Management of Frost Injury, Fire blight, and Fruit Russetting 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 3 commercial pear orchards. Both 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 orchards. Total indigenous bacterial populations associated with pear in the early spring in 2003 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 1000 to 100,000. Generally, a majority of the bacteria associated with buds were external, while the proportion that was internal to pear tissue increased in developing flower and tissues after bud break. The fraction of the total bacterial population associated with pear buds that was "internal" was generally less than 10% in all orchards indicating that they are superficial colonists and probably not systemic in the trees. Bacterial populations generally increased rapidly after bud enlargement. Since most spring rain occurred after bud break in 2003, it did not have a major effect on bud populations. 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 most effective colonization of flowers from the single early-season spray application of antagonist with surfactant occurred in the two orchards in which most of the flowers, while not yet open, were poised to open; much less colonization occurred in the orchard which was sprayed at an earlier stage of bloom. 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 2003 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 2003 we monitored the process of colonization of pear buds and the flowers that emerged from these 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 two commercial pear orchards in Lake County, and one orchard in 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. 1-3). Total indigenous bacterial populations associated with pear in the early spring in 2003 was higher than in the past several years, reflecting the somewhat more normal winter rainfall. Average total bacterial populations per bud ranged from about 1000 to 100,000 cells in the various orchards. Since the sampling period was not associated with frequent rainfall, the numbers of bacteria on buds in these orchards did not change appreciably during the spring, and actually decreased with time in some orchards (Figs. 1-3). The fraction of the total bacterial population associated with

pear samples that was "internal" was generally about 10% or less in all of the orchards before bud break (Figs. 1-3). 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 2003 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 determine the populations of bacteria that will develop on newly forming flowers and fruit than weather conditions before buds open. Unfortunately, relatively dry weather occurred at this time in 2003, so we could not ascertain weather effects after flower opening. This study is 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. 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 2003. 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. 4). In this plot the cooperator accidentally oversprayed the entire plot with Blightban A506 on about April 10. For this reason, a high proportional off all flowers in the orchard were colonized by strain A506, even on trees treated previously only with antibiotics after this date (Fig. 4). The effects of early season application of Blightban A506 thus should be considered only for dates before about April 10. 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. 4). 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. 4). 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*. While we did not observe sufficient fire blight in our pear trials to evaluate the effect of iron addition on fire blight control, we did find that iron did not increase nor inhibit the colonization of flowers with strain A506. In a large-scale trial in Sacramento County, we also observed that addition of iron chelate to Blightban A506 sprays did not alter the colonization of flowers by strain A506; a similarly large proportion of flowers were colonized by strain A506 irrespective of whether sequestrene was added to the weekly applications of Blightban A506 (Fig. 4). The application of iron chelate to trees also did not alter the fruit russet of pear in either trial (Table 1). The possibilities if enhancing fire blight control with iron additions is intriguing and we will continue to evaluate it in trials in 2003.

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 alone (Fig. 5) or a mixture of Blightban A506 and Sequestrene 138 (Fig. 6) the great majority of cells of strain A506 was associated with the pistil. To determine the location of bacteria in flowers, they were dissected to remove the pistil form the remainder of the flower. Generally from 90 to 99.9% of all of the cells of strain A506 on a given flower were on the pistil (Figs. 5 and 6). 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 10^6 cells/pistil. This is generally considered to be the so-called carrying capacity for bacteria on the pistilate surface. We thus conclude that the pistil was colonized 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 many pear flowers on trees that had been treated a single time with Blightban A506 + 0.5% Breakthru at first bloom that were sampled during the main bloom (in mid to late March) were colonized by strain A506, the fraction of flowers that emerged later in the growing season that were colonized decreased (Fig. 7). Generally the proportion of flower that harbored detectable numbers of stain A506 on trees treated weekly with Blightban A506 alone or a mixture of Blightban A506 and Sequestrene 138 were substantially than on trees treated at first bloom only with Blightban A506 (Fig. 7). We attribute the lesser colonization of flowers from the single application of Blightban + Breakthru compared to the trial in Lake County to the fact that it was applied at an earlier stage of flower development. While many flowers were poised to open when this treatment was applied in the Lake County trial, many flowers in the Sacramento trial were relatively tightly closed when the very first flowers emerged and when the treatment was applied. It was also surprising to see that a lower proportion of flowers on trees treated weekly with Blightban A506 were colonized in the Sacramento trial (Fig. 7) compared to the Lake County trial. This is unexpected since we have nearly always seen a very high proportion

of flowers to be colonized in such treatments, much like that observed in the Lake County trial in 2003 (Fig. 4). The reason for this is not clear, and we are investigating whether another pesticide might have been applied to these trees that interfered with flower colonization.

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 (Fig. 8). The pistilate populations on these trees represented on average about 90% of all of the bacteria recovered from these flowers (Fig. 8). While the populations of bacteria on the pistils of flowers treated a single time at first bloom with Blightban A506 and Breakthru were much lower than on trees treated weekly with Blightban A506 (Fig. 9), the pistilate populations of strain A506 also represented about 90% of all of the bacteria recovered from dissected flowers (Fig. 10). This is an important finding 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 1). Given that relatively little rainfall occurred during March when most flowers had opened at this site, the relatively low severity of fruit russet even on trees that received only antibiotic sprays can likely be explained by the fact that indigenous bacterial populations on the flowers and developing fruit that normally contribute to russet formation were relatively low during this period. Since russet is apparently induced within the first few weeks of flowering, the rains that were prevalent later in April may have come too late to increase indigenous bacterial populations sufficiently early to achieve russet induction.

The results of large-scale field tests of early season applications of Blightban A506 in 2003 support the idea that the timing of such a treatment is very important to maximize the effectiveness of biological fireblight control. Since flower buds do not open simultaneously, and since the bacteria will gain entry into the more open flower buds more efficiently than to closed buds, it is likely that more of the flower buds were sufficiently open to allow colonization by strain A506 in the Lake County trial than at Sacramento County at the time of the single spray. Thus it is possible that if sprays are applied too early reduced colonization can result. Our earlier 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 nearly as high as or higher than that on trees treated weekly with Blightban A506 in water alone at all sampling times (Fig. 11). Both the proportion of flowers colonized by strain A506 as well as the population sizes of strain A506 on flowers treated with the Blightban A506 + 0.5% Breakthru mixture were often higher at a given date than that on flowers treated repeatedly with Blightban alone (Figs. 11 and 12). 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. 11). It is important to note, that when Blightban A506 was applied to trees a single time at first bloom but without Breakthru surfactant, that colonization of flowers was much less than when it was applied with Breakthru (Fig. 11). This points out the importance of the penetrating surfactant in making this strategy of biological control possible. While nearly all apple flowers sampled during the bloom period were colonized by strain A506 irrespective of whether Blightban A506 was applied frequently without surfactant or was applied only at first bloom with surfactant, those few flowers that emerged after mid-May were not frequently colonized. As with pear, the buds and those few flowers that emerge late in the spring bloom period are somewhat different from main bloom flowers morphologically and were thus not sufficiently open at "first bloom" for strain A506 to penetrate into the flower bud when sprayed with Breakthru. Likewise, these flowers emerged after the last weekly sprays of Blightban in water alone were applied, and thus escaped inoculation.

Dissection of apple flowers revealed that application of Blightban A506 by a variety of methods always resulted in high relative populations of *P. fluorescens* strain A506 on the pistil of flowers compared to the rest of the flower (Figs. 13-14). Average population sizes of strain A506 on the pistils of flowers from trees treated weekly with Blightban A506 alone were about 10^4 to 10^5 cells, which represented about 90% of all bacteria found on that flower (Fig. 13). Likewise, about 10^4 to 10^5 cells/pistil were found on flowers on trees treated a single time at first bloom with Blightban A506 with 0.5% Breakthru (Fig. 14). These populations represented the majority of the bacteria on flowers at a given sampling time. 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.

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. 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 2004 with the hope that we will encounter sufficient fireblight in our test plots to demonstrate that alternative application strategies of Blightban A506 can yield satisfactory disease control.

Table 1
Severity of pear fruit russeting at harvest from trees treated with Blightban A506 in different ways before and during bloom – Sacramento County, 2003

Treatment	Fruit Russet (% of surface)	
	Upper Lake	Sacramento Co.
Antibiotics only	4.20 a	1.69 b
Blightban A506 weekly	3.95 a	1.90 b
Blightban A506 1st bloom + 0.5% Breakthru	2.49 a	3.38 a
Blightban A506 1st bloom + 0.2% Breakthru		2.09 b
Blightban A506 weekly + Sequestrene 138	2.37 a	2.26 ab
Blightban A506 1st bloom – no surfactant		1.61 b

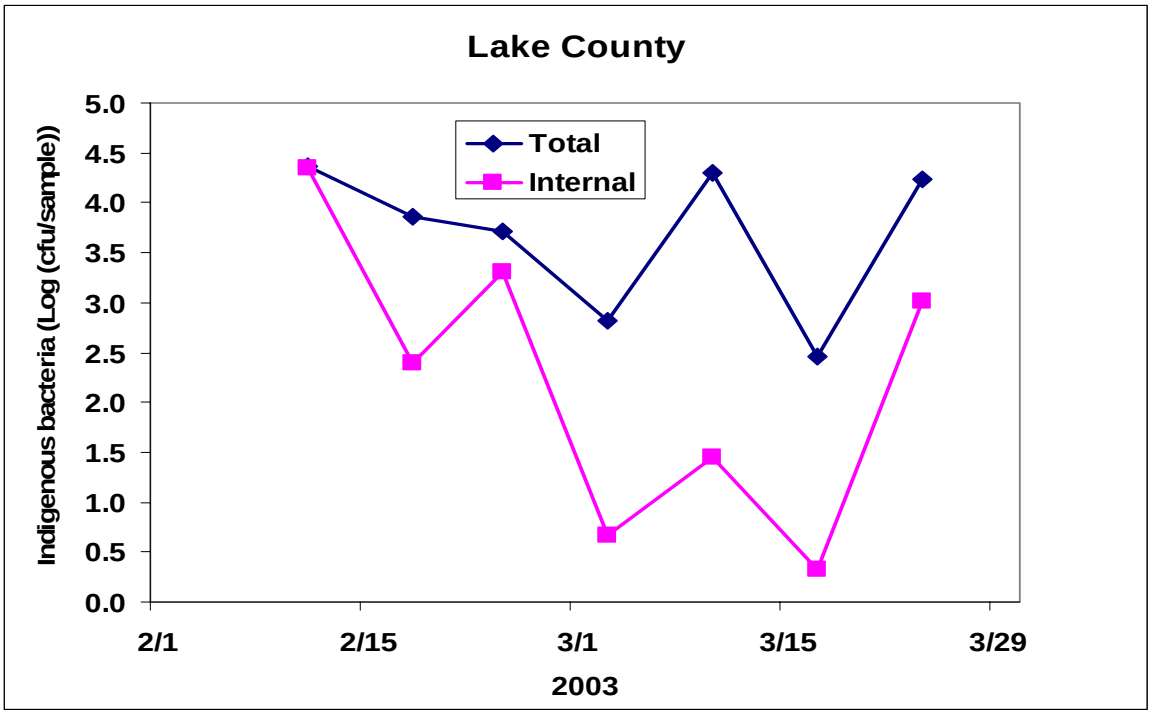

Figure 1. Total bacterial populations (diamonds) and "internal" bacterial populations remaining in buds and clusters of pear that were surface sterilized with bleach (squares) that were collected from a commercial Bartlett pear orchard in Lake County in the spring of 2003.

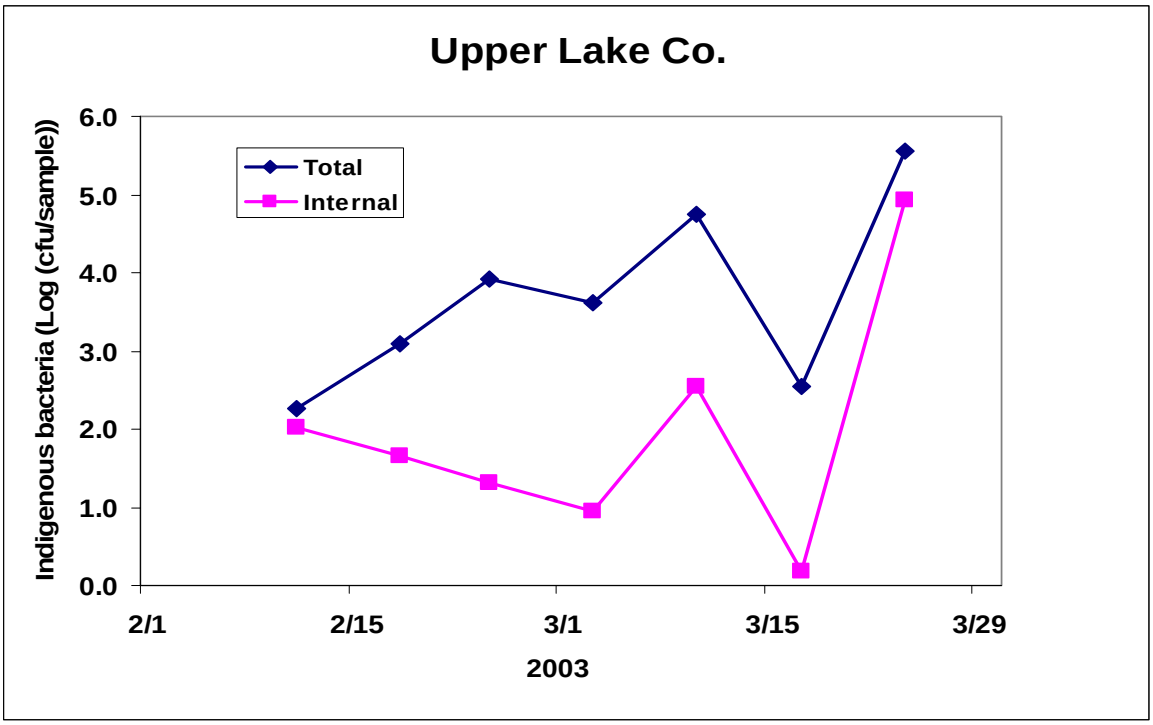

Figure 2. Total bacterial populations (diamonds) and "internal" bacterial populations remaining in buds and clusters of pear surface sterilized with bleach (squares) that were collected from a commercial Bartlett pear orchard in Upper Lake in the spring of 2003.

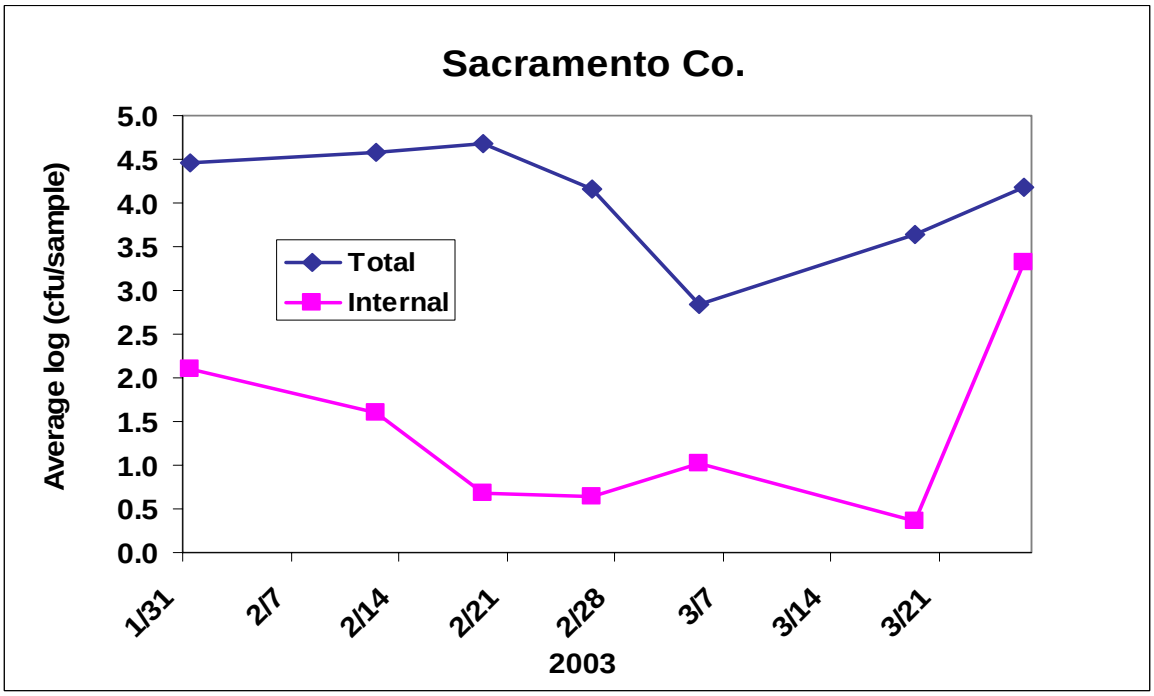

Figure 3. Total bacterial populations (diamonds) and "internal" bacterial populations remaining in buds and clusters of pear that were surface sterilized with bleach (squares) that were collected from a commercial Bartlett pear orchard in Sacramento County in the spring of 2003.

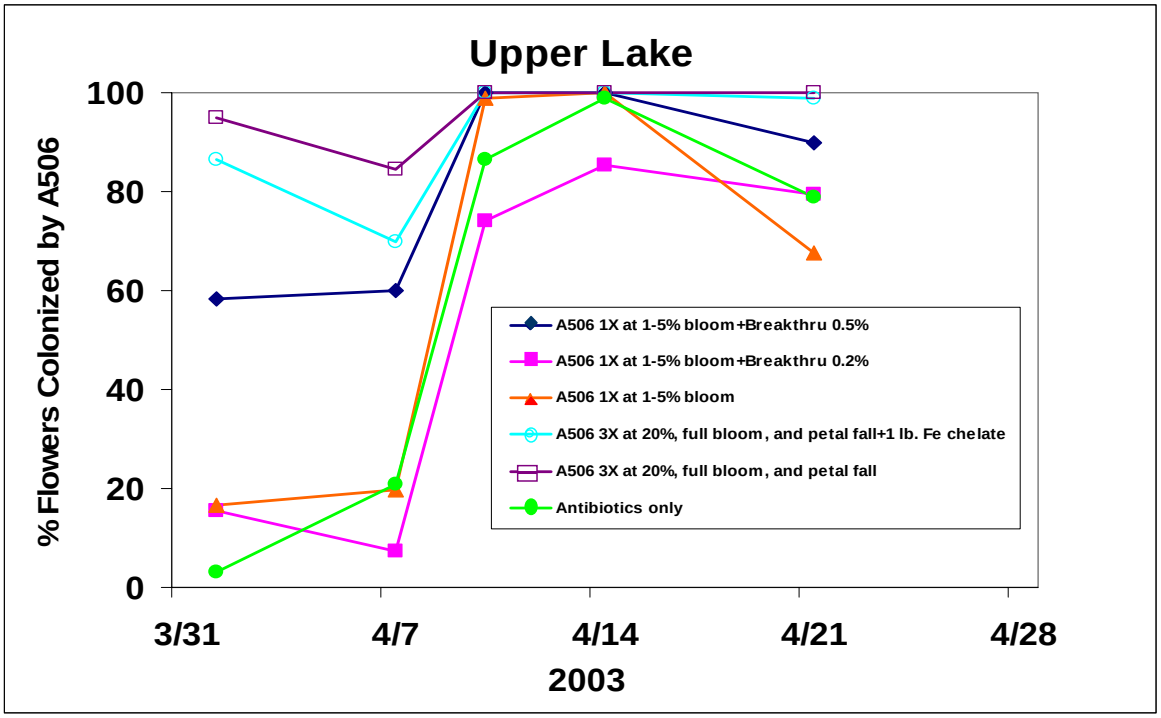

Figure 4. Percent of flowers colonized with *Pseudomonas fluorescens* strain A506 on Bartlett pear trees treated once only at the "first bloom" stage of growth [when only a few flowers were observed in an orchard] with a label rate of Blightban A506 in water alone (triangles) or in 0.5% Breakthru (diamonds) or 0.2% Breakthru (circles) 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 (grey diamonds) in a Lake County plot in 2003.

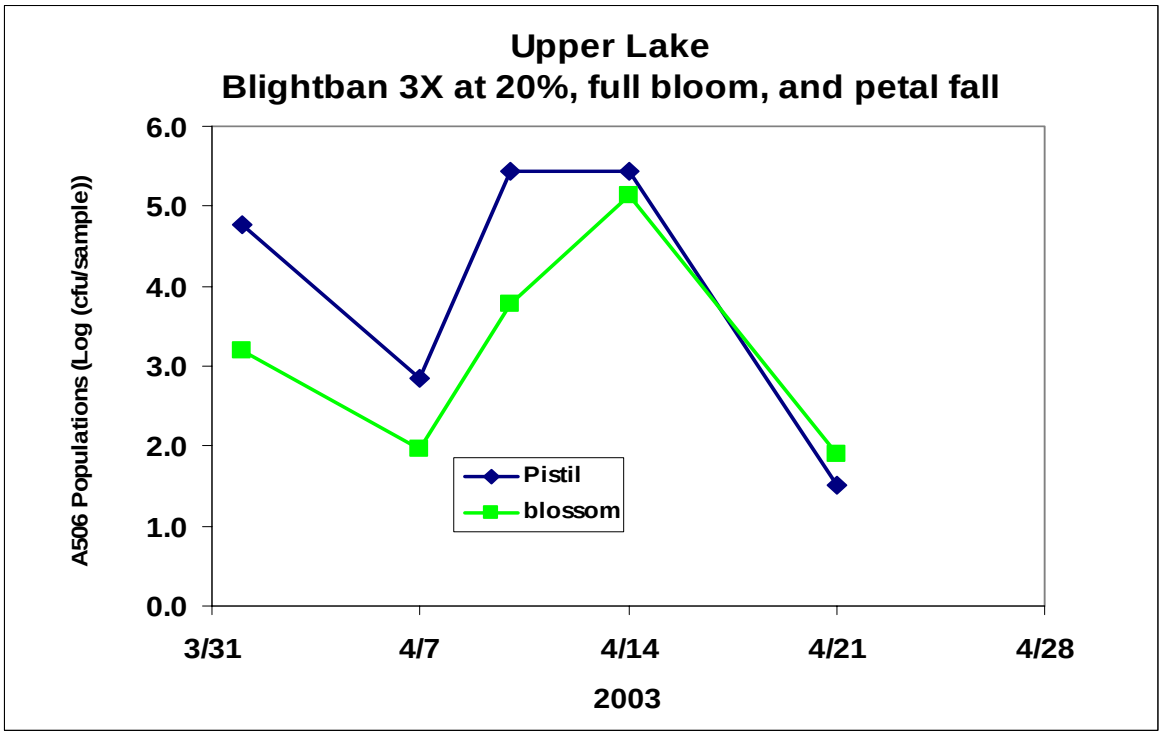

Figure 5. Population size of *Pseudomonas fluorescens* strain A506 on the pistils (diamonds) or remainder of the flower (squares) of Bartlett pear trees treated weekly with a label rate of Blightban A506 in water alone in a Lake Country trial in 2003.

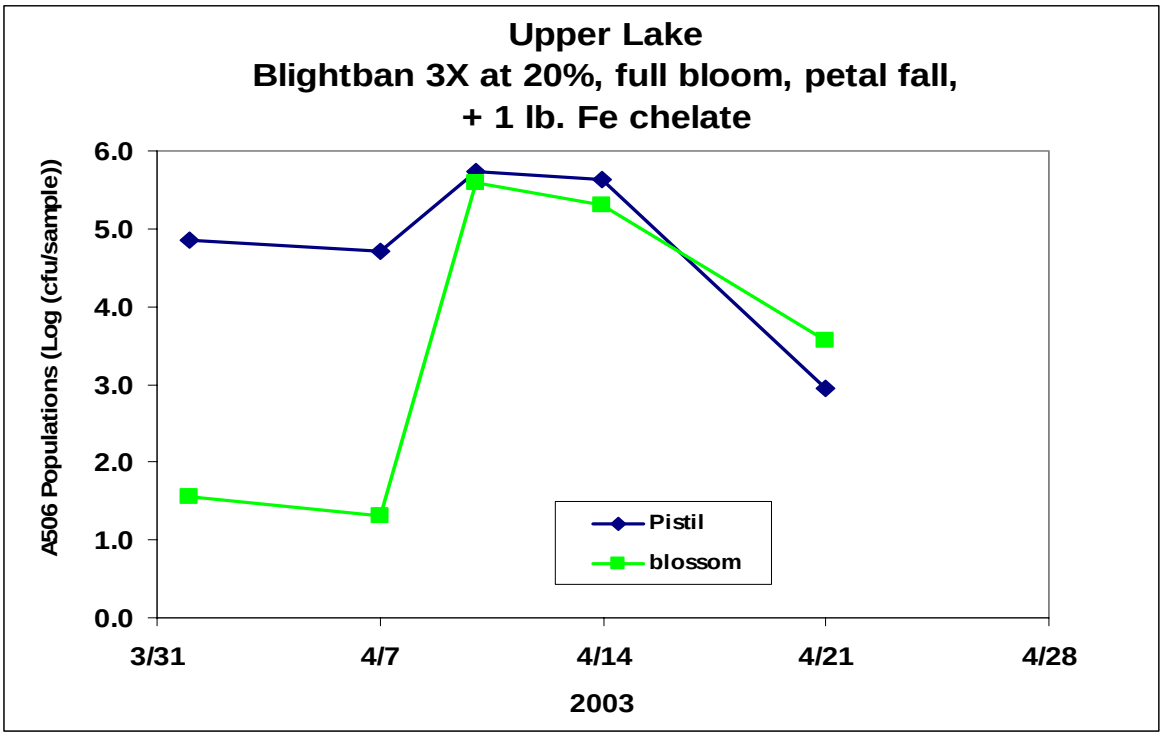

Figure 6. Population size of *Pseudomonas fluorescens* strain A506 on the pistils (diamonds) or remainder of the flower (squares) of Bartlett pear trees treated weekly with a mixture of a label rate of Blightban A506 and 1 lb/100 gal Sequestrene 138 (FeEDDHA).

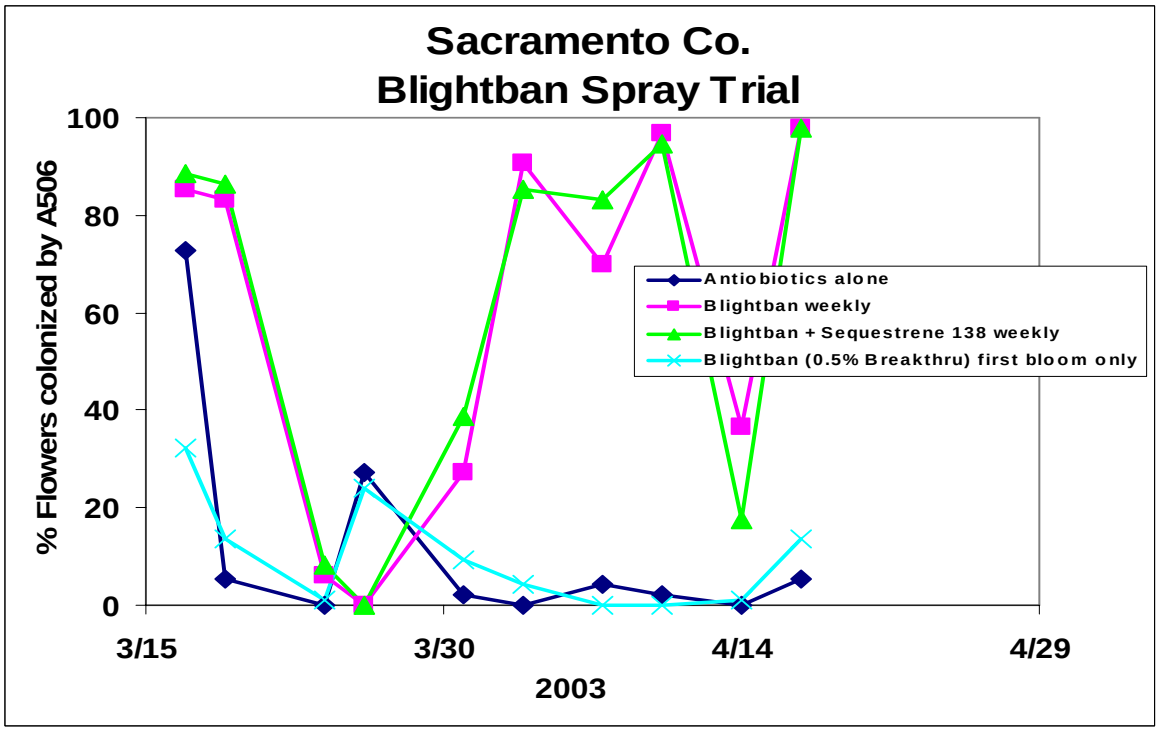

Figure 7. Percent of flowers colonized with *Pseudomonas fluorescens* strain A506 on Bartlett pear trees treated once only at the "first bloom" stage of growth [when only a few flowers were observed in an orchard] with a label rate of Blightban A506 in 0.5% Breakthru (x's), compared with weekly applications of Blightban A506 in water (squares) or weekly applications of Blightban and 1 lb/100 gal Sequestrene 138 (triangles) or with antibiotics alone (diamonds) in a Sacramento County plot in 2003.

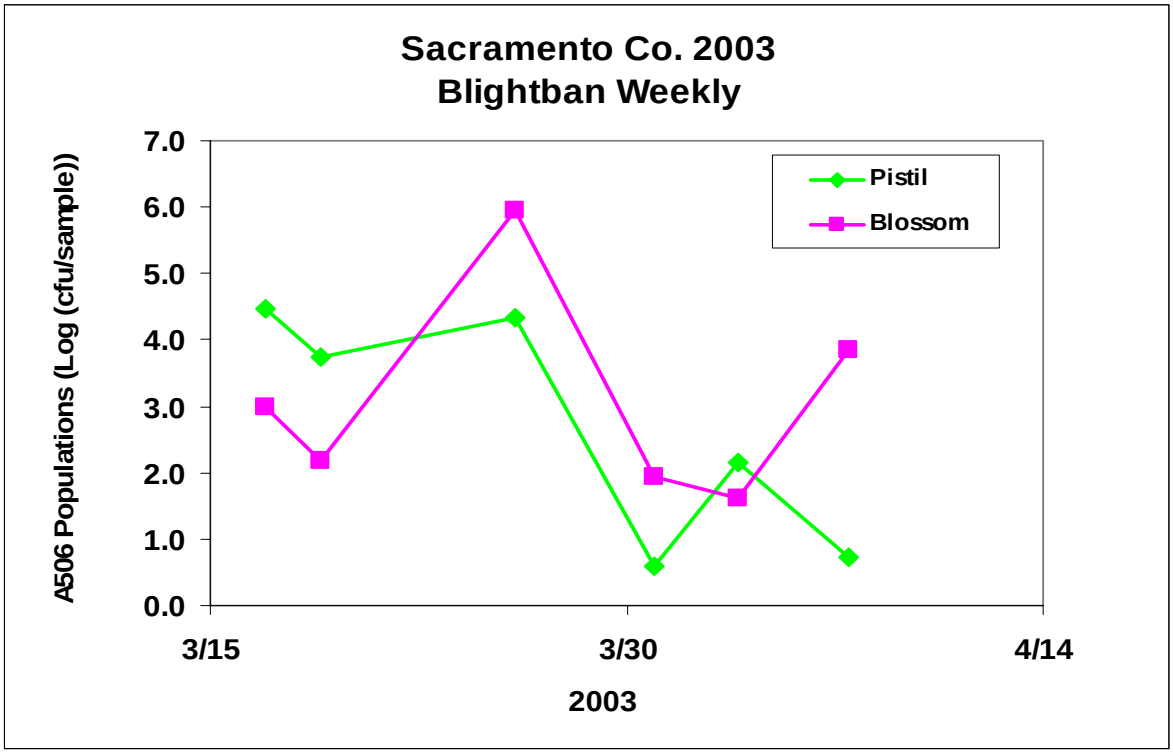

Figure 8. Population size of *Pseudomonas fluorescens* strain A506 on the pistils (diamonds) or remainder of the flower (squares) of Bartlett pear trees treated weekly with a label rate of Blightban A506 in water alone in a Sacramento County trial in 2003.

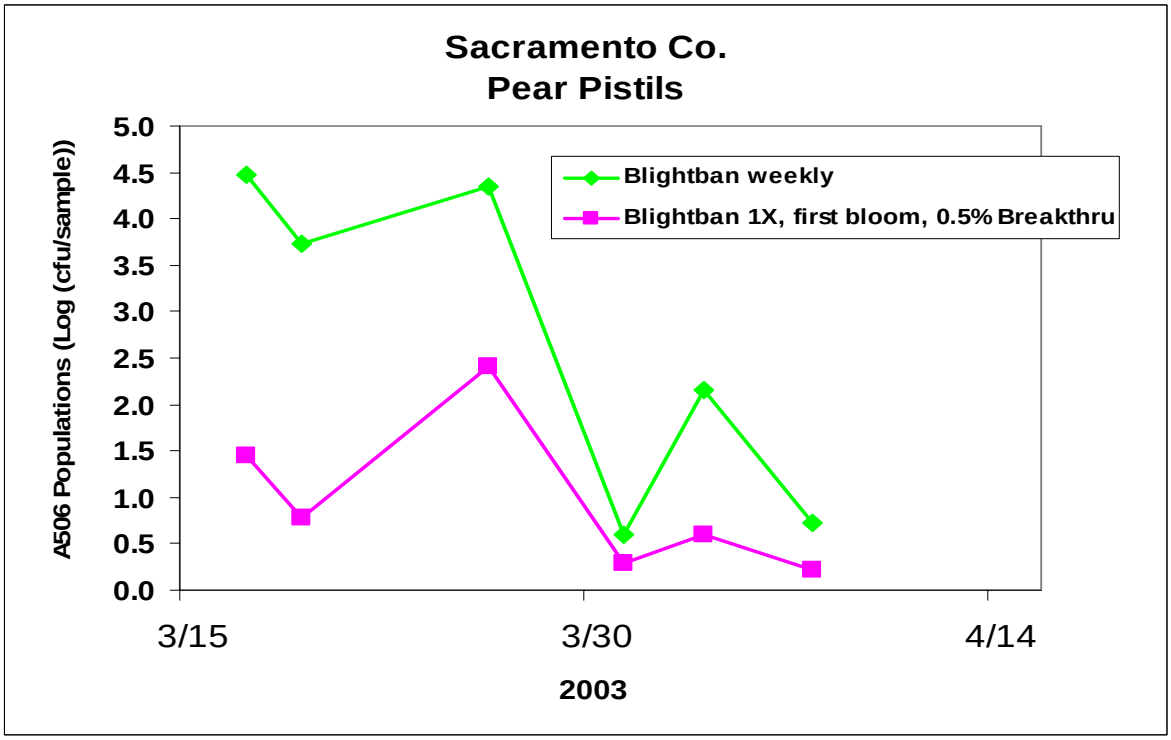

Figure 9. Population size of *Pseudomonas fluorescens* strain A506 on the pistils of flowers of Bartlett pear trees treated weekly with a label rate of Blightban A506 in water alone (diamonds) or once at first bloom with Blightban A506 in 0.5% Breakthru (squares) in a Sacramento County trial in 2003.

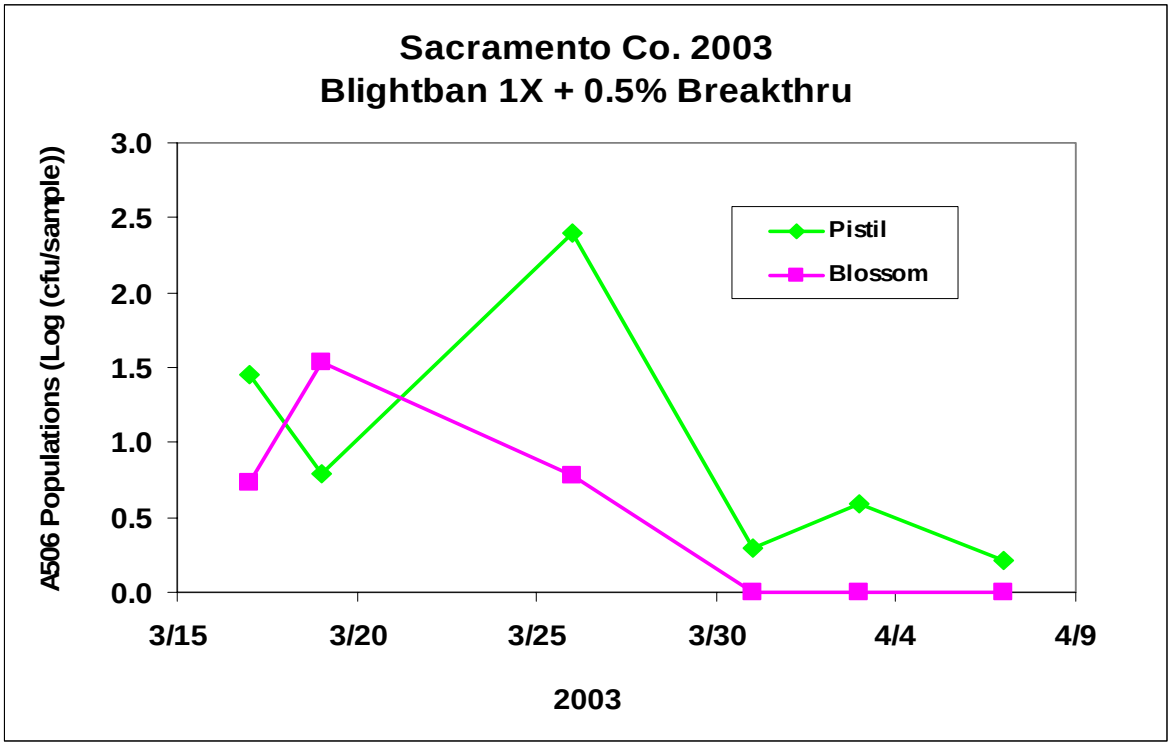

Figure 10. Population size of *Pseudomonas fluorescens* strain A506 on the pistils (diamonds) or remainder of the flower (squares) of Bartlett pear trees treated once at first bloom with a label rate of Blightban A506 in 0.5% Breakthru in a Sacramento County trial in 2003.

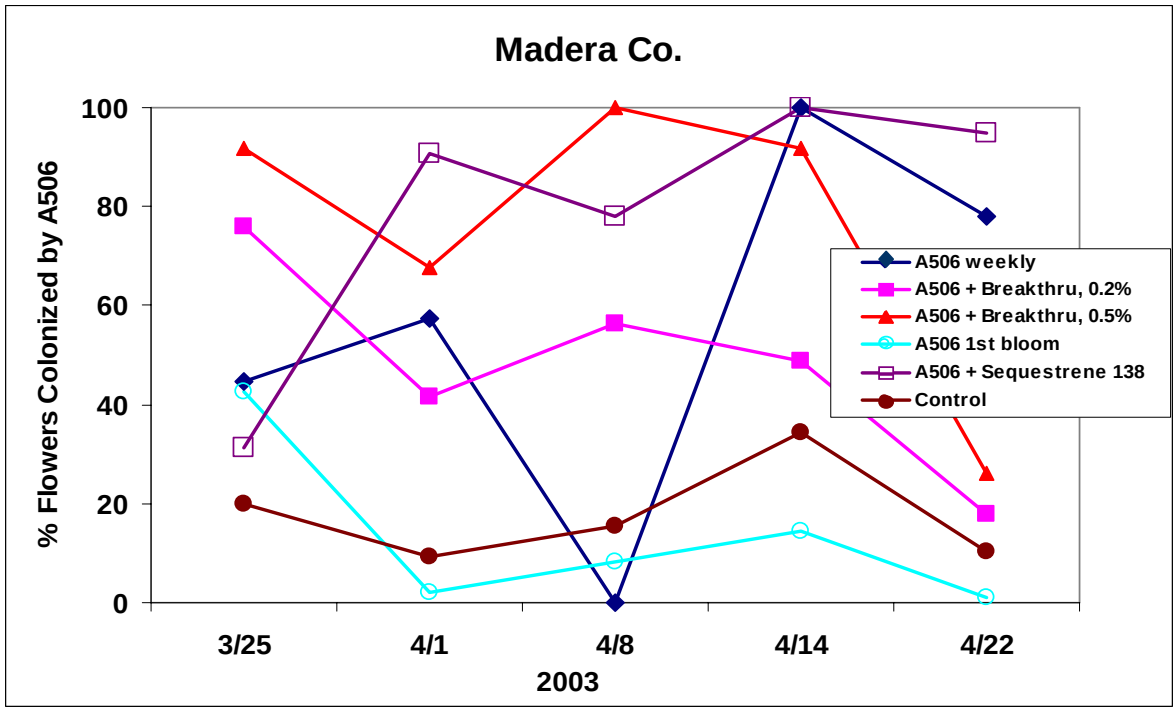

Figure 11. Percent of flowers colonized with *Pseudomonas fluorescens* strain A506 on Pink Lady apple trees treated once only at the "first bloom" stage of growth [when only a few flowers were observed in an orchard] with a label rate of Blightban A506 in water alone (x's) or in 0.5% Breakthru (triangles), 0.2% Breakthru (squares), compared with weekly applications of Blightban A506 in water (diamonds) or weekly applications of Blightban and 1 lb/100 gal Sequestrene 138 (stars) or with antibiotics alone (circles) in a Madera County plot in 2003.

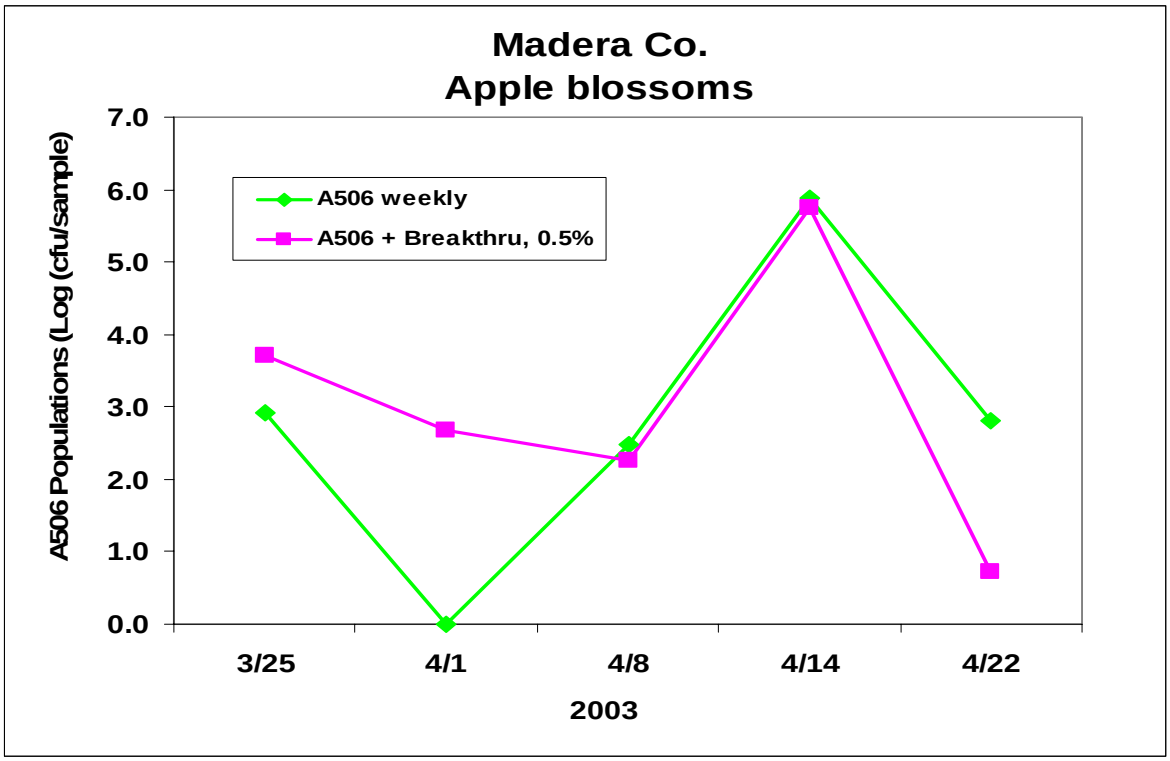

Figure 12. Total populations of Pseudomonas fluorescens strain A506 on entire flowers of Pink Lady apple trees treated weekly with Blightban A506 in water alone (diamonds) or treated once at first bloom with Blightban A506 in 0.5% Breakthru (squares) in a Madera County trial in 2003.

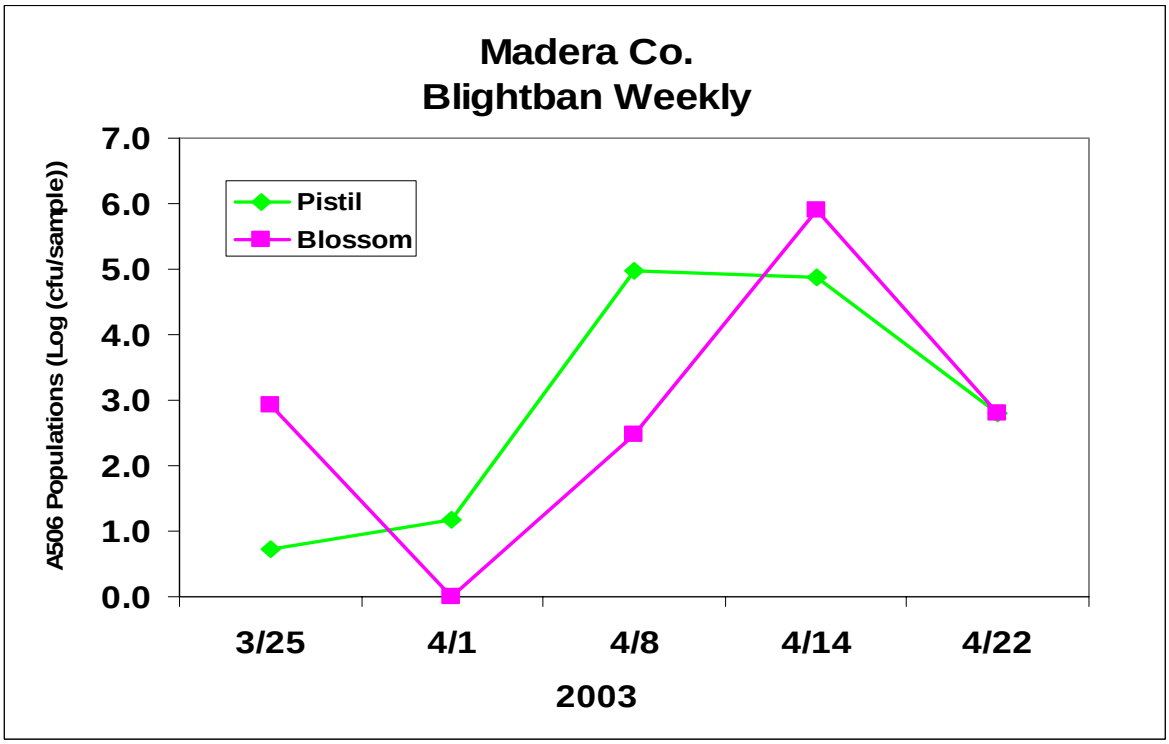

Figure 13. Population size of *Pseudomonas fluorescens* strain A506 on the pistils (diamonds) or remainder of the flower (squares) of Pink Lady apple on trees treated weekly with a label rate of Blightban A506 in water alone in a Madera County trial in 2003.

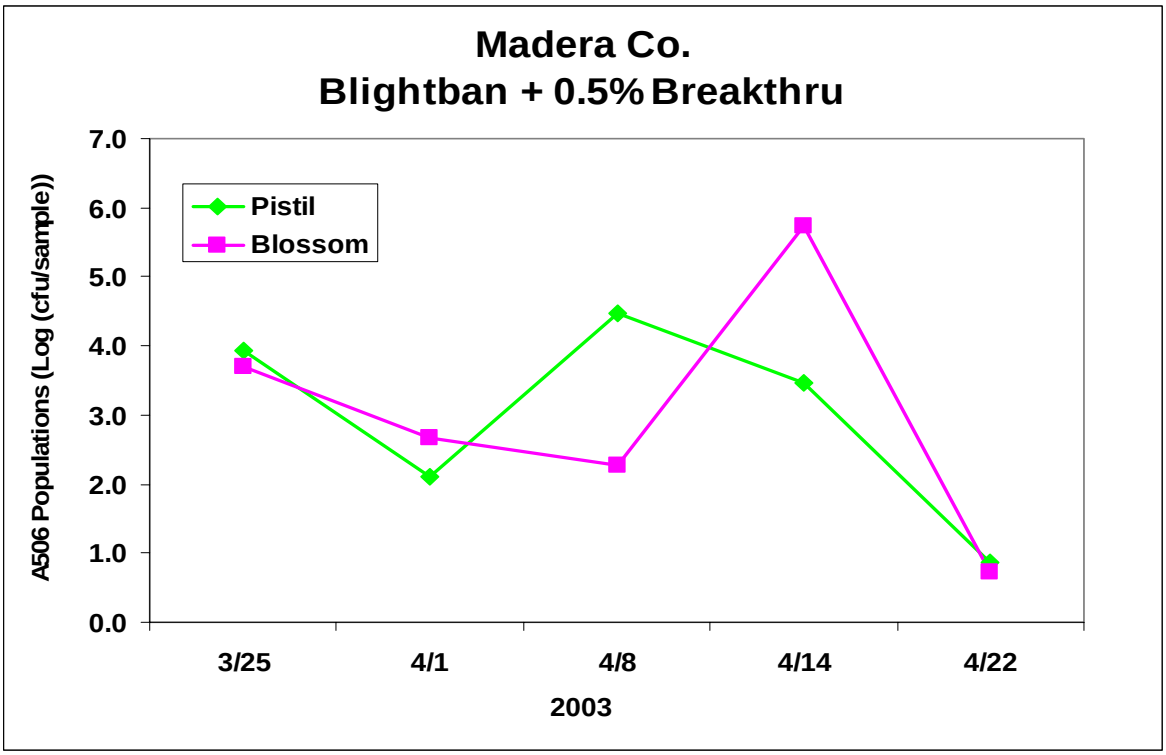

Figure 14. Population size of *Pseudomonas fluorescens* strain A506 on the pistils (diamonds) or remainder of the flower (squares) of Pink Lady apple on trees treated once at first bloom with a label rate of Blightban A506 in 0.5% Breakthru in a Madera County trial in 2003.