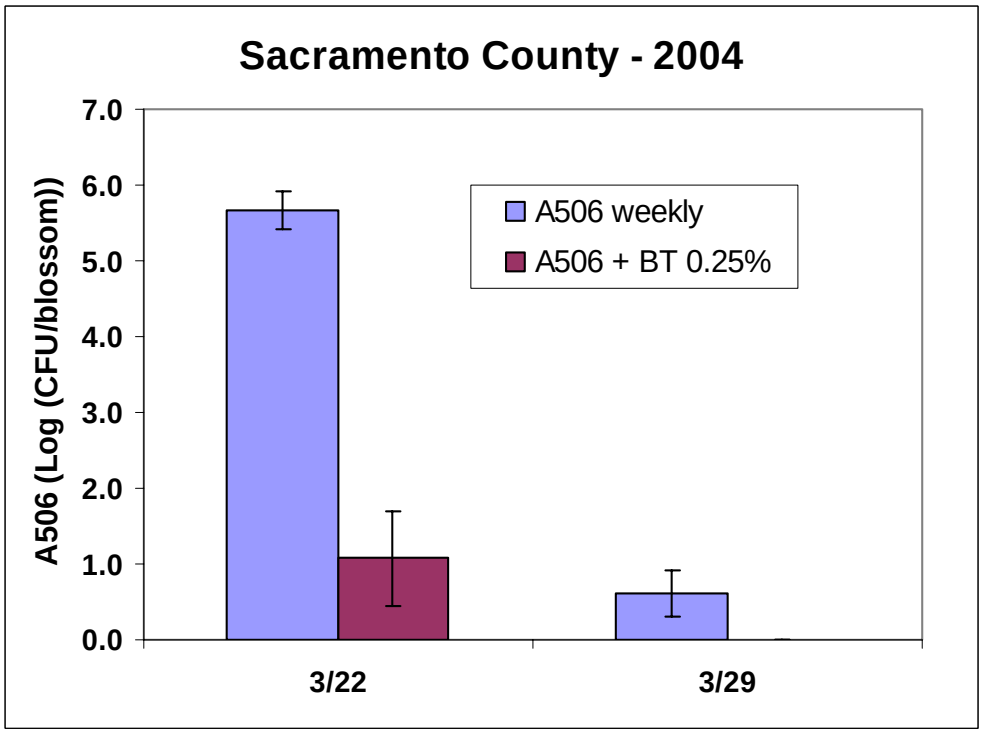

Figure 8 Population size of *Pseudomonas fluorescens* strain A506 on the remainder of the flower (after removal of the pistil) of flowers from Bartlett pear trees treated weekly with a label rate of Blightban A506 in water alone or treated a single time at 10% bloom with Blightban A506 in 0.25% Breakthru in a Sacramento County trial in 2004.

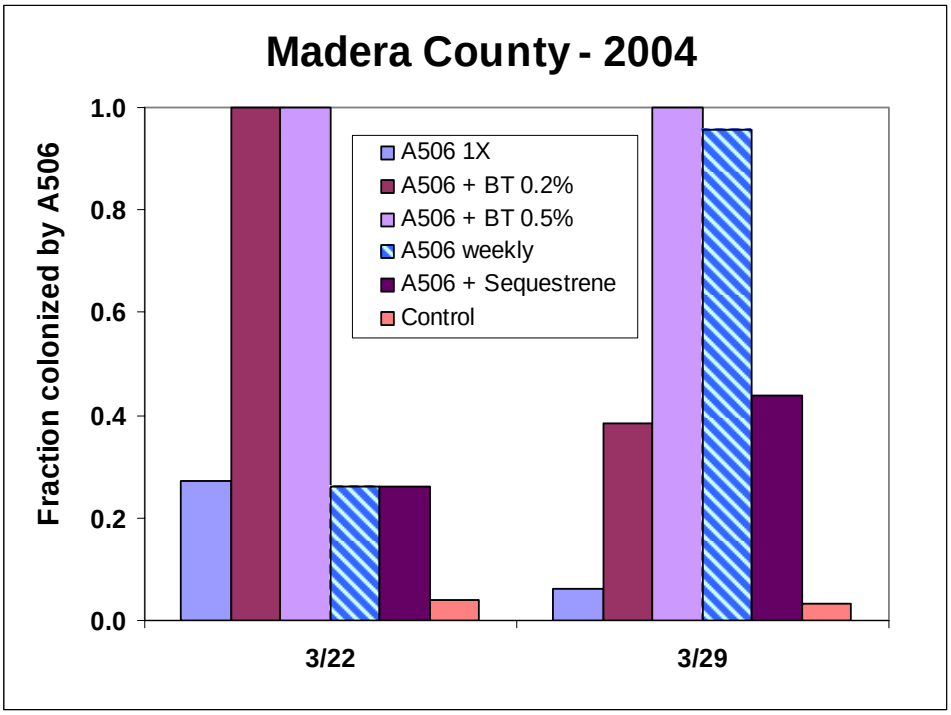

Figure 9. Fraction of flowers colonized with *Pseudomonas fluorescens* strain A506 on Pink Lady apple trees treated once only at the "first bloom" stage of growth [when only a few flowers were observed in an orchard] with a label rate of Blightban A506 in water alone or in 0.5% Breakthru, 0.2% Breakthru , 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.

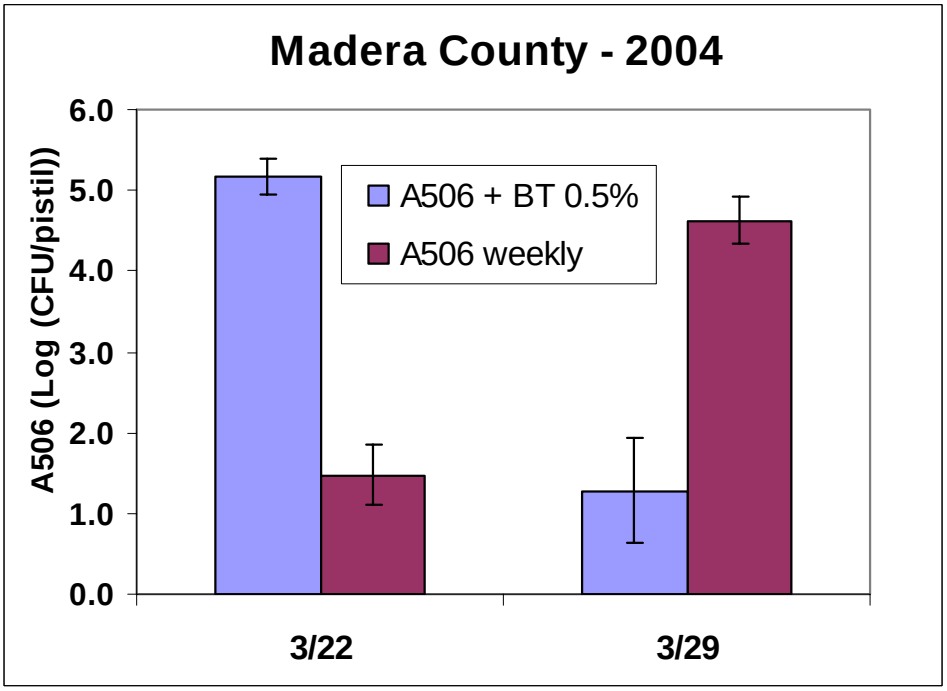

Figure 10. Population size of *Pseudomonas fluorescens* strain A506 on the pistils of Pink Lady apple flowers on trees treated weekly with a label rate of Blightban A506 in water alone or with a single application of Blightban A506 in 0.5% Breakthru at 1% bloom in a Madera County trial in 2004.

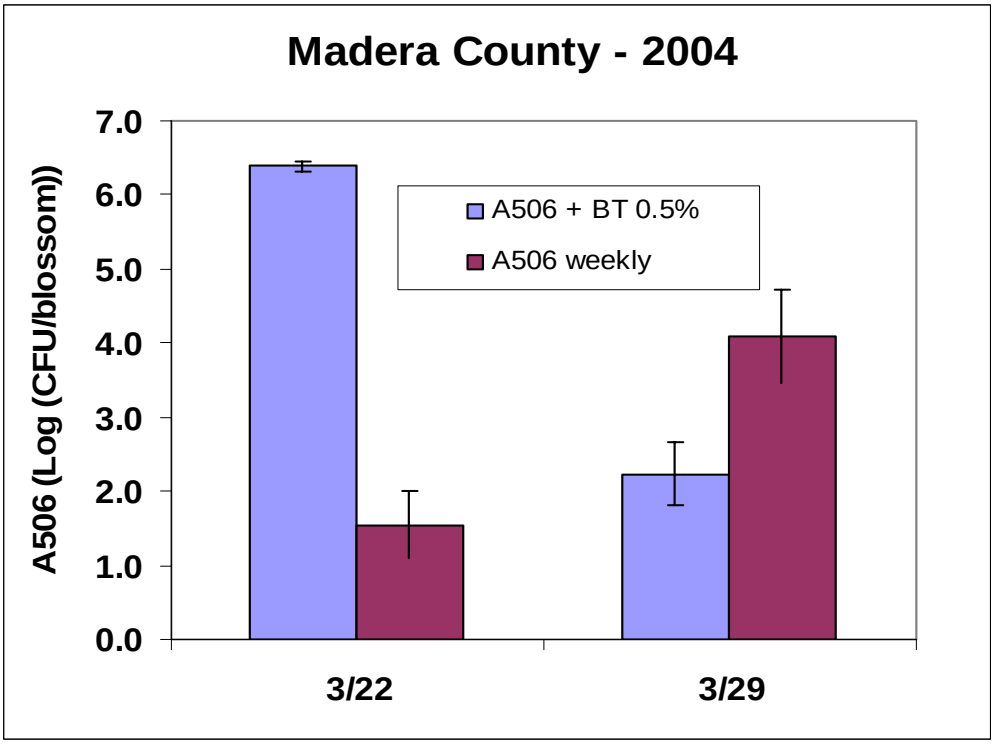

Figure 11. Population size of *Pseudomonas fluorescens* strain A506 on the remainder of flowers (after removal of the pistil of Pink Lady apple on trees treated weekly with a label rate of Blightban A506 in water alone or with a single application of Blightban A506 in 0.5% Breakthru at 1% bloom in a Madera County trial in 2004.