Epidemiology and Control of Bacterial Spot of Almond in California

Project No.: **14-PATH5-Adaskaveg**

Project Leader: J. E. Adaskaveg
Department of Plant Pathology and Microbiology
UC Riverside
Riverside, CA 92521
951.827.7577
jim.adaskaveg@ucr.edu

Project Cooperators and Personnel:
R. Duncan, UCCE – Stanislaus County
B. Holtz, UCCE – San Joaquin County
D. Doll, UCCE – Merced County
H. Förster, D. Thompson, S. Haack, and D. Cary, UC Riverside
L. Wade, ArystaLifeSciences

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 *Xanthomonas arboricola* pv. *Pruni (Xap)* strains using specific PCR primers
   c. Determination of the genetic variability by molecular methods

II. 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

III. Management of bacterial spot in the field
   a. Dormant applications will be done in early winter (2014) and late winter (2015) 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, and the bacterial membrane disrupter Ceragenin. Mixtures of copper-mancozeb (or other fungicides with bactericidal properties) and antibiotic-mancozeb combinations will also be evaluated.
   c. The biocontrol treatments Serenade Optimum (*Bacillus* sp.), Actinovate (*Actinomyces* sp.), and Botector (*Aureobasidium* sp.) with and without growth enhancers will be evaluated.

Interpretive Summary:

Bacterial spot was found in California in 2013 at high incidence at some locations in Colusa, San Joaquin, Stanislaus, Merced, and Madera Co., especially on cv. Fritz. Other cultivars such as Nonpareil, Aldrich, Butte, Carmel, and Price also had disease but at lower incidence and severity levels. The pathogen was identified as *Xanthomonas arboricola* pv. *pruni* (*Xap*) that commonly causes bacterial spot of peach and cherry in the eastern United States. The disease was found again, often at high levels, at previous and additional locations in 2014 and 2015. Due to drier weather conditions in the spring of 2015, however, the incidence of bacterial spot was generally lower. Still, there were exceptions where high levels of disease occurred in orchards with high humidity or with high-angle sprinklers. The pathogen was again isolated from overwintering symptomatic fruit mummies, indicating their role as primary inoculum sources during spring infection periods. Mummies still contained viable inoculum when collected in early July. Bacterial inoculum levels can increase exponentially within a short time period, and therefore, with viable inoculum present, conducive conditions (wetness and warm temperatures) may provide infection periods during almond development over a long spring season. Field trials on cv. Fritz on the management of the disease were conducted and included dormant and in-season applications. In contrast to 2014, none of the dormant treatments (applied either in mid-December or in late January) with copper or copper-mancozeb resulted in a significant reduction of bacterial spot. This was likely due to the very dry winter that resulted in limited inoculum production during this time. In-season treatments, however, that started at full bloom or soon after (petal fall) significantly reduced the disease when timed around rain events and before temperatures started to rise in the springtime. The most effective and consistent treatments included copper (Kocide 3000, Badge, ChampION^{++}) and copper mixed with mancozeb or with kasugamycin (e.g., Kasumin). Copper phytotoxicity was observed on leaves after four or five applications even when copper rates were successively reduced for the second and subsequent sprays, and minor leaf tip necrosis was present after four or more successive Kasumin applications. Other treatments that significantly reduced the disease included Mycoshield and the experimental USF2018A. Field inoculation studies with the pathogen were only successful when done during bloom or on young, immature fruitlets. The disease was then observed on developing fruit after four to six weeks. Inoculations of older fruit were unsuccessful. This, in addition to our field timing studies, indicates the importance of bactericide applications at bloom time. Based on our results from two years of field studies we conclude that with a wet winter season the most effective management program for bacterial spot likely will include a late dormant application (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, dormant treatments may not be needed, and applications should be timed around rain events starting at bloom time. Additional field trials will be conducted in the coming season to validate these conclusions.

Materials and Methods:

Surveys on the distribution of bacterial spot in California almond orchards and genetic variability of pathogen populations. Samples of diseased almond tissues were submitted by farm advisors and PCAs. *Xap* was isolated from symptomatic almond fruit on yeast extract-dextrose-calcium carbonate agar using standard procedures. The identity of the pathogen was verified by cultural morphology and by using specific PCR primers that have been developed by others.

In vitro sensitivity of* Xap *against copper, mancozeb, and antibiotics. Copper sensitivity was evaluated by streaking bacteria on nutrient agar media amended with 20 ppm or 30 ppm copper ion. Bacterial growth was evaluated after 2 days of incubation.

Management of bacterial spot in the field. Seven field trials were conducted on cv. Fritz orchards in San Joaquin Co. where bacterial spot occurred at high incidence in 2013 and 2014. Treatments were applied using an air-blast sprayer during the dormant period (i.e., mid-December or mid-January) and during the growing season between late-February (petal fall) and late April or mid-May at selected timings around rain events. Bactericides used are indicated in the Results section. Two split-plot trials were done with dormant treatments as main plots and in-season treatments as sub-plots. For each treatment, there were four single-tree replications. Trees were evaluated periodically for disease in the springtime. Final evaluations were done in early July, and the number of fruit infections was counted for each tree. Data were evaluated using analysis of variance and mean separation procedures for split-plot trials using SAS.

Results and Discussion:

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 none of the leaf lesions evaluated (possibly copper damage) resulted in positive isolations. The pathogen was also isolated from overwintering symptomatic fruit mummies collected on trees between December 2014 and early July 2015. This indicates survival of the pathogen for over a year and the role of mummies as primary inoculum sources during infection periods in the spring and possibly early summer time. Molecular analysis of isolates to obtain information on population structure is pending.

Table 1. Effect of early dormant and timing of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2015

Disease incidence (%)		Timing 1		Timing 2		Timing 3		Timing 4			
		D: 12/18/14		D: 12/18/14		D: 12/18/14		D: 12/18/14			
		IS: 3/11/15		IS: 4/6, 4/24		IS: 3/11, 4/6, 4/24, 5/13		IS: none		Treatment Avg	
Dormant treatment	In-season	Disease^	LSD^^	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD
Control	ChampION++ + Manzate	2.0	B a	4.3	AB a	2.5	B a	10.3	A a	4.8	a
Kocide 3000 6 lb	ChampION++ + Manzate	2.3	A a	1.8	A a	1.0	A a	4.5	A a	2.4	a
Kocide 3000 6 lb + Manzate 6 lb	ChampION++ + Manzate	0.8	A a	2.0	A a	1.0	A a	3.3	A a	1.8	a
	Timing Avg	1.7	B	2.7	B	1.5	B	6.0	A		

Copper phytotoxicity rating on leaves		Timing 1		Timing 2		Timing 3		Timing 4			
		D: 12/18/14		D: 12/18/14		D: 12/18/14		D: 12/18/14			
		IS: 3/11/15		IS: 4/6, 4/24		IS: 3/11, 4/6, 4/24, 5/13		IS: none		Treatment Avg	
Dormant treatment	In-season		LSD		LSD		LSD		LSD	Disease	LSD
Control	ChampION++ + Manzate	1.5	B a	0.8	C a	2.8	A a	0.0	C a	1.3	a
Kocide 3000 6 lb	ChampION++ + Manzate	1.0	B a	1.0	B a	2.5	A a	0.0	C a	1.2	a
Kocide 3000 6 lb + Manzate 6 lb	ChampION++ + Manzate	1.0	B a	0.8	B a	2.3	A a	0.0	D a	1.0	b
	Timing Avg	1.2	B	0.83	C	2.6	A	0	D		

* D= dormant treatment. IS = in-season treatments with ChampION++ + 3.5 lb. Manzate 75DF/A. Rates for ChampION++ and Manzate were: 3.3 lb. + 96 fl oz., 1.6 lb. + 64 fl oz., 0.8 lb. + 64 fl oz., and 0.8 lb. + 64 fl oz. for 3-11, 4-6, 4-24, and 5-13 applications, respectively.

^ Fruit were evaluated for the presence of bacterial spot on 7-1-15. 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. Treatment averages are values for treatments over all timings and are statistically compared by column. Timing averages are values "for each timing" for all treatments and are statistically compared within the row.

Table 2. Effect of late dormant applications and different in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2015.

Disease incidence (%)	Control		ChampION^{++} + Manzate		ChampION^{++} + Kasumin		ChampION^{++}			
	D: 1/27/15		D: 1/27/15		D: 1/27/15		D: 1/27/15			
	IS: no applications		IS: 4 applications		IS: 4 applications		IS: 4 applications		Treatment Avg	
Dormant treatment	Disease^	LSD^^	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD
Control	4.5	A a	0.8	B a	0.5	B a	1.3	B a	1.8	a
Kocide 3000 6 lb	2.0	A b	0.5	A a	1.3	A a	1.0	A a	1.2	a
Kocide 3000 6 lb + Manzate 6 lb	2.3	A b	0.0	B a	0.5	B a	0.8	AB a	0.9	a
	2.9	A	0.4	B	0.75	B	1.0	B		

Copper phytotoxicity rating on leaves	Control		ChampION^{++} + Manzate		ChampION^{++} + Kasumin		ChampION^{++}			
	D: 1/27/15		D: 1/27/15		D: 1/27/15		D: 1/27/15			
	IS: 4 applications		IS: 4 applications		IS: 4 applications		IS: 4 applications		Treatment Avg	
Dormant treatment	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD
Control	0.00	B a	1.00	A a	0.75	A a	0.75	A a	0.6	b
Kocide 3000 6 lb	0.00	B a	1.00	A a	1.00	A a	1.00	A a	0.8	ab
Kocide 3000 6 lb + Manzate 6 lb	0.00	B a	1.25	A a	1.00	A a	1.00	A a	0.8	a
	0	B	1.1	A	0.9	A	0.92	A		

* D= dormant treatments, IS = in-season treatments. In-season treatments were done on 2-25, 3-6, 3-17, and 4-6-15. Treatments were: ChampION^{++} + 3.5 lb. Manzate 75DF/A (rates for ChampION^{++} and Manzate were: 3.3 lb. + 96 fl oz., 1.6 lb. + 64 fl oz., 0.8 lb. + 64 fl oz., and 0.8 lb. + 64 fl oz. for the first, second, third, and forth applications, respectively); ChampION^{++} + Kasumin 64 fl oz. (ChampION^{++} rates were the same as for the previous treatment), ChampION^{++} (rates were the same as for the two previous treatments); and untreated.

^ Fruit were evaluated for the presence of bacterial spot on 7-1-15. 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. Treatment averages are values for treatments over all timings and are statistically compared by column. Timing averages are values "for each timing" for all treatments and are statistically compared within the row.

In vitro sensitivity of* Xap *against copper, mancozeb, and antibiotics. All isolates evaluated to date all 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.). Additional isolates are currently being tested, also against other bactericides to develop baseline levels of sensitivity.

Management of bacterial spot in the field. Following a dry winter and spring, disease symptoms on fruit were first observed in early June, and final evaluations were done in early July. Under these environmental conditions, the incidence of disease was lower than in the two previous seasons. No disease was observed on leaves. In seven field trials, the efficacy and timing of dormant and in-season treatments was evaluated.

In contrast to 2014, none of the dormant treatments (applied either in mid-December or in late January) with copper or copper-mancozeb resulted in a significant reduction of bacterial spot. This was likely due to the very dry winter that resulted in limited inoculum production and dispersal. Data of the split-plot analyses are presented in **Tables 1** and **2**. Dormant treatments (columns in shaded areas of tables) were compared for each of the in-season timings with ChampION^{++}/Manzate (**Table 1**) or in-season applications with different treatments (**Table 2**).

All dormant treatments were found to be statistically non-significant, although differences in numerical disease incidence were present.

In-season applications, however, that started at petal fall or soon after resulted in significant differences in disease incidence. Thus, in the first trial, in-season treatments with ChampION^{++}/Manzate reduced the incidence of disease as compared to the dormant treatment alone, and all timings (timings 1,2,3) were statistically similar. This is shown in **Table 1** in the row named "Timing Avg" where in-season treatments were compared for each timing, and timing 4 (where no in-season applications were done) had significantly more disease than timings 1, 2, or 3. Similarly in **Table 2**, the row named "Treatment Avg" indicates that four applications of the three treatments used resulted in lower disease than when no in-season treatments were done. There was no difference in efficacy among treatments consisting of ChampION^{++}/Manzate, ChampION^{++}/Kasumin, or ChampION^{++} by itself.

In four 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

Table 3. Effect of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2015

			Disease inc. fruit on tree**		Disease inc. fruit on tree and ground		Phytotoxicity on leaves***	
No.	Treatment*	Rate(/A)	%	LSD^	%	LSD	Rating	LSD
1	Control	---	14.6	a	28.8	a	0	b
2	Kasumin	64 fl oz	1.3	bc	3.5	cd	0.5	ab
3	Mycoshield	12 oz	2.8	bc	8.5	bcd	1	a
4	Kocide 3000 + Kasumin	8 oz + 64 fl oz	3.3	bc	7.3	bcd	1	a
5	Kocide 3000 + Mycoshield	12 oz + 12 oz	0.3	c	1.0	d	1	a
6	Quintec	12 fl oz	16.0	a	25.0	ab	0.5	ab
7	Quintec + Manzate Max	12 fl oz + 96 fl oz	6.5	abc	11.3	abcd	0	b
8	Ceragenin	5 fl oz	7.5	ab	17.8	abc	0	b

* Applications were done using an air-blast sprayer at 100 gal/A on 2-25 (petal fall), 3-6, 3-16, 3-26, 4-6, and 4-24-15.
** Fruit on the tree and on the ground were evaluated for the presence of bacterial spot on 7-1-15. 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 ($P > 0.05$).

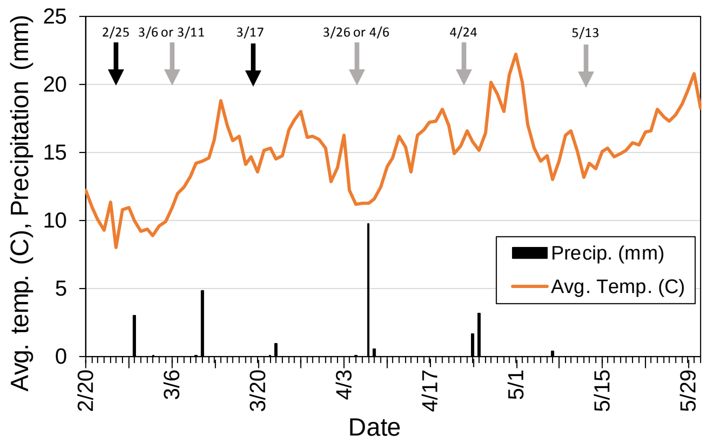

Figure. 1. Environmental conditions near field trial locations in Ripon, CA in the spring of 2015. Arrows indicate bactericide timings in several studies where applications were done based on rain events (*see* **Tables 1** and **5**, gray arrows) or calendar-based in 10- to 14- day intervals (*see* **Tables 2, 3, 4,** and **6** gray and black arrows).

Table 4. Effect of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2015.

			Disease inc. fruit on tree**		Disease inc. fruit on tree and ground		Phytotoxicity on leaves***	
No.	Treatment*	Rate(/A)	%	LSD^	%	LSD	Rating	LSD
1	Control	---	6.7	a	18.6	a	0	a
2	Kocide 3000 + Serenade Opti + NuFilm P	3.3 to 0.8 lb + 8 oz + 8 fl oz	0.5	d	3.3	cd	0.5	a
3	Serenade Opti + NuFilm P	8 oz + 8 fl oz	2.5	abc	11.3	abc	0	a
4	Serenade Opti + NuFilm P	16 oz + 8 fl oz	5.5	a	14.3	ab	0	a
5	USF2018A	7 fl oz	0.8	d	7.8	abc	0	a
6	USF2018A	5.3 fl oz	3.0	bcd	5.0	bcd	0	a
7	Badge X2	3.3 to 0.8	0.0	d	1.3	d	0	a
8	Badge X2 + Vintre	3.3 to 0.8 lb + 64 fl oz	0.8	cd	4.3	cd	0.75	a
9	Actinovate	8 oz	4.5	a	15.0	ab	0	a
10	Actinovate + Nutrient	8 oz + 2 lb	3.8	abc	7.8	abc	0	a

* Applications were done using an air-blast sprayer at 100 gal/A on 2-25 (petal fall), 3-6, 3-17, 3-26, and 4-6-15. Copper rates in treatments 2, 7, and 8 were 3.3, 1.6, 0.8, 0.4, and 0.8 lb./A for the five timings, respectively.

** Fruit on the tree and on the ground were evaluated for the presence of bacterial spot on 7-1-15. 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 ($P > 0.05$).

Table 5. Effect of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2015.

Treatment*	Rate/A	Disease incidence** %	LSD^
Control	---	20.8	a
Kasumin	64 fl oz.	3.5***	b
Kasumin + Manzate Max	64 fl oz. + 64 fl oz.	2.8	b

* Applications were done using an air-blast sprayer at 100 gal/A around rain events on 3-11, 4-6, 4-24, and 5-13-15.

** Fruit on the tree were evaluated for the presence of bacterial spot on 7-1-15. Disease values are the number of diseased fruit counted per tree.

*** There was some minor phytotoxicity with leaf tip necrosis in the Kasumin treatment.

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

(*see* **Figure 1** for environmental conditions at trial site and timings). The most effective and consistent treatments included copper (Kocide 3000, Badge, ChampION++) and copper-containing mixtures as well as Kasumin (**Tables 3** to **6**). Disease incidence was reduced to very low levels by these treatments. Other treatments that significantly reduced the disease included Mycoshield and the experimental USF2018A. These data indicate that effective treatments for bacterial spot are currently available. As also indicated in the results tables, copper phytotoxicity was observed on leaves after four or five applications even when copper rates were successively reduced for the second and subsequent sprays. Still, on a scale of 0 to 4, phytotoxicity ratings were always less than 1.5. For commercial management of the disease, only one or two in-season applications may be needed (this will be evaluated in future trials) and this will reduce the risk of copper injury. Minor leaf tip necrosis was present after four or five successive Kasumin treatments. In the trial of **Table 4**, no phytotoxicity was observed on the Badge treatment, however Badge with oil (Vintre) caused some minor phytotoxicity.

Table 6. Effect of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2015.

			PF	1-wk	3-wk	5-wk	7-wk	Disease incidence**		Phytotoxicity on leaves***	
No.	Treatment*	Rate(/A)	2/25	3/6	3/17	3/26	4/6	%	LSD^	Rating	LSD
1	Control	---	---	---	---	---	---	17.4	a	0	c
2	Mycoshield	16 oz	@	@	@	@	@	8.8	ab	0	c
3	ATD	13 oz	@	@	@	@	@	5.3	abc	0	c
4	ATD + Kasumin	13 oz + 64 fl oz	@	@	@	@	@	4.0	bc	0.13	bc
5	Kasumin	64 fl oz	@	@	@	@	@	3.8	bc	0.75	b
6	ChampION++	3.3 to 0.8 lb	3.3	1.6	0.8	0.8	0.8	1.8	bc	1.75	a
	Mycoshield	16 oz	@	@	@	@	@				
7	ChampION++	3.3 to 0.8 lb	3.3	1.6	0.8	0.8	0.8	1.3	bc	1.75	a
	Kasumin	64 fl oz	@	@	@	@	@				
8	ChampION++	3.3 to 0.8 lb	3.3	---	1.6	---	0.8	1.0	bc	0.87	b
	Mycoshield	16 oz	@	@	@	@	@				
	Manzate	4 lbs/A	---	@	---	@	---				
9	ChampION++	3.3 to 0.8 lb	3.3	---	---	---	---	0.5	c	0.75	b
	Mycoshield	16 oz	@	@	@	@	@				

* Applications were done using an air-blast sprayer at 100 gal/A. Copper rates were reduced with each treatment.

** Fruit on the tree were evaluated for the presence of bacterial spot on 7-1-15. 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 ($P > 0.05$).

Field inoculation studies with the pathogen were only successful when done during bloom or early fruit development, but not when done during mid-fruit development. The disease was then observed on fruit after four to six weeks. This, in addition to our application timing studies, indicates the importance of bactericide applications early in the season. Further field studies will be needed to confirm this.

Based on our results from two years of field studies we conclude that with a wet winter season the most effective management program for bacterial spot likely will include a late dormant application (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, dormant treatments may not be needed, and applications should be timed around rain events starting at bloom time. Additional field trials will be conducted in the coming season to validate these conclusions.