Epidemiology and Control of Bacterial Spot of Almond in California

Project No.: 13-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. In vitro sensitivity of *Xap* against copper, mancozeb, and antibiotics
   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 were done in 2013 and will be repeated in late fall 2014 using copper and copper-mancozeb combinations.
   b. Spring-time applications will include traditional and new formulations of copper with low phytotoxicity potential, and the antibiotics kasugamycin, oxytetracycline, and streptomycin. Mixtures of copper-mancozeb (or other fungicides with bactericidal properties) and antibiotic-mancozeb combinations will also be evaluated.

Interpretive Summary:

In the spring of 2013, bacterial spot was found in California at high incidence at some locations in Colusa, San Joaquin, Stanislaus, Merced, and Madera counties, especially on cv. Fritz, but at lower severity also on cvs. Nonpareil, Butte, Carmel, and Price. The pathogen was identified as *Xanthomonas arboricola* pv. *pruni* (*Xap*) that is commonly causing bacterial spot of peach in the eastern United States. In 2014, we verified the presence of the disease at previous and additional locations. The pathogen was also isolated from overwintering symptomatic fruit mummies between December 2013 and February 2014, indicating their role as primary inoculum sources during infection periods. Isolates evaluated to date were all rated as copper-

sensitive with growth occurring at 20 ppm copper, but not at 30 ppm. Field trials on the management of the disease were conducted that included dormant and in-season applications. Late dormant treatments (late January) with copper, copper-mancozeb, or copper-mancozeb-captan significantly reduced the incidence of disease, but not early dormant treatments that were applied in mid-November and mid-December. In-season treatments were most effective when timed around rain events and before temperatures started to rise in the springtime. Thus, the major infection period was identified to have occurred between mid- and late March 2014. All copper products significantly reduced disease. Among products evaluated, however, Kocide 3000, Badge, Champ-Ion^{2+}, and Cuprofix were more effective than Cueva. No copper phytotoxicity was observed after four applications when copper rates were successively reduced for the second and subsequent sprays. Other treatments with high efficacy included Kasumin-Manzate, Kasumin-Captan, Kocide-Tanos, copper-Manzate (several copper products), Mycoshield/Fireline (oxytetracycline; federally registered on peach for this disease), Serenade Optiva, and the new bacterial membrane disruptor Cerogenin. Based on our results, the most effective management program for bacterial spot likely will include a late dormant bactericide application to reduce inoculum and at least one in-season application around rainfall events and rising temperatures to prevent new infections. To validate this, field trials will be conducted again in the coming trial season.

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 County where bacterial spot occurred at high incidence in 2013. Treatments were applied using an air-blast sprayer during the dormant period (i.e., early dormant, mid-dormant, or late dormant applications) and during the growing season between mid-February (bloom) and late April at selected timings around rain events. Bactericides used are indicated in the Results and Discussion section below. 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 late May and the number of fruit infections was counted for a specified evaluation time. 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 (San Joaquin, Stanislaus, and Merced Co.) and northern districts (Colusa Co.). Still, the pathogen was not isolated from some samples with apparently typical symptoms of bacterial spot. Therefore, correct diagnostic procedures are important for disease verification. None of the leaf lesions evaluated resulted in positive isolations. The pathogen was also isolated from overwintering symptomatic fruit mummies collected between December 2013 and February 2014 indicating their role as primary inoculum sources during infection periods in the springtime. Molecular analysis of isolates to obtain information on population structure is pending.

In vitro sensitivity of* Xap *against copper, mancozeb, and antibiotics. 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 based on other *Xanthomonas* spp.). Isolates are currently being tested against other bactericides including mancozeb and cerogenin, as well as the antibiotics kasugamycin and oxytetracycline.

Management of bacterial spot in the field. First disease symptoms were observed in early May, which was later than in 2013 when the disease was first observed in April. By the end of May, disease on fruit was present at all our field sites, but not on leaves. In seven field trials, the efficacy and timing of dormant and in-season treatments was evaluated. Dormant treatments with copper, copper-mancozeb, or copper-mancozeb-captan when applied at late dormancy (late January) significantly reduced the incidence of disease, most likely by reducing the amount of inoculum (split-plot field trials in **Table 1** and **2**; shaded area of timing 6 or 4, respectively, = comparison of dormant treatments only, no in-season applications). In contrast, dormant treatments that were applied in mid-December were not effective (split-plot field trial in **Table 3**; shaded area of timing 6 = comparison of dormant treatments only, no in-season applications). This is probably because temperatures are lower at this time of the year and the pathogen is inactive in overwintering diseased tissue and has not started to re-colonize infected tissues and produce new inoculum**.** Moreover, by the time conducive conditions occurred for disease, the bactericidal residues from early and mid-dormant treatments were depleted on the tree from rainfall and/or new tree growth in the spring.

Table 1. Effect of late dormant and timing of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin County 2014.

Dormant treatment	In-season treatment	Timing 1		Timing 2		Timing 3		Timing 4		Timing 5		Timing 6		Treatment Avg	
		D: 1/24/14*		D: 1/24/14		D: 1/24/14		D: 1/24/14		D: 1/24/14		D: 1/24/14			
		IS: 2/19, 4/24		IS: 3/5/14		IS: 3/18/14		IS: 3/31/14		IS: 2/19, 3/5, 3/18, 3/31, 4/24		IS: none			
		Disease^	LSD^^	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD
Control	Kas-Man	12.3	a	18.8	a	8	a	19	a	4.3	a	37.8	a	16.7	A
Kocide 3000 6 lb	Kas-Man	12.8	a	31.3	a	7.3	a	7	a	6	a	16.8	b	13.5	AB
Kocide 3000 6 lb + Manzate 6 lb	Kas-Man	11	a	14.8	a	7.5	a	11.8	a	3.8	a	9.5	b	9.7	B
	Timing Avg	12.0	B	21.6	A	7.6	BC	12.6	B	4.7	C	21.3	A		

* D= dormant treatment, IS = in-season treatment with 64 fl oz Kasumin + 3.5 lb Manzate 75DF/A

^ Fruit were evaluated for the presence of bacterial spot on 5/21/14. Disease values are the number of diseased fruit counted per tree for a specified evaluation time.

^^ Values followed by the same letter 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 are by column using lower 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 and timing of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin County 2014.

Dormant treatment	In-season treatment	Timing 1		Timing 2		Timing 3		Timing 4		Treatment Avg	
		D: 1/24/14*		D: 1/24/14		D: 1/24/14		D: 1/24/14			
		IS: 2/19, 3/31		IS: 3/5, 4/24/14		IS: 3/19/14		IS: none			
		Disease ^	LSD^^	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD
Control	Cap - Man	13.8	a	33.5	a	18.3	a	36.3	a	25.4	A
Kocide 3000 6 lb + Manzate 6 lb + Captan 80 5 lb	Cap - Man	20.5	a	23.5	a	4.5	b	14.7	b	15.8	A
	Timing Avg	17.1	AB	28.5	A	11.4	B	25.5	AB		

* D= dormant treatment, IS = in-season treatment with 3 lb Captan 80WDG + 3 lb Manzate 75DF/A (2/19/14 was 70% bloom, 3/5/14 was petal fall).

^ Fruit were evaluated for the presence of bacterial spot on 5/27/14. Disease values are the number of diseased fruit counted per tree for a specified evaluation time.

^^ Values followed by the same letter 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 are by column using lower 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 3. Effect of mid-dormant and timing of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin Co. 2014

Dormant treatment**	In-season treatment	Timing 1		Timing 2		Timing 3		Timing 4		Timing 5		Timing 6		Treatment Avg	
		D: 12/17/13*		D: 12/17/13		D: 12/17/13		D: 12/17/13		D: 12/17/13		D: 12/17/13			
		IS: 2/19, 4/24		IS: 3/5/14		IS: 3/20/14		IS: 3/31/14		IS: 2/19, 3/5, 3/21, 3/31		IS: none			
		Disease^	LSD^^	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD	Disease	LSD
Control	Control	55.5	a	50.5	a	19.5	ab	45.8	a	52.8	a	37.5	a	43.6	A
Kocide 3000 6 lb + 1% oil	Kocide 3000	6.5	b	5	b	7.3	b	15	b	3.8	b	29.3	a	11.2	B
Kocide 3000 6 lb (no oil)	Champ-Ion[2+]	21.3	b	10.5	b	4.8	b	16.8	ab	1.8	b	28.3	a	13.9	B
Cueva 2 gal + 1% oil	Cueva	24.8	b	61.8	a	33.5	a	38.3	ab	10.8	b	24.5	a	32.3	A
Badge 7 pt + 1% oil	Badge X2	9.3	b	8.5	b	2.3	b	15.8	b	2.8	b	19.3	a	9.7	B
Cuprofix 4.5 lb+ 1% oil	Cuprofix	6	b	19.5	b	3.5	b	11	b	3	b	24.5	a	11.3	B
	Timing Avg	20.6	AB	26.0	A	11.8	B	23.8	A	12.5	B	27.2	A		

* D= dormant treatment, IS = in-season treatment

** Rates for in-season treatments were for Kocide 3000: 3.3 lb, 1.6 lb, 0.8 lb; for ChampIon: 3.3 lb, 1.6 lb, 0.8 lb; for Cueva: 64 fl oz, 32 fl oz, 16 fl oz; for Badge X2: 3.7 lb, 1.9 lb, 0.05 lb; and for Cuprofix: 2.5 lb, 1.25 lb, 0.62 lb for the first, second, and following applications, respectively.

^ Fruit were evaluated for the presence of bacterial spot on 5/27/14. Disease values are the number of diseased fruit counted per tree for a specified evaluation time.

^^ Values followed by the same letter 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 are by column using lower 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.

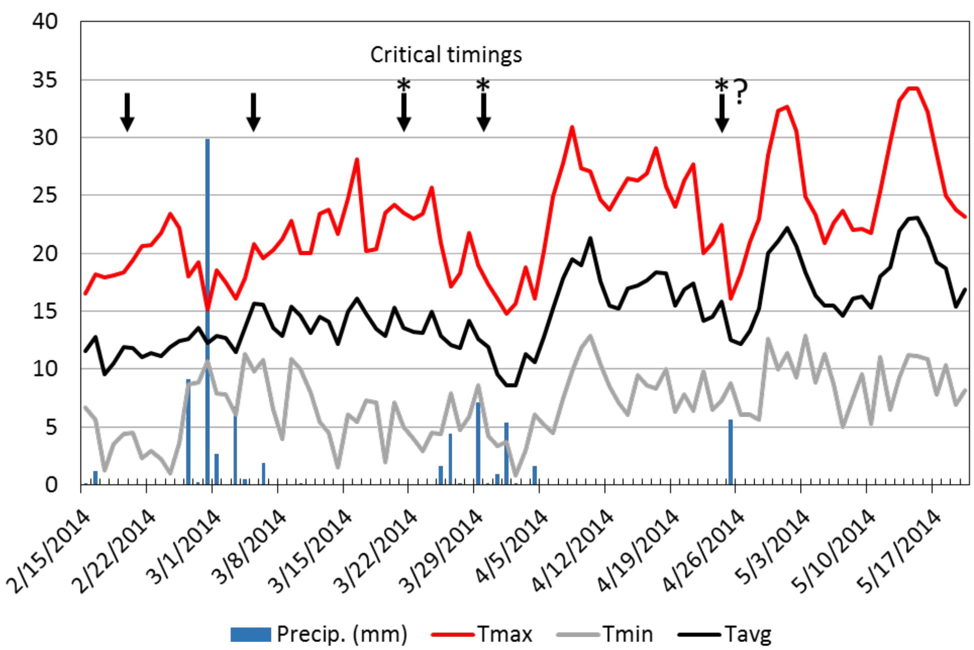

Figure 1. Environmental conditions near field trial locations (Ripon, CA).

In-season treatments were most effective when timed around rain events and before temperatures started to rise in the springtime. In the three trials discussed above, timings that involved an application on March 19 or 20 were most effective in reducing the amount of disease. The March 31 application was also critical in one trial (**Table 1**) but not in another one (**Table 3**).Similarly, the late April application was effective in one trial (**Table 3**) but not in another one (**Table 1**). These data are shown in **Tables 1 - 3** in the row named "Timing Avg" where all in-season treatments were compared for each timing. Weather data for the trial area are presented in **Figure 1** and indicate the critical timings with an asterisk before and during a period with several rain events just before an increase in temperatures. Thus, a major infection period was identified to have occurred between mid- and late March. Still, it took over five weeks for first symptoms to develop.

Table 4. Efficacy of in-season bactericide treatments for management of bacterial spot of cv. Fritz almond in San Joaquin County 2014.

			Application timing					
Program	Treatment*	Rate/A	Full bloom 2/18	Petal fall 3/5	2 wk APF 3/20	4 wk APF 4/3	Incidence of bacterial spot on fruit (%)**	LSD^
---	Control	-----	-----	-----	-----	-----	12.0	a
Single treatments	Kphite + Widespread	96 fl oz + 8 fl oz	@	@	@	@	11.0	ab
	Actinovate	12 oz	@	@	@	@	8.0	abcd
	Actinovate + Buffer	12 oz + 86 oz	@	@	@	@	6.3	bcd
	Serenade Optiva	20 oz	@	@	@	@	4.3	cd
	Taegro	5 oz	@	@	@	@	9.5	abc
Mixture treatments	MycoShield + Champ-Ion^{2+}	16 oz + 3.3 lb	@	---	---	---	8.3	abcd
		16 oz + 1.6 lb	---	@	---	---		
		16 oz + 0.8 lb	---	---	@	@		
	Kasumin 2L + Manzate 75DF	64 fl oz + 6 lb	@	---	---	---	3.0	d
		64 fl oz + 4 lb	---	@	@	@		
	Kocide 3000 + Manzate 75DF	3.3 lb + 6 lb	@	---	---	---	6.0	bcd
		1.6 lb + 4 lb	---	@	---	---		
		0.8 lb + 4 lb	---	---	@	@		
	Kocide 3000 + Tanos	3.3 lb + 10 oz	@	---	---	---	4.8	cd
		1.6 lb + 10 oz	---	@	---	---		
		0.8 lb + 10 oz	---	---	@	@		
	Magna Bon + Manzate 75DF	64 fl oz + 6 lb	@	---	---	---	5.3	cd
		27 fl oz + 4 lb	---	@	@	@		

* Treatments were applied at a rate of 100 gal/A using an air-blast sprayer. APF = after petal fall.

** Fruit were evaluated for the incidence of bacterial spot on 5/21/14.

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

Among copper products evaluated, Kocide 3000, Badge X2, Champ-Ion2+, and Cuprofix were more effective than Cueva (**Table 3**; in column "Treatment Avg" treatments are compared for all timings). No copper phytotoxicity was observed after four applications when copper rates were successively reduced for the second and subsequent sprays. Other treatments with high efficacy identified included Kasumin-Manzate, Kasumin-Captan, Kocide-Tanos, copper-Manzate (several copper products), Mycoshield (oxytetracycline; federally registered on peach for this disease), Serenade Optiva, and the new bacterial membrane disruptor Cerogenin (**Tables 4** and **5**). In an additional trial with in-season treatments only, Kasumin and Kasumin-Manzate were also very effective (**Table 6**).

Table 5. Efficacy of in-season bactericide treatments for management of bacterial spot of cv. Fritz almond in San Joaquin County 2014.

			Application timing						
No.	Treatment*	Rate/A	Full bloom 2/19	Petal fall 3/6	2 wk APF 3/20	4 wk APF 3/31	6 wk APF 4/18	Incidence of bacterial spot on fruit (%)	LSD^
1	Control	---	---	---	---	---	---	6.5	a
2	Quintec	6 fl oz	@	@	@	@	@	5.8	abc
3	Kasumin 2L + Kocide 3000	64 fl oz + 3.3 lb	@	---	---	---	---	3.5	abc
	Kasumin 2L	64 fl oz	---	@	@	@	@		
4	Kasumin 2L	64 fl oz	@	---	---	---	---	3.3	abc
	Kasumin 2L + Quintec	64 + 6 fl oz	---	@	@	@	@		
5	Mycoshield	16 oz	@	@	@	@	@	3.0	bcd
6	Kasumin 2L + Manzate 75DF	64 + 6 lb	@	---	---	---	---	2.3	cd
	Kasumin 2L + Manzate 75DF	64 fl oz + 4 lb	---	@	@	@	@		
7	Quintec + Manzate 75DF	6 fl oz + 6 lb	@	---	---	---	---	1.8	cd
	Quintec + Manzate 75DF	6 fl oz + 4 lb	---	@	@	@	@		
8	Mycoshield + Champ-Ion^{2+}	16 oz + 3.3 lb	@	---	---	---	---	1.5	cd
	Mycoshield	16 oz	---	@	@	@	@		
9	Cerogenin + NuFilmP	6 fl oz	---	@	@	@	@	0.8	d

* A commercial treatment with 8 lb/A NuCop was done to the orchard by the grower in early November 2013. In-season treatments were applied at a rate of 100 gal/A using an air-blast sprayer. APF = after petal fall.

** Fruit were evaluated for the incidence of bacterial spot on 5/14/14.

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

Table 6. Effect of in-season treatments on the incidence of bacterial spot of cv. Fritz almond in San Joaquin County 2014.

Treatment	Application timings 2/19	3/5	3/18	3/31	4/24	Disease Incid. (%)^	LSD^^
Control	-----	-----	-----	-----	-----	7.7	a
Kasumin 2L 64 fl oz	@	@	@	@	@	2.8	b
Kasumin 2L 64 fl oz + Manzate 75DF 6 lb	@	---	---	---	---	0.5	b
Kasumin 2L 64 fl oz + Manzate 75DF 4 lb	---	@	@	@	@		

^ Fruit were evaluated for the presence of bacterial spot on 5/27/14. Disease values are the number of diseased fruit counted per tree for a specified evaluation time.

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

In summary, in the first year of our project we obtained a large amount of valuable information on the management of bacterial spot of almond. Based on our results, the most effective management program likely will include a late dormant (delayed dormant) bactericide application to reduce inoculum and at least one in-season application during the period after petal fall around rainfall events and rising temperatures to prevent new infections. To validate this, field trials will be conducted again in the coming field season.