Epidemiology and Control of Bacterial Spot of Almond in California

Project No.: **15-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 bacterial spot (*Xap)* using specific PCR primers
   c. Determination of the genetic variability by molecular methods

II. Disease epidemiology
   a. Determine time of infection by the pathogen (i.e., host phenological stage) by inoculation of almond flowers and fruit at different stages of development
   b. Determine pathogen survival and inoculum sources in the spring by isolating the pathogen from overwintering fruit mummies and from buds.

III. In vitro sensitivity of *Xap* against copper, mancozeb, antibiotics, and selected biologicals (e.g., Serenade)
   a. The sensitivity will be evaluated using agar dilution plating and the spiral gradient dilution method
   b. Selected materials will be evaluated alone or in combination

IV. Management of bacterial spot in the field
   a. Dormant applications will be done in early (late 2015) and late winter (early 2016) using copper and copper mancozeb combinations.
   b. Spring-time applications will include traditional and new formulations of copper with low phytotoxicity potential, the antibiotics kasugamycin, oxytetracycline, and streptomycin. Mixtures of copper-mancozeb (or other fungicides with bactericidal properties) and antibiotic-mancozeb combinations will also be evaluated.
   c. Selected biocontrol treatments such as Serenade Optimum (*Bacillus* sp.), Actinovate (*Actinomyces* sp.), and/or Botector (*Auereobasidium* sp.) will be evaluated and we will focus on Botector with and without growth enhancers.

Interpretive Summary:

Bacterial spot caused by *Xanthomonas arboricola* pv. *pruni* (*Xap*) continues to be a problem in California since the initial identification in 2013. The disease can be found on cultivar (cv.) Fritz, as well as, other cultivars such as Nonpareil, Aldrich, Butte, Carmel, and Price in Colusa, San Joaquin, Stanislaus, Merced, and Madera Co. The disease can cause epidemics in wet springs or in orchards with high humidity or with high-angle sprinklers that wet the foliage of the tree. No resistance to copper was detected among strains collected during surveys in 2015 and 2016. The pathogen was again isolated from overwintering symptomatic fruit mummies throughout the spring and early summer (June/July). The pathogen was not detected in buds, flowers, or twig lesions and thus, mummies are the primary inoculum sources during spring infection periods. Bacterial inoculum levels can increase exponentially within a short time period under conducive conditions (wetness and warm temperatures) during the spring season. Field inoculation studies on cv. Fritz on blossoms, young fruitlets, and on older immature fruit were successful. The disease was then observed on developing fruit after four to six weeks. Field trials on cv. Fritz on the management of the disease were conducted and included dormant and in-season applications. Delayed dormant copper-mancozeb resulted in a significant reduction of bacterial spot as compared to the untreated control similar to 2014. In-season treatments that were applied at full bloom or petal fall significantly reduced the disease when timed around rain events and before temperatures started to rise in the springtime. This, in addition to our field inoculation timing studies, indicates the importance of bactericide applications at bloom time and petal fall. The most effective and consistent treatments included copper (e.g., NuCop, ChampION^{++}) and copper mixed with mancozeb. Copper phytotoxicity was observed on leaves after a single application of 1 lb MCE/A at petal fall or two 1 lb MCE/A applications at full bloom and petal fall. Symptoms included leaf tip necrosis and necrotic circular lesions. Minor tip necrosis occurred only after multiple Kasumin applications. Other treatments that significantly reduced the disease included Mycoshield and the experimental ATD. In an organic product trial, Serenade+copper was an effective treatment under low disease pressure. Based on our results from three years of field studies, with a wet winter season the most effective management program for bacterial spot include a delayed dormant bactericide application to reduce inoculum and at least one or two in-season applications around rainfall events and rising temperatures to prevent new infections. In drier winter seasons, dormant treatments may not be beneficial, and applications should start at bloom time and subsequently be timed around rain events during fruit development. Effective biological treatments were identified for organic growers. In the future, alternative treatments based on antibiotics or experimental compounds will be hopefully registered and can be used in rotation or mixture to prevent resistance development to any one mode of action.

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 from overwintering diseased mummies and newly infected fruit using standard microbiological methods. *Xap* was initially identified by yellow colony morphology and subsequently by PCR using species-specific primers. Several strains were collected from each orchard location. The

genetic variability (population structure) of the pathogen within and among orchards was determined using PCR with primers targeting repetitive DNA sequences that often show a high degree of variability among strains of a species (i.e., rep-PCR primers BOX and ERIC; Louws et al., 1994). Banding patterns of amplification products in agarose gels were compared.

II. Epidemiology

Inoculation timing study. Inoculations were done in the early growing season to determine susceptibility of selected fruit and leaf development stages to infection by *Xanthomonas arboricola* pv. *pruni* (*Xap*). At one site in Stanislaus Co., three inoculations were conducted on cv. Fritz almonds at full bloom (2/16/16) and at two fruit developmental stages, i.e., shuck split (3/8/16), and 7-wk-after petal fall (4/14/16). At a second site in Solano Co., only full bloom inoculations were conducted on cvs. Fritz and Wood Colony trees. *Xap* strains 942 obtained from cv. Nonpareil and 1789 obtained from cv. Fritz were used alone or in mixtures in equal proportions. High (OD_{600} 80% transmission) and low (OD_{600} 70% transmission diluted 1:10) concentrations were used, providing inoculum concentrations of about 1×10^7 cfu/ml and 2×10^6 cfu/ml respectively that were applied using a hand-sprayer to run-off. Inoculum was prepared in water or in water-surfactant (Triton X-100 at 0.1%). Treated branches were covered with white plastic bags sprayed with water for 12-18 h, and inoculations were evaluated for disease after 4-5 weeks. Eight replications per treatment on 8 different trees per inoculation date were used.

Disease evaluation. Ratings were conducted as the disease developed during the spring season. All Solano Co. inoculations were evaluated on 4/14/16; whereas Stanislaus Co. inoculations were evaluated on 4/14/16, 5/11/16, and 6/3/16. For disease rating, the incidence of infected nuts per total nuts per branch was determined. Severity on fruit was rated using a scale of 0 to 4, with 0 = healthy, 1 = sunken lesion(s), 2 = 1 gumming lesion, 3 = 2 gumming lesions, and 4 = 3 or more gumming lesions/fruit. Disease on leaves was rated using a scale of 0 = healthy, 1 = >2 to < 25%, 2 = 25 to 50%, 3 = 51 to 75%, 4 = 76 to 94%, and 5 = >95% leaves/branch with lesions. Re-isolations were conducted from a subset of leaves and fruit to confirm the presence of *Xap*. For fruit evaluations, incidence data were arcsine-transformed for each replication and averaged for the treatment. For leaf and fruit evaluations, disease severity ratings were determined based on the sum of the number of leaves or fruit in each category multiplied by the rating value and divided by the total number of leaves or fruit evaluated. Values of each replicate were then averaged for the treatment. Data were then analyzed using a general linear model with treatment as the single factor. Multiple comparisons were conducted using Fisher's Least Significant Difference test at $\alpha=0.05$ (SAS version 9.4).

Pathogen survival. To determine pathogen survival sites over the winter and inoculum sources in the spring, isolations were done from symptomatic overwintering fruit mummies from Dec. 2015 to June 2016, from buds before bloom, and from flowers. Standard microbiological methods were used for bacterial isolation. *Xap* was initially identified by yellow colony morphology and subsequently by PCR using species-specific primers.

III. In vitro sensitivity of *Xap* against copper, mancozeb, antibiotics, and biologicals. For determination of the in vitro sensitivity against antibiotics and mancozeb, we used the spiral gradient dilution assay where a gradient of bacterial 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 sensitivity, serial dilution

plating with selected copper concentrations was done, and copper mixture evaluations were conducted on 10 ppm MCE.

VI. Management of bacterial spot in the field. In studies in commercial cv. Fritz orchards where the disease is known to occur, the relative efficacy of dormant and spring-time applications was evaluated. Treatments were applied using an air-blast sprayer at 100 gal/A. One trial was done as a split-plot design with dormant applications in the main plots and spring-time applications in the sub-plots. For dormant applications ChampION or ChampION-mancozeb were used and were applied in December or late-January. Copper was also applied in mixtures with an agricultural oil or with an adjuvant.

In-season applications were initiated at bloom and continued prior to rain events in the spring. Treatments included copper products, antibiotics (kasugamycin, oxytetracycline, streptomycin), and biological controls such as Serenade Optimum (*Bacillus subtilis* strain QST 713), Actinovate (*Streptomyces lydicus*), or Botector (*Aureobasidium pullulans*). Biological controls were also applied in a mixture with a nutrient solution to increase growth because this benefited activity in some of our trials on other fruit crops in 2015. Multiple applications were required based on springtime conditions (i.e., March-June). In treatments with standard copper (e.g., ChampIon++, Kocide 3000), rates were decreased from 1, to 0.5, to 0.25, and to 0.2 lb MCE/A for each application timing. Rates for other coppers were based on registrant recommendations. Fungicide and antibiotic rates were based on their current labels on almond or other crops, respectively. For each treatment, there were four single-tree replications. Disease was evaluated in late spring and the incidence was calculated based on the number of diseased fruit of the total number of fruit evaluated.

Results and Discussion:

I. Surveys on the distribution of bacterial spot in California almond orchards and genetic variability of pathogen populations. *Xap* was identified from lesions on almond fruit from several locations in California representing two major production areas in the central and northern districts. Still, the pathogen was not isolated from some fruit samples with apparently typical symptoms of bacterial spot (possibly insect damage) and from leaf lesions (possibly copper damage). Molecular analysis of isolates to obtain information on population structure is pending.

II. Epidemiology

Inoculation timing. All *Xap* inoculation timings and techniques resulted in disease development with symptoms similar to those observed in natural infections for fruit and leaves (**Tables 1, 2**). Some water controls showed low levels of disease, likely due to inoculation drift or presence of natural inoculum within orchards. Water controls using a surfactant did not show any phytotoxic effects on fruit, whereas early fruit inoculations containing surfactant resulted in more lesions and more severe gumming compared to non-surfactant inoculations (**Table 1**); whereas no difference was observed on leaves. Inoculations of different phenological stages all resulted in disease, and no significant differences were observed in disease incidence and severity among phenological stages. Fruit inoculations, however, generally resulted in more disease than blossom inoculations. Additionally, low- and high-inoculum concentrations resulted in similar disease incidence and severity on fruit and leaves. This was likely due to only a 1.1-log difference in concentration. Flower inoculations of cv. Wood Colony resulted in lower levels of disease on leaves as compared to cv. Fritz (data not

shown). Very few nuts were available for evaluation due to windy conditions during the inoculation procedures that probably caused the flowers to abscise. Re-isolations from fruit and leaves confirmed *Xap* infection, and cultures were positively identified using species-specific primers Y17CoF/Y17CoR.

***Survival of the pathogen*.** Xap was isolated from approximately 50% of sampled overwintering symptomatic fruit mummies collected on trees or on the orchard floor between December 2015 and early June 2016. This indicates that for a second year we were able to demonstrate survival of the pathogen for over a year in fruit mummies and the role of these mummies as primary inoculum sources during infection periods in the spring and possibly early summer. Bud and flower isolations were negative, and twig lesions were not found. Although these latter survival mechanisms may occur and have been reported, we were unable to confirm these mechanisms on cv. Fritz.

Table 1. Inoculation of cv. Fritz almond with *Xanthomonas arboricola* pv. *pruni* at selected phenological stages.

Treatment	Fruit Incidence*		Leaf Severity	
	Average	LSD	Average	LSD
Bloom inoculations: 2/16/16				
Water control	1.5	C	0.1	C
Xap 942	35.0	B	1.2	AB
Xap 1789	28.8	B	0.9	AB
Early fruit stage inoculations: 3/8/16				
Water control	5.6	C	0.1	C
Xap mixture^	26.6	B	1.0	AB
Xap mixture + surfactant^^	73.2	A	1.5	A
Late fruit stage inoculations: 4/14/16				
Water control	0.0	C	0.0	C
Xap mixture	77.7	A	1.1	AB
Xap mixture + surfactant	39.3	B	0.4	BC

* See text for incidence and severity evaluation methods. Values in columns followed by the same letter are not significantly different based on analysis of variance and LSD mean separation ($P > 0.05$) procedures.

^ *Xap* mixture inoculations containing approximately equal amounts of strains *Xap* 942 and *Xap* 1789.

^^ Surfactant treatments contained 0.1% Triton X-100 in water or inoculum mixture. No phytotoxicity was observed.

Table 2. Inoculation of cv. Fritz almond flowers with *Xanthomonas arboricola* pv*. pruni*.

Treatment	Fruit Incidence (%)*		Fruit Severity Rating		Leaf Severity Rating	
	Average	LSD	Average	LSD	Average	LSD
Water control	37.5	B	0.8	B	0.9	B
Xap mixture-high concentration	90.0	A	3.1	A	4.1	A
Xap mixture-low concentration	96.4	A	2.6	A	3.3	A

* See text for disease incidence and severity evaluation methods. Values in columns followed by the same letter are not significantly different based on analysis of variance and LSD mean separation ($P > 0.05$) procedures.

^ Inoculum contained approximately equal portions of strains Xap 942 and Xap 1789. The high inoculum concentration was OD_{600} 80% transmission; whereas the low inoculum concentration was OD_{600} 70% transmission followed by 1:10 dilution (ca. 1 and 2 x 10^7 cfu/ml).

III. In vitro sensitivity of Xap against copper, mancozeb, and antibiotics. All isolates evaluated to date grew at 20 ppm copper, but not at 30 ppm, and therefore were rated as copper-sensitive (>50 ppm is considered copper-resistant for other *Xanthomonas* spp.). Baseline levels were determined using 72 strains for each antimicrobial. Minimum (MIC) and lowest (LIC) inhibitory values to oxytetracycline and kasugamycin, mancozeb, and selected copper mixtures are shown in **Table 3.** Oxytetracycline was most inhibitory to the pathogen, and antibiotic-copper mixtures generally were more inhibitory than the active ingredients by themselves. Data for ATD are pending.

Table 3. Sensitivities (minimum inhibitory concentration - MIC and lowest inhibitory concentration - LIC) of selected bactericides to *Xanthomonas arboricola* pv*. pruni*.

	Inhibitory values (mg/L)									
	Oxytetracycline		Kasugamycin		Mancozeb		Cu + Mancozeb		Cu + Kasugamycin	
	MIC	LIC	MIC	LIC	MIC	LIC	MIC	LIC	MIC	LIC
Ave	0.07	0.03	30.61	13.33	2.18	1.13	0.05	0.02	25.16	9.75
Min	0.04	0.00	24.95	5.67	0.76	0.29	0.02	0.01	8.30	2.88
Max	0.10	0.04	39.05	16.62	3.57	1.94	0.79	0.36	60.14	17.29

* Inhibitory values for the antibiotics were determined using the SGD method and for copper using serial dilutions.

VI. Management of bacterial spot in the field. Following a high-rainfall winter and scattered showers in the spring, disease symptoms on fruit were first observed in April, and final evaluations were done in early June. Under these environmental conditions, the incidence of disease was higher and developed sooner than in the previous season. In four field trials, the efficacy and timing of dormant and in-season treatments was evaluated (**Tables 4-7**). Data of the split-plot analysis are presented in **Table 4**. Mean values of the dormant treatments are shown in the far right column. Similar to 2014 data, the delayed dormant copper-mancozeb treatment (applied in late January) resulted in a significant reduction of bacterial spot from that of the untreated control. The dormant treatment applied in December numerically reduced the disease and formed an intermediate statistical group. Thus, the delayed dormant treatment was efficacious in a winter with higher precipitation than in 2015.

Disease incidences for the in-season treatments with ChampION^{++}/ Manzate applied at bloom, petal fall, or at both phenological stages (i.e., Timings 1,2,3, respectively) were significantly lower than when no in-season treatments were applied (Timing 4 - shown in the bottom row of **Table 1**). Disease incidences were compared for each of the in-season timings by row and for each dormant treatment by column. Both dormant treatments were effective in the in-season timings for Timings 1 and 2, but not Timing 3. This is because the two in-season applications in Timing 3 were highly effective in reducing the disease even when no dormant application of copper-mancozeb was done. In contrast, when a delayed dormant application was made, no significant difference was obtained between in-season timings because the delayed dormant treatment was highly effective. Phytotoxicity was significantly higher with Timings 2 and 3 where copper (1 lb MCE at each application) was applied at petal fall or at full bloom and petal fall. Thus, one strategy would be to apply a delayed dormant treatment of copper-mancozeb and if no additional rainfall is forecasted, then in-season treatments may not be needed or can be delayed until rain events are forecasted in late spring; whereas if high rainfall is forecasted during bloom and petal fall, additional in-season treatments should be applied with copper rates reduced by ½ with each additional application.

An additional study of delayed dormant or in-season applications of copper, copper-oil, copper-mancozeb, or copper-mancozeb-oil is shown in **Table 5**. In most cases, delayed dormant applications of copper or copper-oil by themselves and delayed dormant treatments combined with in-season applications of copper or copper-mancozeb significantly reduced bacterial spot from that of the untreated control.

Table 4. Effect of early dormant and timing of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2016

		Timing 1		Timing 2		Timing 3		Timing 4		Dormant			
Treatments		IS: 2/16 (FB)		IS: 3/7 (PF)		IS: 2/16; 3/7 (FB,PF)		IS: none		Treatment Avg.		Phytotoxicity	
Dormant*	In-Season	Disease^	LSD^^	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD	Severity	LSD
Control	ChampION^{++} + Manzate	7.5	AB a	5.0	AB a	1.0	B a	21.8	A a	8.8	a	1.5	a
Early Dormant (12/4)	ChampION^{++} + Manzate	0.5	B b	0.3	B b	4.5	A a	4.3	A ab	2.4	ab	1.5	a
Delayed Dormant (1/28)	ChampION^{++} + Manzate	0.8	A b	0.5	A b	1.5	A a	0.8	A b	0.9	b	1.6	a
	Timing Avg.	2.9	B	1.9	B	2.3	B	9.0	A				
Phytotoxicity	Severity Avg.	0.86	B	2.57	A	2.86	A	0.00	C				

* Dormant treatments with 6 lb (2 lb MCE) ChampION^{++} + 6 lb Manzate 45DF/A were applied 12-4-2015 (Early dormant) and 1-28-2016 (Delayed Dormant). IS = in-season treatments with 3 lb (1 lb MCE) ChampION^{++} + 3.5 lb Manzate 75DF/A.

^ Fruit were evaluated for the presence of bacterial spot on 6-15-15. Disease values are the number of diseased fruit counted per tree. Phytotoxicity on leaves was evaluated using a rating scale from 0 (= no phytotoxicity) to 4 (= severe).

^^ Values followed by the same number are not significantly different based on an analysis of variance and LSD mean separation ($P > 0.05$). Statistical comparisons for values in the shaded area by column are with lower case letters, those by row are with upper case letters. Dormant treatment averages over all timings are in the right columns. Timing averages over all dormant treatments are in the bottom rows.

Table 5. Effect of late dormant applications and selected in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2016.

Delayed Dormant*	In-season^			
(1-28-16)	FB (2-16-16)	PF (3-8-16)	Disease	LSD^^
NuCop-Man	---	---	8	A
UTC	---	---	7.25	AB
NuCop-Man-Oil	---	---	4.5	AB
NuCop-Man	NuCop-Man	---	2.5	BC
NuCop-Oil	---	---	2.25	BC
NuCop	NuCop	---	1.5	C
NuCop-Oil	---	NuCop-Man	1.25	C
NuCop	---	---	1.25	C
NuCop-Man-Oil	---	NuCop	0.5	C

* Delayed dormant applications with NuCop, Manzate, and oil (Dormant oil 440) were applied at 6 lb,128 fl oz, and 3%v/v per Acre, respectively.

^ In-season applications of NuCop or NuCop-Manzate were applied at 2 lb and 64 fl oz/A, respectively.

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

In two additional field trials, different in-season treatments were compared without any dormant application. Treatments started at petal fall or shortly after and were mostly timed around rain events (*see* **Figure 1** for environmental conditions at trial site and timings). In comparisons of experimental and antibiotic treatments with and without copper or mancozeb, all treatments significantly reduced bacterial spot from that of the control for diseased fruit evaluated in the tree or for the total diseased fruit found on the ground and in the tree (**Table 6**). The most effective and consistent treatments included Kasumin (kasugamycin) and Mycoshield (oxytetracycline); either antibiotic mixed with ChampION^{++} (copper); Kasumin mixed with Manzate Max (mancozeb); and ATD used alone or in mixtures with ChampION^{++} or Kasumin. Disease incidence was reduced to very low levels by these treatments. No phytotoxicity was observed on all treatments except for the copper treatment. Minor phytotoxicity developed even with the reduced rates of copper (e.g., 3.3 to 0.8 lb) applied with each application.

Table 6. Effect of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2016

Treatment	Rate(/A)	Diseased Fruit-Tree	LSD	Diseased Fruit-Total	LSD
Control	---	6.25	A	9.25	A
Mycoshield + Manzate Max	16 oz + 64 fl oz	3.25	B	4.75	B
ATD	500 ppm	1.25	C	3.00	BC
Kasumin + ChampION	64 + 3.3-0.8 lb	0.75	C	2.5	BC
Mycoshield + ChampION	16 oz + 3.3-0.8 lb	0.75	C	1.5	C
Kasumin	64 fl oz	0.5	C	1.75	C
Kasumin + Manzate MAX	64 + 64 fl oz	0.5	C	3.25	BC
Mycoshield	16 oz	0.5	C	1.5	C
ATD + ChampION	500 ppm + 3.3-0.8 lb	0.5	C	1.5	C
ATD + Kasumin	500 ppm + 64 fl oz	0	C	1.25	C

* Applications were done using an air-blast sprayer at 100 gal/A on 2-23 (petal fall), 3-8, 3-30, and 4-21-16.

** Fruit on the tree and on the ground were evaluated for the presence of bacterial spot on 6-6-16. Disease values are the number of diseased fruit counted per tree.

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

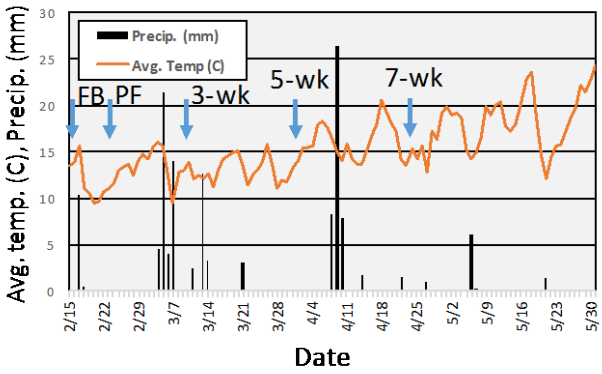

Figure 1. Environmental conditions near field trial locations in Ripon, CA in the spring of 2016. Arrows indicate bactericide timings in several studies where applications were done based on host phenology, rain events, or calendar-based intervals (*see* **Tables 4, 5, 6, and 7**).

In a trial to evaluate organic treatments, all treatments significantly reduced disease from the untreated control (**Table 7**). The mixture of Serenade + NuFilm-P + ChampION++ reduced the disease to zero. Other treatments that reduced the disease to low levels (<1 diseased fruit per tree) included Actinovate, Actinovate + Molasses, Badge, and Botector. These treatments were similar to copper-mancozeb (commercial standard). Results of biocontrol efficacy studies over the past three years have been consistent under low disease pressure (i.e., < 10% incidence), but as disease pressure increases, the biological controls generally are not highly effective. Still, for organic growers of almonds, alternatives to copper were identified.

Table 7. Effect of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2016.

No.	Treatment	Rate(/A)	PF 2/23	3-wk 3/7	5-wk 3-30	Disease^	LSD
1	Control	---	@	@	@	5.5	A
2	Serenade Opti+NuFilm-P	16 oz + 8 fl oz	@	@	@	2.25	B
3	Botector+Molasses	10 oz + 86 fl oz	@	@	@	1.50	B
4	Actinovate	12 oz	@	@	@	1	B
5	Actinovate+Molasses	12 oz + 86 fl oz	@	@	@	0.75	B
6	Botector	10 oz	@	@	@	0.75	B
7	Badge-Manzate	3.7-0.93 lb + 64 fl oz	@	@	@	0.5	B
8	Badge	3.7-0.93 lb	@	@	@	0.25	B
9	Serenade Opti+NuFilm-P-ChampION^{++}	16 oz + 8 fl oz + 3.3 - 0.8 lb	@	@	@	0	B

* Applications were done using an air-blast sprayer at 100 gal/A.

** Fruit on the tree and on the ground were evaluated for the presence of bacterial spot on 6-6-16. Disease values are the number of diseased fruit counted per tree.

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

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. With a wet winter season, the most effective management program for bacterial spot management likely will include a delayed dormant application of copper or copper-mancozeb (to reduce inoculum production and dispersal) and one to two in-season applications around rainfall events and rising temperatures (to prevent new infections). In drier winters or springs, dormant and in-season treatments may not be needed. On cv. Fritz, copper-mancozeb applications should be timed around rain events starting at bloom time through the weeks following petal fall. Effective biological treatments were identified for organic growers. In the future, alternative treatments based on antibiotics or experimental compounds will be hopefully registered and can be used in rotation or mixture to prevent resistance development to any one mode of action.