Epidemiology and Control of Bacterial Spot of Almond in California

| Project No.: | 17-PATH5-Adaskaveg |
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Objectives:

- I. Surveys on the distribution of bacterial spot in California almond orchards and genetic variability of pathogen populations
 - a. Collection of almond fruit with symptoms resembling bacterial spot in collaboration with farm advisors and PCAs throughout California almond growing areas
 - b. Isolation of the pathogen and identification of Xap using specific PCR primers
 - c. Determination of the genetic variability by molecular methods

II. Disease epidemiology

- a. Time of infection by the pathogen (i.e., host phenological stage)
- b. Pathogen survival and inoculum sources in the spring
- III. In vitro sensitivity of *Xap* against copper, mancozeb, antibiotics, and selected biologicals
 - a. Evaluation using agar dilution plating and the spiral gradient dilution methods
 - b. Selected materials will be evaluated alone or in combination
- IV. Management of bacterial spot in the field
 - a. Early and late dormant applications with copper and copper mancozeb mixtures.
 - b. Spring-time applications with copper, kasugamycin, oxytetracycline, streptomycin, selected mixtures
 - c. Selected biocontrol treatments: Serenade ASO (*Bacillus subtilis*) and Botector (*Aureobasidium pullulans*) with and without growth enhancers.

Interpretive Summary:

Bacterial spot caused by Xanthomonas arboricola pv. pruni (Xap) continues to be a problem and has spread throughout the Central Valley of California since the initial detection in 2013. Wet springs are highly favorable for the disease. Bacterial spot can develop throughout the growing season in orchards with high humidity or where foliage is frequently wet from high-angle sprinklers. It is most serious on cv. Fritz, but is also found on Nonpareil, Aldrich, Butte, Carmel, NePlus Ultra, and Price in Butte, Colusa, Kern, San Joaquin, Merced, Madera, and Stanislaus, Co. No copper resistance in the pathogen populations was detected during surveys in 2015 to 2018. Molecular comparisons of strains showed little genetic diversity, and this could suggest a recent introduction of a homogeneous population. In the spring, the pathogen was again isolated from overwintering symptomatic fruit mummies, but also from healthy flower buds, as well as from emerging leaves and spurs that were collected in close proximity of mummies in the tree. This confirmed that mummies act as the primary inoculum source, spreading inoculum by rain splash to newly emerging surrounding tissues. Spur isolations represent the first recovery of Xap from a woody tissue in California, suggesting that infected spurs remaining on the tree after drop or removal of a symptomatic mummy may be an inoculum source. Field inoculations of flowers, young fruitlets, and immature fruit of cv. Fritz were successful, indicating a long period where susceptible almond tissues are available. Flower inoculations may not directly result in an infection, but only contribute inoculum for fruit infections later in the season. Field trials on the management of the disease on cv. Fritz were conducted. In-season treatments at full bloom, petal fall, or after petal fall reduced the disease to very low levels, whereas applications after petal fall were less effective. This, in addition to our field inoculation timing studies, indicates the importance of bactericide applications from bloom through petal fall. The most effective and consistent treatments included copper (e.g., Badge X2, ChampION⁺⁺) mixed with mancozeb, kasugamycin mixed with mancozeb or copper, oxytetracycline, and selected experimentals. Copper phytotoxicity was observed on leaves at lower levels than in 2016 and 2017 probably because of rainfall in spring 2018 that removed some of the copper residues. In a trial using organic products, Serenade ASO mixed with sugar as a nutrient source for the biocontrol agent, Blossom Protect+buffer, and Champ were effective under moderate disease pressure and represent options for organic growers. The experimental nanoparticle Zinkicide was also moderately effective and could represent a new direction of bactericide development. Based on our results from several years of field studies we conclude: in wet winter/spring seasons, a delayed dormant bactericide application to reduce inoculum should be followed by a bloom or petal fall treatment around rainfall events and rising temperatures to prevent new infections. Blossom applications with copper cause minimal phytotoxicity. In drier winter seasons, only a dormant treatment or a bloom/petal fall application may be necessary for effective disease management. In the future, alternative treatments based on antibiotics and new compounds will be hopefully registered and can be used in rotation or mixture to prevent resistance development to any one mode of action and reduce copper phytotoxicity.

Materials and Methods:

I. Surveys on the distribution of bacterial spot in California almond orchards and genetic variability of pathogen populations. Almond fruit with symptoms of bacterial spot were collected in collaboration with farm advisors and PCAs. Isolations were done using

standard microbiological methods. *Xap* was initially identified by yellow colony morphology and subsequently by PCR using species-specific primers Y17CoF and Y17CoR. Several strains were collected from each orchard location (**Table 1**).

Repetitive element sequence-based polymerase chain reaction (rep-PCR) was performed on 134 isolates using primers REP1R-I and REP2-I, ERIC1R and ERIC2, or BOXA1R that have been used to demonstrate variability within several other bacterial species. A *Pseudomonas savastanoi* pv. *savastanoi* (*Psv*) strain was included as an outgroup. Amplification products were separated in agarose gels containing ethidium bromide.

II. Epidemiology

<u>Pathogen survival.</u> To determine pathogen survival sites over the winter and inoculum sources in the spring, symptomatic mummies were collected from the tree and from the orchard floor. In addition, buds, flowers at popcorn stage, open flowers, emerging leaves

| Sample | No. of isolates | Year | Tissue | Almond cultivar | Location (County) |
|--------|-----------------|------|-------------|--------------------|----------------------|
| 1 | 6 | 2012 | Green shoot | Unknown | Placer |
| 2 | 5 | 2013 | Fruit | Fritz | Merced |
| 3 | 2 | 2013 | Fruit | Fritz | Stanislaus |
| 4 | 2 | 2013 | Leaf | Nonpareil | Stanislaus |
| 5 | 1 | 2013 | Fruit | Fritz | Stanislaus |
| 6 | 1 | 2013 | Fruit | Nonpareil | San Joaquin |
| 7 | 1 | 2013 | Fruit | Fritz | San Joaquin |
| 8 | 3 | 2013 | Fruit | Nonpareil | San Joaquin |
| 9 | 3 | 2013 | Fruit | Fritz | San Joaquin |
| 10 | 6 | 2013 | Fruit | Fritz | Colusa |
| 11 | 5 | 2013 | Fruit | Padre | San Joaquin |
| 12 | 2 | 2013 | Fruit | Fritz | San Joaquin |
| 13 | 12 | 2014 | Fruit | Unknown | San Joaquin |
| 14 | 3 | 2014 | Fruit | Unknown | San Joaquin |
| 15 | 5 | 2014 | Fruit | Unknown | San Joaquin |
| 16 | 5 | 2014 | Fruit | Unknown | Stanislaus |
| 17 | 9 | 2015 | Fruit | Unknown | San Joaquin |
| 18 | 3 | 2017 | Fruit | UCD 1-271 | Butte |
| 19 | 9 | 2017 | Fruit | P13-019 | Butte |
| 20 | 3 | 2017 | Leaf | Unknown | Placer |
| 21 | 4 | 2017 | Green shoot | Unknown | Placer |
| 22 | 3 | 2017 | Fruit | Fritz | Butte |
| 23 | 10 | 2017 | Fruit | Unknown | Merced |
| 24 | 8 | 2017 | Fruit | Fritz | Butte |

Table 1. General background information of 113 *Xanthomonas arboricola* pv. *pruni* isolates used in genetic diversity studies with rep-PCR.

from areas in the tree within 1 meter of symptomatic mummies, as well as spurs attached to symptomatic mummies were collected. All collections were done in San Joaquin Co. from cv. Fritz almond. For spurs, isolations were made from the outer surface and from internal tissues. Standard microbiological methods were used for isolation and identification of *Xap* colonies.

III. In vitro sensitivity of Xap against copper-SBH or -ATD/ZTD. For determination of the in vitro sensitivity to the copper-enhancing compound SBH, the spiral gradient dilution assay was used where a gradient of bactericidal concentrations is established in nutrient agar. Suspensions of *Xap* were streaked radially across the concentration gradient. Inhibitory concentrations were determined using a computer program. For copper-SBH mixtures, the agar was amended with 10 ppm metallic copper equivalent (MCE), and SBH or ATD/ZTD were applied in a concentration gradient.

<u>VI. Management of bacterial spot in the field.</u> In studies in commercial cv. Fritz orchards where the disease is known to occur, the relative efficacy of spring-time applications was evaluated. A delayed-dormant copper treatment was applied to the orchard by the grower where in-season trials were conducted. In-season treatments were applied using an air-blast sprayer at 100 gal/A. In this and all other trials when in-season copper was applied multiple times to the same tree, rates were decreased by half in each subsequent application (for rates see (**Tables 4-6**).

In a timing study of in-season treatments, applications of copper hydroxide (e.g., ChampION++) or copper hydroxide-mancozeb (e.g., Manzate) were done at full bloom (2-13-2018), petal fall (2-28), 3 weeks (3-15), or 5 weeks (3-26) after petal fall. Biological and conventional bactericides were evaluated in three trials in two- or three-application programs based on environmental conditions as shown in (**Figure 2**) for bactericide applications listed in (**Tables 3-6**). Rates were based on their current labels on almond or other crops. For each treatment, there were four single-tree replications. Disease was evaluated in mid-June on 100 fruit for each tree, and the incidence was calculated based on the number of diseased fruit of the total number of fruit evaluated. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures (P > 0.05) of SAS version 9.4.

<u>Efficacy of kasugamycin against bacterial blast.</u> Kasugamycin was applied immediately prior to a frost event in mid-February to Fritz almond for managing bacterial blast caused by *Pseudomonas syringae* pv. *syringae*. Blasted blossoms were evaluated 1 week after the frost and nut counts five weeks after petal fall on lower limbs of trees. Data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures (P > 0.05) of SAS version 9.4.

Results and Discussion:

<u>I. Surveys on the distribution of bacterial spot in California almond orchards and genetic</u> <u>variability of pathogen populations.</u> *Xap* was identified from lesions on almond fruit from several locations in California representing major production areas from the southern to northern growing districts. (e.g., from Kern to Butte Co.). Still, the pathogen was not isolated from some fruit samples that had insect damage or from leaf injuries that were possibly caused by copper treatments.



Figure 1. Agarose gel showing the genetic fingerprints of 7 representative *Xap* isolates using rep-PCR with primers REP1R-I/REP2-I, BOXA1R, or ERIC1R/ERIC2. M, 100-bp DNA marker. Lanes 1-7 are *Xap*: 1, sample 1 (Fritz, Placer Co.); 2, sample 2 (Fritz, Merced Co.); 3, sample 3 (Fritz, Stanislaus Co.); 4, sample 4 (Nonpareil, Stanislaus Co.); 5, sample 11 (Padre, San Joaquin Co.); 6, sample 18 (UCD-1-271, Butte Co.); 7, sample 19 (P13-O19, Butte Co.); and 8, *Psv* from olive (used as an outgroup).

For the 113 *Xap* isolates used in this study, a limited number of intense bands were consistently amplified in rep-PCR ranging in size from 100 to 1,500 bp (**Figure 1**). Fingerprint patterns were identical among isolates indicating a high degree of genetic homogeneity based on this method, and only the *Psv* isolate produced a different fingerprint pattern. The primers employed have been widely used for other bacterial pathogens and are a standard tool to detect variability within bacterial populations including *X. arboricola* pv. *juglandis* where a high degree of genetic heterogeneity was demonstrated by us and others.

Thus, bacterial spot of almond in California is caused by a clonal population of *Xap* and may have originated from a single introduction of the pathogen. *Xap* populations from other *Prunus* spp. studied by others in different parts of the world also showed very limited or no genetic variation. It remains to be determined if variability exists among worldwide populations of *Xap*, and this might help to elucidate the origin of the pathogen.

II. Epidemiology

Survival of the pathogen. For isolations of *Xap* from 2015 to 2018, more than 500 samples were used with at least 30 for each tissue type. *Xap* was recovered from all tissue types collected (**Table 2**), however, not from asymptomatic mummies, flowers, or buds that were more than 20 cm distant from symptomatic mummies. Of the positive tissues, new or emerging leaves close to infected mummies had the lowest levels of detection (average 3.4%). In 2018, there were numerous symptomatic mummies remaining in the trees from the 2017 season. This suggests that mummies are the major primary inoculum source, spreading inoculum by rain splash to newly emerging surrounding tissues, but we also recovered *Xap* from spurs. Therefore, spurs remaining on the tree after drop or removal of a symptomatic mummy may still act as an inoculum source. The pathogen is reported to cause woody twig lesions in Australia, but this has not been confirmed in California (although green shoot lesions have been found).

| | | | Percent recovery | | |
|---|-----------------|-----------------------|------------------|-------------|--|
| Structure/tissue | Years collected | No. samples | Mean | Range | |
| Symptomatic mummy | 2013 - 2018 | 201 | 40.0 | 10.0 - 67.7 | |
| Asymptomatic mummy ^a | 2015, 2016 | 30 | 0.0 | 0.0 - 0.0 | |
| Spur of a symptomatic mummy | 2017, 2018 | 44 | 51.4 | 42.9 - 60.0 | |
| Dormant buds ^a | 2016, 2018 | 66 (132) ^c | 0.0 | 0.0 - 0.0 | |
| Flowers ^a | 2016, 2018 | 46 (66) | 0.0 | 0.0 - 0.0 | |
| Flowers (<u><</u> 20 cm of a mummy ^b) | 2017, 2018 | 56 (138) | 19.4 | 11.5 - 46.7 | |
| New leaves (<20 cm of a mummy ^b) | 2016, 2017 | 71 (175) | 3.4 | 0.0 - 6.7 | |

Table 2. Isolation of Xap from selected tissues of cv. Fritz almond in San Joaquin Co.

a Asymptomatic tissues collected more than 2 m from a symptomatic mummified fruit or from a tree without mummified fruit.

b Mummies showed symptoms of bacterial spot.

c Values in parenthesis represent the number of individual units of tissue that were used for composite samples.

<u>III. In vitro sensitivity of Xap against copper-SBH or -ATD/ZTD.</u> All isolates collected from new locations were sensitive to copper (growth at 20 ppm MCE and no growth at 30 ppm MCE). Baseline sensitivity levels to a new copper enhancing product, SBH, in the presence of 10 ppm MCE was determined for 72 *Xap* strains. The average minimum inhibitory concentration (MIC) was 0.31 ppm with a range from 0.15 to 0.94 ppm. The average lowest inhibitory concentration (LIC) mean was 0.07 ppm with a range from 0.03 to 0.14 ppm. SBH by itself had a MIC >90 ppm. Sensitivities of these isolates to mancozeb, oxytetracycline, kasugamycin, and copper mixtures were previously reported. In-vitro toxicity of the experimental bactericide ATD/ZTD was low.



Figure 2. Environmental conditions in San Joaquin Co. in the winter and spring of 2018 when field trials were conducted on the timing of inseason bactericide applications. All trees also had received a delayed-dormant copper application.

<u>VI. Management of bacterial spot in the field.</u> Following the high-disease year 2017, the incidence of disease at our trial site was moderate to high in 2018. Disease symptoms on fruit were first observed in April, and final evaluations were done in mid-June. Phytotoxicity from copper injury was low in 2018. Ratings for full bloom and petal fall applications were less than 1 on a scale from 0 (no phytotoxicity) to 4 (injury resulting in defoliation).

Table 3. Evaluation of timing of in-season treatments with copper-mancozeb for management of bacterial spot of cv. Fritz almond in San Joaquin Co. in 2018.*

| | | Incidence of | |
|------------------------------|-------------|--------------------|------|
| Phenological stage | Date | diseased fruit (%) | LSD^ |
| Control | | 23.3 | А |
| Full bloom | 2-13-18 | 7.0 | В |
| Petal fall | 2-28-18 | 5.4 | BC |
| 3 weeks after petal fall | 3-15-18 | 3.5 | BC |
| Full bloom + petal fall | 2-13 + 2-28 | 2.4 | С |
| 3 + 5 weeks after petal fall | 3-15 + 3-26 | 2.8 | BC |

* Treatments were applied using an air-blast sprayer at 100 gal/A. Champ 50WDG was applied at 3.3 lb/A, and Manzate at 4 lb/A.

 Values followed by the same number are not significantly different based on an analysis of variance and LSD mean separation (*P* > 0.05).

A comparison of in-season treatment timings was done in several trials. Applications were done at full bloom, petal fall, or 3 or 5 weeks after petal fall. All timing treatments significantly decreased disease as compared to the control (**Table 4**). The combination of full bloom and petal fall had a significantly lower incidence than the full bloom application alone; whereas all petal fall treatments were not significantly different from each other with disease incidence ranging from 2.5 to 5.4% as compared to 28.4% incidence in the control. Most of the infections occurred during the petal fall period with favorable environmental conditions with rainfall and warming temperatures (**Figure 2**).

New bactericides were evaluated in two studies with three applications starting at petal fall. In comparisons of experimental antibiotic treatments with and without copper or mancozeb and of new copper-enhancing treatments, most treatments significantly reduced bacterial spot of fruit from that of the control (**Table 4**). Oxytetracycline (Mycoshield) significantly reduce disease incidence, but efficacy was significantly improved with the addition of copper (ChampION⁺⁺). Other treatments including oxytetracycline+mancozeb (Manzate), kasugamycin (Kasumin), kasugamycin+copper or mancozeb, mancozeb alone, copper, copper+DAS-1 (an experimental copper enhancer based on SBH – *see* in vitro studies above), and copper+mancozeb were also very effective reducing disease to 1.5 to 7.5% as compared to 42.5% in the control (**Table 4**). No phytotoxicity was observed for all treatments except for copper. Minor phytotoxicity developed even when reduced rates of copper (e.g., 3.3 to 0.8 lb) were applied with each application.

| | | | PF 3-wk 5-wk | | Disease** | | |
|-----|----------------------------------|-------------------------|--------------|------|-----------|------------|------|
| No. | Treatment* | Rate(/A) | 2-28 | 3-16 | 3-26 | Incid. (%) | LSD^ |
| 1 | Control | | | | | 42.5 | А |
| 2 | Mycoshield | 16 oz | @ | @ | @ | 9 | В |
| 3 | Mycoshield + Manzate | 16 oz + 4 lb | @ | @ | @ | 7.5 | BC |
| 4 | Kasumin + ChampION** | 64 fl oz + 4 lb | @ | @ | @ | 4 | BC |
| 5 | Manzate | 4 lb | @ | @ | @ | 3.5 | BC |
| 6 | DAS-1 + ChampION ⁺⁺ | 56 fl oz + 3.3 - 0.8 lb | @ | @ | @ | 3.5 | BC |
| 7 | Kasumin + Manzate | 4 fl oz + 4 lb | @ | @ | @ | 3.3 | BC |
| 8 | ChampION ⁺⁺ + Manzate | 3.3 - 0.8 lb + 4 lb | @ | @ | @ | 3.0 | BC |
| 9 | Kasumin | 64 fl oz | @ | @ | @ | 2.8 | BC |
| 10 | ChampION** | 3.3 - 0.8 lb | @ | @ | @ | 1.8 | BC |
| 11 | Mycoshield + ChampION** | 16 oz + 3.3 - 0.8 lb | @ | @ | @ | 1.5 | С |

Table 4. Effect of new bactericides as in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. in 2018.

Applications were done using an air-blast sprayer at 100 gal/A. The copper rate was reduced by half with each subsequent application from an initial rate of 3.3 lb/A.

** Disease on fruit was evaluated on 6-20-18. Values are the incidence of diseased fruit of 100 fruit for each of four single-tree replications.

 Values followed by the same number are not significantly different based on an analysis of variance and LSD mean separation (P > 0.05).

In a second trial, food-grade anti-bacterial products such as nisin and ε -poly-L-lysine were used alone or in combination with conventional treatments including kasugamycin and zinc oxide, copper, and mancozeb (**Table 5**). All of the treatments significantly reduced bacterial spot to <16.3% as compared to 41% in the untreated control. Nisin mixed with kasugamycin, zinc oxide, or copper hydroxide reduced disease incidence to less than 8.8%. The two best treatments with less than 4.3% and 1.3% incidence were copper mixed with ε -poly-L-lysine or nisin, respectively. Zinc oxide, nisin, and ε -poly-L-lysine will be pursued in additional research as we identify potential registrants that will submit registration petitions to regulatory agencies. These materials were most effective in combinations with conventional bactericides. The food-grade products are readily degraded in the environment and therefore, formulations to prevent physical, chemical, or biological degradation need to be developed and evaluated.

| | | | PF | 3-wk | 5-wk | Disease** | |
|-----|----------------------------|-----------------------|------|------|------|------------|------|
| No. | Treatment* | Rate(/A) | 2-28 | 3-16 | 3-26 | Incid. (%) | LSD^ |
| 1 | Control | | | | | 41.0 | А |
| 2 | Kasumin+Nisin+Nufilm-P | 16 oz | @ | @ | @ | 16.3 | В |
| 3 | Nisin | 16 oz + 4 lb | @ | @ | @ | 14.8 | BC |
| 4 | Kasumin+Nisin+ZnO+Nufilm-P | 64 fl oz + 4 lb | @ | @ | @ | 11.8 | BCD |
| 5 | Nisin+Manzate+NuFilm-P | 4 lb | @ | @ | @ | 8.8 | BCDE |
| 6 | Nisin+ZnO+Nufilm-P | 56 fl oz + 3.3-0.8 lb | @ | @ | @ | 5.8 | BCDE |
| 7 | Kasumin+ZnO | 4 fl oz + 4 lb | @ | @ | @ | 5.0 | CDE |
| 8 | Badge+ ε -poly-L-lysine | 3.3-0.8 lb + 4 lb | @ | @ | @ | 4.3 | DE |
| 9 | Badge+Nisin+NuFilm-P | 64 fl oz | @ | @ | @ | 1.3 | Е |

Table 5. Effect of new experimental bactericides as in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2018.

Applications were done using an air-blast sprayer at 100 gal/A. The copper rate was reduced by half with each subsequent application from an initial rate of 3.3 lb/A.

** Disease on fruit was evaluated on 6-20-18. Values are the incidence of diseased fruit of 100 fruit for each of four single-tree replications.

 Values followed by the same number are not significantly different based on an analysis of variance and LSD mean separation (P > 0.05).

In the study with biologicals, treatments were compared to copper (Champ) (**Table 6**). Disease incidence was 28.4% in the untreated control; whereas the copper treatment was significantly lower at 7.3%. Zinkicide (nanoparticles of zinc), Serenade ASO-sugar, and Blossom Protect significantly reduced the disease to the lowest levels (6.8%, 2.4%, and 2.8%, respectively) among all treatments evaluated. Serenade ASO by itself, however, was less effective than when the product was used with sugar as a nutrient source (**Table 6**). Perhaps sugar enhances growth of the living biocontrol organism in the Serenade formulation. *Aureobasidium pullulans* is the biological agent in Blossom Protect and the treatment includes a "buffer" that also provides a nutrient source for growth of the organism. Therefore, we have identified these products as non-copper alternatives for managing bacterial spot for organic growers of almonds.

Based on our results from three years of field studies, we conclude that copper-mancozeb is an effective treatment for managing the disease on almond. No resistance to copper was detected in surveys and the mixture should provide resistance management. Still, other products with different modes of action need to be developed to have a sustainable disease management program. In the future, alternative treatments such kasugamycin, nisin, and ϵ -poly-L-lysine, as well as copper-enhancing compounds (e.g., SBH, DAS-1), hopefully will be available to be used in rotations or mixtures to prevent resistance development to any one mode of action and to reduce phytotoxicity from repeated copper applications. Zinkicide, a nano-particle zinc product, has regulatory hurdles due to unknown impacts on the environment or worker safety. Still, we also identified effective biological treatments, Serenade ASO and Blossom Protect used as we described, for organic growers.

| | | | PF | 3-wk | Disease** | |
|-----|----------------------------------|----------------------|------|------|------------|------|
| No. | Treatment* | Rate(/A) | 2-28 | 3-15 | Incid. (%) | LSD^ |
| 1 | Control | | | | 28.4 | а |
| 2 | Vacciplant | 64 fl oz | @ | @ | 15.5 | ab |
| 3 | Nisin + Zinkicide | 13.5 oz + 64 fl oz | @ | @ | 12.8 | bc |
| 4 | Serenade Opti + Nufilm-P | 64 + 8 fl oz | @ | @ | 12.0 | bc |
| 5 | Champ 50WDG | 2-0.5 lb | @ | @ | 7.3 | bcd |
| 6 | Zinkicide | 64 fl oz | @ | @ | 6.8 | bcd |
| 7 | Serenade Opti + Nufilm-P + Sugar | 64 + 8 fl oz + 32 oz | @ | @ | 4.0 | cd |
| 8 | Blossom Protect + Buffer | 20 oz + 143 oz | @ | @ | 2.8 | d |

Table 6. Effect of in-season biological treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2018.

Treatments were applied using an air-blast sprayer at 100 gal/A. The copper rate was reduced by half with each subsequent application from an initial rate of 2 lb/A.

** Disease on fruit was evaluated on 6-20-18. Values are the incidence of diseased fruit of 100 fruit evaluated for each of four single-tree replications.

 Values followed by the same letter are not significantly different based on an analysis of variance and least significant difference (LSD) mean separation (P > 0.05) procedures.

Reviewing the efficacy data for the last several years, under wet winter/spring seasons, the most effective management program for bacterial spot management should consist of a delayed dormant or a petal fall treatment, or of a two-spray program using both timings. The dormant treatment will reduce inoculum production and dispersal, whereas the inseason application will protect almond tissues from new infections. In-season applications should be done around rainfall events and rising temperatures which comprise infection events for the pathogen. In drier winters or springs, a single delayed dormant or petal fall treatment will be sufficient, provided that cultural practices such as high-density orchards creating high humidity and precipitation from resulting dews, high-input farming, or high-angled irrigation creating excessive canopy wetness are avoided.

Efficacy of kasugamycin against bacterial blast. In a study on bacterial blast caused by *Pseudomonas syringae* pv. *syringae*, kasugamycin applied immediately prior to a frost event, decreased flower blast by 70% as compared to the control. Nut counts five weeks after petal fall on lower limbs of treated trees were increased by 60% as compared to untreated trees. This efficacy in managing bacterial blast indicates that in addition to bacterial spot caused by *Xap*, bacterial blast should also be included on the Kasumin label when the bactericide is registered on almond.