Department of Plant Pathology
Relevant AES/CE Project No.: 11103864

University of California
Division of Agricultural Sciences
ANNUAL RESEARCH PROGRESS REPORT
California Olive Committee/California Olive Oil Commission
Project Year: 2017

Project Leader:
Dr. J. E. Adaskaveg, Professor
Department of Plant Pathology
University of California, Riverside
Riverside, CA 92521
(O) 951-827-3880 FAX: 951-827-7577 (M) 951-288-9312
jim.adaskaveg@ucr.edu

Title: Epidemiology and management of olive knot caused by *Pseudomonas savastanoi* pv. *savastanoi (Psv)*

Introduction

Olive knot caused by the bacterium *Pseudomonas savastanoi pv. savastanoi* (Psv) is a serious disease of olives (*Olea europaea*) worldwide (7). The pathogen enters through wounds causing outgrowths (knots, tumors, galls) on branches and twigs, and infrequently on leaves and fruit. Olive knot is one of the most economically important diseases of olives as infection may lead to tree defoliation, dieback, and reduced tree vigor, which ultimately lowers fruit yield and quality (5). *Psv* can survive epiphytically on olives, but the main sources of inoculum are bacteria living within knots (6). Large quantities of bacterial ooze can be exuded upon wetting knots. This exudate is disseminated by rain, wind, insects, birds, as well as human activity. The opportunistic pathogen takes advantage of wounds caused by natural leaf abscission (3), frost, hail, as well as cultural practices such as pruning and harvesting. These latter practices also lead to direct mechanical damage of the knots, exposing and spreading inoculum to healthy tissue. In California, infections occur mostly during the rainy season (late fall, winter, and spring) but knots do not develop until new growth starts in the spring. Infections can occur at low temperatures (-5°C) and thus, wetness is the main limiting factor for the disease. Historically, the most susceptible olive cultivars are Manzanillo, Sevillano, Ascolano, and Mission. None of the currently grown cultivars is resistant to the pathogen (4).

Control of olive knot is difficult, and growers rely on applications of copper-based bactericides as the only effective foliar treatment. Reliance on a single active ingredient has led to our detection of copper resistance in *Psv* strains in two olive orchards. These strains were highly virulent when inoculated to Arbequina and Manzanillo olive wounds, and application of copper provided reduced control as compared to inoculation with a sensitive strain. Still, the incidence of copper resistance is very low, however, the occurrence of resistance necessitates the development of alternatives.

We have been instrumental in the development of the new agricultural antibiotic kasugamycin (commercial name Kasumin) for several bacterial diseases of agronomic crops in the United States. Kasugamycin has high activity against *Erwinia* and *Pseudomonas* (2) and moderate activity against *Xanthomonas* species and other plant pathogenic bacteria. We found it to be the most promising new treatment for preventing olive knot in our extensive field studies. Registration of kasugamycin through the IR-4 project is expected in 2020. Kasugamycin would greatly complement current copper sprays and could be used in rotation or mixtures with copper. New antibiotic registrations, however, find little acceptance with regulatory agencies, and we are currently in discussion with EPA to develop a science-based approach on the use of antibiotics in plant agriculture.

In addition to developing conventional chemical compounds, research on alternative materials such as SDH, biopesticides, and food additives may provide new modes of action for managing olive knot. Salicylidene benzoylhydrazone (SBH) was recently discovered to display synergism when combined with copper. We performed preliminary tests with a derivative of this molecule with very promising results using several genera of phytopathogenic bacteria including *Psv*. Low concentrations of metallic copper combined with SBH were highly inhibitory in vitro against a copper-resistant *Psv* strain, while copper or SBH by themselves at the same concentrations were not effective. Field trials will be necessary to support these initial in vitro findings.

Biopesticides such as Serenade and several food additives that are 'generally recognized as safe' (GRAS) have antimicrobial properties. They are often naturally produced molecules of gram-positive *Streptomyces* species. Although these compounds are typically applied to food products as preservatives, they may have potential for controlling plant diseases when applied as a foliar treatment. Integration of these alternative materials with conventional treatments may improve disease control, reduce the risk of resistance development, and provide olive growers with more resources for managing olive knot. Therefore, we evaluated nisin, epsilon-poly-L-lysine, and lactic acid.

Objectives

1) **Develop novel chemicals to improve performance of copper-based bactericides against *Psv***
   a) In-vitro sensitivity of *Psv* to copper in the presence of SBH (and potential derivatives) using selected copper/SBH ratios.
   b) Efficacy of copper-SBH mixtures for the management of olive knot caused by copper-sensitive and -resistant strains of *Psv* in field studies.
      i) Evaluate selected copper/SBH ratios with the goal to minimize the amount of copper applied while maintaining good disease control.

2) **Evaluate a biopesticide and several food additives for the control of olive knot**
   a) Determine the efficacy of the bio-pesticide Serenade (*Bacillus subtilis* strain QST 713) in field studies for the management of olive knot.
   b) Determine the efficacy of the GRAS food additives nisin, epsilon-poly-L-lysine, and lactic acid in field studies for the management of olive knot.

Materials and methods

1) Develop novel chemicals to improve performance of copper-based bactericides against *Psv*.

1a. To evaluate the toxicity of copper-SBH mixtures against *Psv*, a dilution plate method was combined with the spiral gradient endpoint (SGE) method. Agar media were amended with fixed concentrations of copper. Subsequently, SBH was spiraled onto the copper-amended plates producing a SBH concentration gradient in combination with a fixed copper concentration. Suspensions of *Psv* strains were streaked onto the amended media. This allowed the determination of minimal inhibitory values for *Psv* at different ratios of copper and SBH. These data were used to calculate appropriate field rates.

1b. Copper-SBH mixtures were tested in the field on Arbequina and Manzanillo olives at UC Davis. Plants were wounded with lateral and leaf scar wounds. Lateral wounds on 1-2-year-old twigs were made using a scalpel by removing the bark and exposing cambial tissue. Leaf scars were made by pulling leaves off the same twigs. Additionally, a natural leaf scar study was done in the spring when leaves naturally fall. Copper and copper-SBH treatments were sprayed onto wounds before inoculation with a suspension of copper-sensitive or -resistant *Psv* strains. The efficacy of treatments was assessed as the incidence of knots forming on treated, inoculated wounds as compared to wounds that were treated with water and inoculated (i.e., control). SBH was applied using rates based on the laboratory tests and also at the equivalent rate of mancozeb (1.8 lb a.i./A or 2.4 lb/A Manzate Prostick). Copper was also used at different rates.

1c. Similar in vitro and efficacy studies for zinc thiadiazole as copper-SBH evaluations in 1a and 1b.

2) Evaluate a biopesticide and several food additives for the control of olive knot

2a and b. Field tests were conducted on Arbequina and Manzanillo olives to evaluate the efficacy of Serenade, nisin, epsilon-poly-L-lysine, and lactic acid against *Psv*. The same wounding, treatment, and inoculation procedures were used as described above.

3) Continue to support the registration of the antibiotics kasugamycin and oxytetracycline (newly added) - UV-blockers and stabilizers are being evaluated to improve the performance of antibiotics and copper. For this, Raynox Plus (Valent USA) at a rate of 5% and similar products that prevent sun damage by blocking UV light are being used with both antibiotics. Oxytetracycline is especially vulnerable to UV-degradation and new adjuvants (e.g., Tactic) are being tested.

Additionally, an inter-commodity and industry group is working with the Minor Crop Farmer Alliance to recommend an EPA policy change towards the use of antibiotics in plant agriculture. Specifically, a new internal EPA Guidance Document (GD) for use of antibiotics in plant agriculture needs to be developed based on science. Historically, EPA GD 152 for registration of antibiotics in animal husbandry is used for all requests in agriculture.

Results and Discussion

1a. In-vitro sensitivity of* Psv *to copper in the presence of SBH (and potential derivatives). Eight Cu-sensitive (growing at ≤25 mg/L MCE) or -resistant (growing at ≥50 mg/L MCE) *Psv* strains were exposed to a SBH concentration gradient from 0.3 to 31 mg/L in absence of presence of 10, 25, or 50 mg/L metallic copper equivalent (MCE). SBH by itself was not inhibitory at ≤31 mg/L to any of the strains. When the SBH gradient was combined with 10 mg/L MCE, inhibition was observed for all strains. The range of minimal inhibitory concentrations (concentration that reduces bacterial growth by ≥95%) for SBH against *Psv* was between 1.4 and 4.7 mg/L in the presence of 10 mg/L MCE. Using 25 mg/L or 50 mg/L MCE in combination with SBH, no growth was observed for all copper-sensitive and moderately-resistant strains. Inhibition against highly copper-resistant strains, however, was not greatly improved as compared to using 10 mg/L MCE.

1b. Efficacy of copper-SBH mixtures for the management of olive knot caused by copper-sensitive and -resistant strains of* Psv *in field studies. Field studies were initiated in the spring of 2017 on two olive cultivars in experimental or commercial plantings, and treatments are shown in Tables 1 and 2. DAS 1 and DAS 2 are SBH derivatives. DAS 2 is pre-formulated and includes copper. ZTD is a derivative of amino-thiadiazole (ATD) containing zinc that we previously tested in-vitro and that enhanced the efficacy of copper. On leaf scars, results indicate that copper-ZTD mixtures reduced disease incidence caused by a copper-sensitive strain from that of the control and copper alone. On lateral wounds, however, this treatment performed similarly to copper in reducing olive knot.

In the second trial in a commercial orchard, kasugamycin and kasugamycin-copper mixtures resulted in the lowest disease on lateral and leaf scar wounds with a >90% reduction in incidence. The mixtures of copper with ZTD or DAS-1 did not improve the performance of copper alone. Although registered in China, ZTD was rejected by the EPA as a biopesticide, and EPA indicated to the registrant that full toxicological and environmental persistence evaluations would need to be done. The registrant stated that this makes registration in the United States economically prohibitive. In contrast, the registrant of DAS-1 and -2 (the SBH products) is willing to test additional derivatives in the coming year.

In additional trials using natural leaf scars, copper and kasugamycin were highly effective reducing disease incidence by >95% when using a copper sensitive strain for inoculation (Table 3). The natural leaf scar as opposed to removing leaves by hand most likely had a natural abscission zone that helped to prevent bacterial ingress.

2a. Determine the efficacy of the bio-pesticide Serenade (*Bacillus subtilis *strain QST 713) in field studies. Serenade and Serenade-copper mixtures were not effective at the rates evaluated in reducing olive knot as compared to the non-treated control (Table 1).

2b. Determine the efficacy of the GRAS food additives nisin, epsilon-poly-L-lysine, and lactic acid in field studies. On leaf scars, lactic acid reduced disease incidence caused by a copper-sensitive strain from that of the control and copper alone. On lateral wounds, however, this treatment was less effective but was statistically similar to copper in reducing olive knot. Using a copper-resistant strain, lysine, nisin, and lactic acid had the lowest disease incidence on leaf scars. On lateral wounds, these treatments were similar in their performance to copper.

3) Continue to support the registration of the antibiotics kasugamycin and oxytetracycline (newly added) - UV-blockers/stabilizers and registrant-recommended adjuvants (e.g., Tactic) are currently being evaluated to improve the performance of antibiotics and copper. This research is ongoing.

Table 1. Evaluation of potential new bactericides for management of olive knot in a UC Davis experimental orchard

		Incidence of knot formation (%)							
	Product	cv. Manzanillo inocul. with Cu-sensitive strain				cv. Arbequina inocul. with Cu-resistant strain			
Treatment	Rate/A	Lateral wounds	LSD	Leaf scars	LSD	Lateral wounds	LSD	Leaf scars	LSD
Untreated	---	82.5	abc	92.5	abc	90	ab	85	abc
DAS 2	128 fl oz	97.5	a	92.5	ab	100.0	a	92.5	ab
ChampION+ DAS 1	2 lb + 64 fl oz	62.9	cde	85.7	abcd	100.0	a	90.0	abc
ChampION + SBH*	2 lb + 24 oz	72.5	bcd	67.5	defg	97.5	a	87.5	abc
Serenade Opti	20 oz	95.0	a	97.5	a	95.0	a	100.0	a
ChampION + SBH*	3.5 lb + 24 oz	35.0	efg	47.5	fghi	92.5	ab	92.5	ab
Serenade Opti + ChampION	20 oz + 3.5 lb	95.0	a	97.5	a	90.0	ab	95.0	ab
ChampION+ DAS 1	3.5 lb + 64 fl oz	11.3	h	45.0	ghi	92.5	ab	92.5	ab
ChampION + ZTD	2 lb + 500 ppm	45.0	def	57.5	efgh	90.0	ab	85.0	abc
DAS 2	64 fl oz	97.5	a	77.5	bcde	87.5	ab	82.5	abc
ChampION + Manzate	2 lb + 2.4 lb	22.5	gh	57.5	efgh	87.5	ab	97.5	ab
Nisin	1%	95.0	ab	75.0	def	87.5	ab	70.0	cde
ChampION	2 lb	60.0	cd	75.0	cdef	77.5	bc	82.5	abc
ChampION + Manzate	3.5 lb + 2.4 lb	12.5	gh	35.0	ij	75.0	bc	80.0	bcd
Lactic Acid	1%	30.0	fgh	27.5	ij	75.0	bcd	65.0	de
ChampION + ZTD	3.5 lb + 500 ppm	10.0	h	20.0	j	67.5	cd	82.5	bcd
Lysine	1%	77.5	abc	75.0	bcde	56.7	cd	56.7	e
ChampION	3.5 lb	10.0	h	37.5	hij	55.0	d	87.5	abc

[1]- Treatments were applied to leaf scar and lateral wounds of using a hand-held sprayer until runoff, allowed to dry, and wounds were inoculated with a copper-sensitive or -resistant *Psv* strain at 10^7 CFU/ml. A total of 50 leaf scar wounds and 50 lateral wounds were used for each treatment. The field study was done as a randomized complete block design and included an untreated-inoculated control.

Table 2. Evaluation of potential new bactericides for management of olive knot in a commercial olive orchard in Yuba City

	Product	% Incidence of knots on treated wounds			
Treatment	Rate (/A)	Lateral wounds	LSD	Leaf Scars	LSD
Untreated	---	74	a	62	a
DAS 2	64 oz	76	a	56	a
ChampION+ DAS 1 high	3.5 lbs + 128 oz	30	b	26	b
ChampION + ZTD	3.5 lbs + 500 ppm	20	b	10	c
ChampION + DAS 1 low	3.5 lbs + 64 oz	18	bc	10	c
ChampION	3.5 lbs	18	bc	6	c
Kasumin	200 ppm	2	cd	6	c
ChampION + Kasumin	3.5 lbs + 200 ppm	0	d	0	c

[1]-Treatments were applied to leaf scar and lateral wounds of Arbequina olive using a hand-held sprayer until runoff, allowed to dry, and wounds were inoculated with a copper-sensitive *Psv* strain at 10^7 CFU/ml. A total of 50 leaf scar wounds and 50 lateral wounds were used for each treatment. The field study was done as a randomized complete block design and included an untreated-inoculated control.

Table 3. Management of olive knot on natural leaf scars using new bactericides

Location	Treatment	Product Rate/A	% Incidence of knots on natural leaf scar wounds*	LSD
UC Davis	Untreated	---	39.4	a
	ChampION	3.5 lbs	0.0	b
	Kasumin	200 ppm	3.8	b
	ChampION + Kasumin	3.5 lbs + 200 ppm	0.0	b
Commercial orchard	Untreated	---	31.1	a
	ChampION	3.5 lbs	3.0	b
	Kasumin	200 ppm	0.0	b
	ChampION + Kasumin	3.5 lbs + 200 ppm	0.0	b

*- Incidence of knots occurring on natural leaf scar wounds made by removing yellow-dying leaves and inoculating the leaf scar after treatment. Experiments done during natural leaf drop in the spring.

A new suggested EPA Guidance Document (GD) for use of antibiotics in plant agriculture was submitted to the EPA through MCFA. Historically, EPA GD 152 for registration of antibiotics in animal husbandry is used for all requests in agriculture. USDA and EPA officials are addressing all uses of antibiotics currently under a CODEX workgroup. Goals including classifying plant agricultural uses as low potential sources of non-target human bacterial pathogen resistance as opposed to animal and human uses.

Benefits to the industry

For management of olive knot, in addition to cultural and sanitation practices, copper materials are currently the only effective treatments. With the detection of low levels of copper resistance in olive knot pathogen populations in California, alternatives are needed for a sustainable and effective management program. We initiated the registration of the new agricultural antibiotic kasugamycin and oxytetracycline for olive knot management through the IR-4 program. Still, new bactericidal products are being evaluated including SBH and other antibacterial food preservatives that potentially can be registered. The registration of several materials will allow the implementation of anti-resistance strategies and will prevent over-use of any single mode of action bactericide. Still, integrated practices will be critical for the successful management of the disease.

Supplemental efforts in 2017.

1) We published one Plant Disease article on sanitizing field equipment using quaternary ammonium (1).
2) We published a second manuscript in Plant Disease on the efficacy of copper and new bactericides for managing olive knot in California (2).
3) A third manuscript on the epidemiology of olive knot has been submitted to Plant Disease.

References

1. Nguyen, K. A., Förster, H., and Adaskaveg, J. E. 2017. Quaternary ammonium compounds as new sanitizers for reducing the spread of the olive knot pathogen on orchard equipment. Plant Dis. 101: 1188-1193.
2. Nguyen, K. A., Förster, H., and Adaskaveg, J. E. 2018. Efficacy of copper and new bactericides for managing olive knot in California. Plant Dis. 102: In Press.
3. Hewitt, W. B. 1939. Leaf scar infection in relation to the olive knot disease. Hilgardia 12:41-66.
4. Penyalver, R., García, A., Ferrer, A., Bertolini, E., Quesada, J.M., Salcedo, C.I., Piquer, J., Pérez-Panadés, J., Carbonell, E.A., del Río, C., Caballero, J.M., López, M.M. 2006. Factors affecting *Pseudomonas savastanoi* pv. *savastanoi* plant inoculations and their use for evaluation of olive cultivar susceptibility. Phytopathology 96:313–319.
5. Schroth, M.N., 1973. Quantitative assessment of the effect of the olive knot disease on olive yield and quality. Phytopathology 63:1064.
6. Wilson, E. E. 1935. The olive knot disease: Its inception, development, and control. Hilgardia 9:233-264.
7. Young, J.M. 2004. Olive knot and its pathogens. Australasian Plant Pathology 33:33–39.