Epidemiology and Management of Bacterial Spot of Almond in California

Project No.: **PATH5**

Project Leader: **J.E. Adaskaveg, University of California, Riverside**

Project Cooperators and Personnel: H. Förster, K. Nguyen (UC Riverside), and S. Haack (FMC Corp), L. Wade (UPL), and R. Duncan, B. Holtz, and L. Milliron (UCCE Stanislaus, San Joaquin, and Butte/Glenn/Tehama Co., respectively)

A. Summary

Bacterial spot is present at many locations from Kern to Butte Co., especially on 'Fritz' almond. The pathogen was found to be genetically homogeneous and overwinters in fruit mummies and adjacent peduncle tissue on the tree. No twig cankers were found. The optimum temperature for growth for California strains is 25°C. Disease symptoms develop from 7 to 25 days depending on temperature. In general disease symptoms develop when daily high temperatures are above 25°C. This information is being used to develop a forecasting model for growers to optimize timing of bactericide treatments. In-season copper + mancozeb treatments at full bloom or petal fall reduced the disease to low levels.The most effective and consistent treatments were: copper, copper-mancozeb. OMRI-approved biologicals (e.g., Serenade, Blossom Protect) were also effective in reducing the disease; whereas the experimentals, Kasumin and Fireline/Mycoshield, were effective in mixture or rotation with mancozeb. New antimicrobials (nisin, poly-L-lysine) approved for food use by FDA are exciting new approaches to bacterial disease control. Kasumin was submitted by IR-4 for full registration to EPA by the registrant in the summer of 2019. The most effective management program for bacterial spot includes a late dormant application to reduce inoculum and one to two early in-season preventative tretaments applied before rainfall and warm temperatures. Kasumin was also shown to be effective against bacterial blast caused by *Pseudomonas syringae* in the last two years of this project. An Emergency registration - Section 18 was submitted to CDPR and was sent to the EPA requesting approval for spring of 2020. Applications of kasugamycin timed within a week of forecasted frost events will be an effective tool in managing bacterial blast of almond.

B. Objectives *(300 words max.)*

I) **Disease epidemiology** – (Adaskaveg, Förster, Nguyen, Haack)
- A. Pathogen identification and characterization of populations
- B. Identify minimum temperature for growth in selected wetness periods
- C. Field evaluation of temperature threshold under different wetness conditions and fruit stages

II) **Management of bacterial spot** – (Adaskaveg, Wade, Förster, Nguyen)
- A. In vitro sensitivity to new food grade biobactericides (e.g., nisin, poly-L-lysine, and activators)
- B. Field testing of dormant and in-season bactericides and biobactericides (EPA biopesticide classification): (coordinated field trials with Duncan, Holtz)
  - i. Kasugamycin, ZTD, and adjuvants
  - ii. Nisin, Ɛ-poly-L-lysine, and activators (biobactericides)
  - iii. Biological controls – Serenade ASO, Blossom Protect, and others

Table 1. Main Goal(s), key objectives, timelines and milestones

Objective(s)	Date to be accomplished	Milestones and deliverables associated to the objective
I A – Pathogen population	2019,2020	Population diversity potential
B - **M**in. temperature for growth	2019,2020	Min/Opti/Max Temperatures
C - **F**ield evaluations	2019-2022	Testing over several seasons
II**.** A - In vitro assays	2019, 2020	Demonstrate activity – 2019-20
B – Field testing –	2019-2021	Registration of Kasumin - 2020
i. Kasumin	2019-2021	Proof of concept – 2019-20
ii. Biobactericides	2022	Identify Registr. & submit to EPA
iii. Field testing-Biocontrols	2019-2021	Biocontrol guidelines
III. All	2022	Integrated Management Program

C. Annual Results and Discussion

Bacterial spot caused by *Xanthomonas arboricola* pv. *pruni* (*Xap*) continues to be a problem and has spread throughout the Central Valley of California (Butte, Colusa, Kern, San Joaquin, Merced, Madera, and Stanislaus, Co.). Wet springs are highly favorable for the disease, but it can develop throughout the growing season in orchards where foliage is frequently wet from dew, rain, or high-angle sprinklers. It is most serious on cv. Fritz, but can also be found on Nonpareil, Aldrich, Butte, Carmel, NePlus Ultra, and Price.

Outbreaks occurred in the spring of 2019 with high rainfall in May. No copper resistance in the pathogen populations was detected during annual surveys in 2015 to 2019. Molecular comparisons of strains collected over several years including 2019 showed little genetic diversity using REP, BOX, and ERIC PCR primers. Thus, bacterial spot of almond in California is caused mostly by a clonal population of Xap that may have originated from a single introduction of the pathogen. A homogeneous population may also suggest a recent introduction of the pathogen.

In the spring, the pathogen was again isolated from overwintering symptomatic fruit mummies, but also from healthy flower buds, as well as emerging leaves and spurs close to infected mummies in the tree. This confirmed that mummies are the major primary inoculum source. Spur isolations were also confirmed for a third year and represent the first recovery of *Xap* from a woody tissue in California, suggesting that infected spurs (and possibly cankers) may be an inoculum source after diseased mummies dehisce.

Field-inoculations of flowers, young fruitlets, and immature fruit of cv. Fritz were successful with symptoms developing when daily high temperatures warmed to above 25˚C. Recent studies reported on peach that at least three successive rainy days with average temperatures between 14 and 19˚C were necessary for primary infections of *X. arboricola* pv. *pruni* in Italy. Still, these temperatures are suboptimal for *X. arboricola* pv*. pruni* growth. Studies in Spain showed optimal growth temperatures of the pathogen between 25 and 30˚C. In our studies with two strains, the optimal temperature for growth was 25˚C. Disease incubation periods were shown to be between 7 and 25 days in warm and cold environments, respectively, with maximum disease severity on peach at 30˚C. The pathogen may also survive as an epiphyte. We demonstrated this by islation of the pathogen from healthy leaves and flowers within 25 cm of a diseased mummified fruit. Thus, our flower inoculations may not directly result in an infection at the time of inoculation but may allow survival of the pathogen until temperatures warm up during leaf and fruit wetness events. This information is being developed into a forecasting model for the disease that could potentially optimize disease management practices to minimize overuse of pesticides.

In-season treatments at full bloom (FB), petal fall (PF), or after petal fall (APF) on cv. Fritz significantly reduced the disease. Two treatments at FB and PF were most effective; whereas later APF applications were less effective. FB and PF applications applied in combination with a dormant application of copper had significantly lower disease than in-season treatments alone. The most effective and consistent treatments included copper (e.g., Badge X2, Champ, ChampION++, Kocide 3000) alone or mixed with mancozeb (Table 1). A manuscript submitted to Plant Disease was recently accepted that describes the optimal timing of dormant and in-season applications of copper and mancozeb for disease management. Other treatments also shown to be effective include kasugamycin mixed with mancozeb or copper; oxytetracycline, and selected experimentals (Fig. 1). Copper phytotoxicity was not observed on leaves in 2019 probably because of high rainfall that removed copper residues.

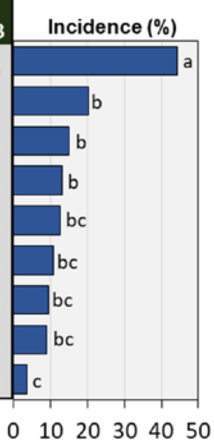
Table 1. Evaluation of dormant and in-season applications of copper-mancozeb for management of bacterial spot of almond in San Joaquinn Co.

In-season treatments *					Incidence of bacterial spot			
	Dates and rates				No dormant application		Dormant applic. Champ 6 lb, 2-6-19	
Treatment	FB 2-21	PF 3-14	LPF 4-3	LLPF 4-25	Dis.**	LSD^	Dis.	LSD
Control	---	---	---	---	60.7	a A	43.3	a A
Copper-Manz.	52/64 oz	---	---	---	32.0	ab A	17.4	bc A
Copper-Manz.	52/64 oz	26/64 oz	---	---	12.0	b A	6.9	c A
Copper-Manz.	---	26/64 oz	---	---	36.5	ab A	17.3	bc A
Copper-Manz.	---	---	13/38 oz	13/38 oz	25.5	b A	30.1	ab A
Copper-Manz.	---	---	---	13/38 oz	42.5	ab A	29.8	ab A
Dormant - No dormant average					33.7	A	24.1	B

* Applications made with an air-blast sprayer at 100 gal/A.
** Fruit were evaluated for bacterial spot.
^ Statistical comparisons by column: lower case letters, by row: upper case letters.

Fig. 1. Efficacy of new antibiotics and biologicals on 'Fritz' almond for management of bacterial spot of almond in San Joaquinn Co.

Treatment	Rate	Applications 3-7	3-19	4-3
Control	---	---	---	---
Kasumin 2L + Manzate	64 fl oz + 64 oz	@	@	@
FireLine + ZnNO3	16 oz + 64 fl oz	@	@	@
Kasumin 4L	32 fl oz	---	@	@
FireLine + LI700	16 oz + 16 fl oz	@	@	@
Mycoshield	16 oz	@	@	@
Mycoshield + LI700	16 oz + 16 fl oz	@	@	@
Serenade ASO + Manzate	96 fl oz + 64 oz	@	@	@
Champ + Manzate	32 - 16 - 8 + 64 oz	@	@	@

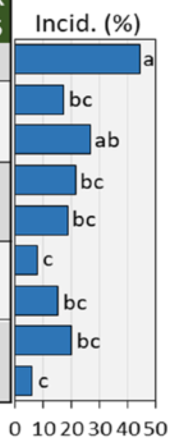


- Kasugamycin and oxytetracycline effectively reduced bacterial spot. None of the additives tested improved efficacy.
- Kasugamycin (Kasumin) is pending registration on almond.

Among organic products, Blossom Protect/buffer performed similar to Champ when applied at FB and PF, and Serenade ASO also significantly reduced the disease from the control (Fig. 2). Applications of copper, Blossom Protect, or Serenade that began at full bloom provided the lowest indence of bacterial spot on fruit. These represent options for organic growers. The experimentals nisin and Ɛ-poly-L-lysine were also effective and potentially represent a new strategy for managing bacterial diseases (Fig. 3). In vitro toxicity was highest with 99% reduction of survival after exposure to 500 ppm nisin mixed with 100 or 500 ppm EDTA for 30 min. For nisin and Ɛ-poly-L-lysine, two registrants have been identified that are interested in developing agricultural formulations of these food-grade bactericides and were are planning to evaluate these in the coming year.

Fig. 2. Efficacy of biologicals and copper applie as in-season treatments for managing bacterial spot on 'Fritz' almond in San Joaquin Co.

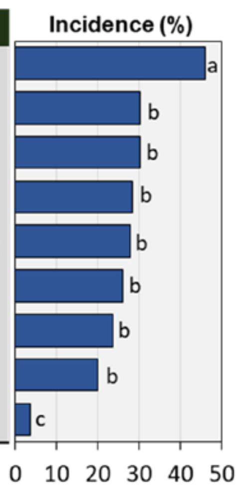
Treatment	Rate	Applications FB 2-20	PF 3-19	LPF 4-3	5 wk 4-25
Control	---	---	---	---	---
Serenade ASO + NuFilm-P	64 fl oz + 8 fl oz	@	---	@	---
		---	@	---	@
Serenade ASO + Nufilm-P + Sugar	64 fl oz + 8 fl oz + 32 oz	@	---	@	---
		---	@	---	@
Blossom Protect + buffer	20 oz + 80 oz	@	---	@	---
		---	@	---	@
Champ	32 - 16 oz	@	---	@	---
		---	@	---	@



- Copper rate was reduced by half with each subsequent application.
- Serenade and Blossom Protect significantly reduced bacterial spot and were sometimes similarly effective as copper. No consistent timing responses.

Fig. 3. Novel 'exempt from tolerance', FDA-approved bactericides evaluated on 'Fritz' almond in San Joaquin Co.

Treatment	Rate
Control	---
Nisin + ZnO + ZnNO3	13.5 oz + 32 oz + 64 fl oz
ε-Poly-L-lysine	3.5 oz
ε-Poly-L-lysine + EDTA	13.5 oz + 500 ppm
Nisin + EDTA + Ca(OH)2	13.5 oz + 500 ppm + 12 oz
Nisin + EDTA	13.5 oz + 500 ppm
ε-Poly-L-lysine	13.5 oz
Nisin	13.5 oz
Champ	32 oz - 16 oz - 8 oz

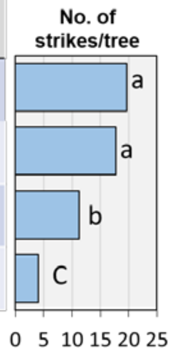


Food-grade anti-bacterial products such as nisin and ε-poly-L-lysine (food preservative formulations) significantly reduced bacterial spot, but their performance still needs to be improved with agricultural formulations.

Based on our results from several years of field studies, in wet winter/spring seasons, a delayed dormant bactericide application to reduce inoculum should be followed by bloom and petal fall treatments around rainfall events and rising temperatures to prevent new infections. Bloom applications with copper cause minimal phytotoxicity. In drier spring seasons, only a dormant treatment or bloom/petal fall applications may be necessary for effective disease management. Based on an IR-4 residue project that we identified and supported, kasugamycin is pending full registration on almond for bacterial spot management. Integration of different compounds should reduce the potential of resistance to any one mode of action and overuse of copper that may cause phytotoxicity.

Fig. 4. Management of bacterial blast in a field trial on ‘Independence’ in Stanislaus Co.

Treatment	Rate/A
Control	---
Mankocide	5 lb
Nisin + EDTA	500 ppm + 8 oz
Kasumin 2L	64 fl oz



- Applications on 2-20-19 (pink bud to 30% bloom) prior to a forecasted frost event.
- Evaluation on 3-7-19 showed high efficacy of kasugamycin.
- No phytotoxicity was observed in any of the treatments.
- An emergency registration was requested for kasugamycin for the spring of 2020 to help manage bacterial blast on almond.

Because of the cold wet environmental conditions duing almond bloom in 2019, we conducted additional trials with kasugamycin for the management of another bacterial disease, bacterial blast of almond. A trials was established in Stanislaus Co. on cv. Independence. A single application of kausgamycin just prior to a forecasted frost event was shown to be highly effective in managing the disease by reducing the number of diseased spurs from 20% to less than 5% (Fig. 4). Similar data was obtained in 2018 as well. Over the course of the summer and fall crop loss data was compiled and an emergency registration petition was submitted for kasugamycin use in the spring of 2020. Thus, this project is demonstrating additional benefits for developing bactericides for the almond industry. Kasugamycin usage in the spring will have zero residues at harvest. The bactericide is a natural fermentation product and belongs to its own FRAC Code 24 indicating a unique mode of action from other fungicides and bactericides. Furthermore, kasugamycin is exclusively used in plant agriculture with no animal or human usage.

This project is accomplishing all objectives and is on track to develop and register new bactericides for not only bacterial spot but also for bacterial blast caused by *Pseudomonas syringae*. This research project can have far reaching benefits to the industry if all objectives including the new bactericides that have been identified could be developed into agricultural formulations and registered as biopesticides. The idneitfication of Blossom Protect and Serenade will also benefit organic gorwers. Integrated bacterial disease management projects could be developed for the first time for the almond industry for endemic bacterial diseases, as well as potential invasive bacterial diseases in the future.

D. Outreach Activities

Dr. Adaskaveg participated at several grower/PCA meetings at different locations in California over the year and gave presentations on almond diseases including the epidemiology of bacterial spot and bacterial blast and their management. At each meeting, there were approximately 50-100 participants.

1) January 2019, Managing Diseases of Almond in California; Bayer Tree, Nut, and Vine Meeting; Organizer: Bayer CropScience; Universal Studios, Universal City, CA 91608

2) January 2019, Almond Diseases: Key Economic Pests, ID, Biology, and Treatments in Almonds; 2019 Independent PCA Symposium; Organizer: Bayer CropScience; Monterey Plaza Hotel and Spa, Monterey, CA 93940
3) January 2019, Almond Disease Management, Colusa Winter Almond Meeting; Organizer: UCCE; Granzella's Banquet Hall, Williams, CA
4) Feb 5, 2019, Bloom and Foliar Diseases; Annual Almond Production Meeting; Organizer: UCCE; Norton Hall, Woodland, CA
5) Nov 8, 2019, Foliage, Blossom and Nut Diseases; Almond Short Course, Organizer UCCE; Visalia Convention Center, Visalia, CA

E. Materials and Methods *(500 word max.)***:**

I. Genetic variability of collected strains of the bacterial spot pathogen. 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). 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*). Sites included Stanislaus Co. and Solano Co. High inoculum concentrations of ca. 1 x 10^7 cfu/ml (OD_{600} 80% transmission) were applied using a hand-sprayer to run-off. Inoculum was prepared in water. Treated branches were covered with white plastic bags sprayed with water for 12-18 h, and inoculations were evaluated for disease weekly. Eight replications per treatment per inoculation date were used.

Disease evaluation. Ratings were conducted as the disease developed during the spring season. 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 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 statistical methods.

Pathogen survival. To determine pathogen survival sites over the winter and inoculum sources in the spring, isolations were done from symptomatic overwintering fruit mummies, peduncles (spurs), 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 growth and sensitivity of *Xap* against copper, mancozeb, antibiotics, and biologicals. For determination of growth at selected temperatures, three replications of two isolates were used at six temperatures (10, 15, 20, 25, 30, 35°C). In vitro sensitivity of isolates against bactericides, was evaluated using direct contact and serial dilution plating with selected concentrations was done, and copper mixture evaluations were conducted on 10 ppm MCE. For each chemical or combination treatment, three replications were used and the experiment was repeated.

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 delayed-dormant treatments, Champ was applied in early February. Copper (Champ)-mancozeb was also applied in mixtures for in-season applications.

In-season applications were initiated at bloom and continued as petal fall applications prior to rain events in the spring. Treatments included copper products, antibiotics (kasugamycin, oxytetracycline), and biological controls such as Serenade ASO (*Bacillus subtilis* strain QST 713), or Blossom Protect (*Aureobasidium pullulans*). Biological controls were also applied in a mixture with a sugar or buffer solution to increase growth because this benefited activity in some of our previous trials. Multiple applications were applied. Copper (e.g., Champ) rates were decreased from 1, to 0.5, to 0.25, and to 0.2 lb MCE/A for each application timing. Bactericide 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 late spring and the incidence was calculated based on the number of diseased fruit of the total number of fruit evaluated.

F. Publications that emerged from this work

1. Haack, S. E., Wade, M. L. Forster, H., and Adaskaveg, J. E. 2020. Epidemiology and Management of Bacterial Spot of Almond Caused by *Xanthomonas arboricola* pv. *pruni*, a New Disease in California. Plant Disease, in press
2. UCANR - Fungicide Efficacy Tables, Almond Pest Guidelines for foliar diseases of almond.
3. Section 18 for Kasugamycin for managing bacterial blast on almonds, 290 pp. Submitted Nov. 2019
4. a) https://www2.ipm.ucanr.edu/agriculture/almond/
   b) http://ipm.ucanr.edu/PDF/PMG/fungicideefficacytiming.pdf