Epidemiology and Control of Almond Scab and Alternaria Leaf Spot

Project No.: **13-PATH3-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: D. Thompson, H. Förster, Y. Luo, D. Cary, UC Riverside
J. Connell, UCCE - Butte County
R. Buchner, UCCE - Tehama County
Craig Kallsen, UCCE - Kern County

Objectives:

I. Etiology
   A. Identify pathogenic species of *Alternaria* using molecular methods.
   B. Determine the *Alternaria* species composition within selected orchards. This objective is contingent on the development of molecular methods for identification of the pathogens.
   C. Determine the *F. carpophilum* population composition within selected orchards and determine if sexual reproduction occurs within orchard populations using molecular methods.

II. Management
   A. Evaluate new and registered fungicides for their efficacy in managing scab, Alternaria leaf spot, and leaf blight. Fungicides to be evaluated by themselves or as components of pre-mixtures include fluopyram - Luna Privilege, fluxapyroxad - Xemium, penthiopyrad - Fontelis, difenoconazole - Inspire, metconazole - Quash, polyoxin-D - Ph-D, Oso, chlorothalonil - Bravo, and dodine - Syllit.
      i. Single-fungicide programs
      ii. Rotation programs of different fungicide chemistries
   B. For scab management, evaluate the effect of dormant applications (Bravo, Manzate, or new formulations of copper used with oil) on sporulation of twig lesions, as well as registered (Bravo, Manzate/Dithane, Ziram) and new fungicides (see above) for in-season use. (Focus on Bravo for extended springtime usage for disease control - i.e., 60 day PHI as the fungicide moves through the IR-4 program for re-registration on almond).
   C. Establish and expand baseline sensitivities and monitor for shifts in sensitivity in populations of *Alternaria* and *Fusicladium* spp. to sub-groups of the SDHIs: pyridine-carboxamides (boscalid), pyrazole-carboxamide (fluxapyroxad), and pyridinyl-ethyl-benzamides (fluxopyroxad), DMIs, polyoxin-D, and QoIs.
      i. Continue to characterize molecular mechanisms for SDHI resistance
   D. Develop and use a modified DSV model based on dew point (instead of leaf wetness) and temperature (i.e., onset of dew formation and rising temperatures during mid-spring) with a goal for county-wide forecasts for the first in-season fungicide application. Time additional applications in 2- to 3- week intervals under low-rainfall conditions.

Interpretive Summary (Note - This report is mainly based on our 2013 data because our 2014 project is ongoing). Scab (caused by *Fusicladium carpophilum;* formerly *Cladosporium carpophilum*) and Alternaria leaf spot (caused by three very closely related species in the *Alternaria alternata* complex) have become of increasing importance in many growing areas in California. In 2013, Alternaria twig infections were identified for the first time. Thus, both diseases include twig infections. Scab lesions, however, occur on many susceptible cultivars; whereas we found Alternaria twig lesions only on cv. Wood Colony at this time. Both diseases are summer diseases that especially occur in locations with high humidity and where air circulation is poor, such as in high-density plantings or in orchards with soils with inadequate drainage or where trees require frequent and extended irrigations throughout the summer. In 2012 and 2013, we evaluated if daily temperatures, daily incidence of dew periods, and daily precipitation can be used as indicators for determining initiation times of fungicide applications for Alternaria leaf spot. These efforts were continued in 2014. Results were inconclusive and thus, either the DSV model or a calendar-based timing that begins in May into late June/early July in approximately three-week intervals are currently recommended for use.

We continued to collaborate with growers, the agrochemical industry, and regulatory agencies to develop and design sustainable treatment programs where several classes of fungicides are mixed or rotated, so that no single class is over-used. Our research on both diseases demonstrates that in the presence of QoI resistance, effective disease and resistance management can be obtained with properly timed applications of currently registered fungicides belonging to three or four FRAC groups. For Alternaria leaf spot, polyoxin-D (Ph-D), several FRAC 3 fungicides (e.g., Quash, Inspire), as well as new pre-mixtures (e.g., Luna Sensation, Luna Experience, Quadris Top, Inspire Super, Merivon) and mixtures (e.g., Ph-D/Quash) are among the most effective treatments. In the management of scab, as in previous years, dormant treatments with chlorothalonil (e.g., Bravo)/oil had a long-lasting effect on the suppression of twig sporulation. This results in a shift to later spring-time application timings (until May-June), resulting in scab timings to coincide with application timings for Alternaria leaf spot and possibly other summer diseases such as rust and hull rot. Effective spring-time fungicides (two applications starting at the onset of twig sporulation) are chlorothalonil (proposed label change to 60 days PHI), FRAC 3 fungicides such as Quash or Inspire Super, Syllit (FRAC U12), compounds containing FRAC 7, FRAC 11 (at locations where the pathogen population has not developed resistance), or Ph-D (FRAC 19). For scab management under high-disease conditions, a three-spray program should include dormant applications with chlorothalonil-oil (or copper-oil) and two petal-fall applications. Under lower disease pressure, a dormant treatment or in-season treatments alone can be considered.

Laboratory evaluations on sensitivity of *Alternaria* and *Fusicladium* spp. to SDHI (FRAC 7) fungicides had revealed several cross-resistance groups among SDHI sub-groups of fungicides. Thus, in field use, in some cases rotations between certain sub-groups could provide a resistance management strategy. Because genotypes of pathogen populations at specific locations, however, are generally not known and genotype composition may change over time, rotation between FRAC groups and not SDHI subgroups is recommended. In *Alternaria* spp., some of the cross-resistance groups correlated with certain mutations in two of the three sub-units of the target gene evaluated. Sequence analysis for isolates of *F. carpophilum* is ongoing. Many isolates of this pathogen are insensitive to SDHI fungicides.

Materials and Methods:

Etiology of scab and Alternaria leaf spot and population structure of scab. The population structure of *Fusicladium carpophilum* is being studied using 30-40 isolates from each of three orchards in different geographic locations. This was done to possibly obtain information on whether the pathogen is sexually reproducing at any of these locations. Fungal populations were evaluated by Amplified Fragment Length Polymorphism (AFLP) analyses and fragment patterns were scored on computer-generated gels. A most parsimonious tree was generated from the AFLP dataset using PAUP (ver. 4.0(Phylogenetic Analysis Using Parsimony)). The Parsimony Tree Length Permutation Test (PTLPT) method was applied to determine if recombination occurred in each population. In collaboration with peach scab researchers, we also compared populations of *Fusicladium* from peach and almond. For this, we used random amplified polymorphic DNA (RAPD) and universally primed polymerase chain reactions (UP-PCR) to identify molecular differences in the two host populations of the fungus.

Fungicide evaluations for management of scab in 2013 and 2014. Data for evaluation of dormant treatments are presented for 2014 and for in-season treatments for 2013. Dormant treatments were applied in a commercial cv. Carmel orchard in Butte Co. in January 2014. Treatments included Bravo WeatherStik (6 pts/A)/3.5% oil, Manzate ProStick 6 lb/3.5% oil, and Captan WDG 5 lb/3.5% oil. Samples of last fall's twigs growth were collected on April 23, 2014, and evaluated in the laboratory for sporulation of overwintering scab lesions. Sporulation was expressed as incidence of sporulating lesions and as severity using a rating scale with 0=no sporulation, 1=very little sporulation, 2=lesion partially covered with sporulation, and 3=lesion completely covered with sporulation or a full concentric ring of sporulation.

In-season treatments were initiated after petal fall (after the onset of twig sporulation) at two locations in Colusa on cv. Monterey. Fungicides used in two applications for each plot included Rhyme (FRAC 3), isopyrazam (FRAC 7), Ph-D (FRAC 19), Syllit (FRAC U12), the pre-mixtures Luna Sensation (FRAC 7/11), Luna Experience (FRAC 3/7), Merivon (FRAC 7/11), and Custodia (FRAC 3/11), the mixtures isopyrazam + Propimax (FRAC 7+3), isopyrazam + Abound (FRAC 7+11), Ph-D + Quash (FRAC 19+3), Syllit + Tebucon or Propimax (FRAC U12+3), and Captan Gold + Custodia (FRAC M4+3/11), and several rotation programs: Bravo (FRAC M5) – Quadris Top (FRAC 3/11), Bravo (FRAC M5) – Inspire Super (FRAC 3/9), and Catamaran (FRAC M5/33) – Viathon (FRAC 3/33). Disease was evaluated on July 16, 2013, based on incidence of fruit with scab lesions and on the number of lesions per fruit (disease severity).

Fungicide evaluations for management of Alternaria leaf spot of almond in 2013. The modified DSV model was used to determine initiation times of spray programs. Two trials were established in Kern Co. (cvs. Monterey and Fritz) and two trials in Colusa Co. (cvs. Carmel and Monterey). Each site received three applications between May 8 and June 30 (Kern Co.) or between May 22 and July 16 (Colusa Co.). Treatments included ARY 0951-001 (a natural product), isopyrazam, Rhyme, Ph-D, the pre-mixtures Custodia, Luna Sensation, Luna Experience, and Merivon, the mixtures Captan Gold + Custodia, isopyrazam + Abound, and Fontelis (FRAC 7) + Tebucon (FRAC 3), as well as several rotation programs. Evaluations were done July 31 in Kern Co., and August 21, 2013, in Colusa Co. For disease incidence 30-40 leaves per single-tree replication were evaluated for the presence of disease. For disease severity (lesions/leaf), a rating was used with a scale from 0 to 4. Trees were also rated for defoliation based on a scale from 0 (= no defoliation) to 4 (= more than 75% of the leaves fallen). Trials were repeated in 2013.

In vitro sensitivity of *Fusicladium carpophilum* and *Alternaria* spp. to selected SDHI fungicides and molecular mechanisms of resistance – 2013 and 2014 Research. Isolates from orchards in Kern, Butte, Colusa, and Stanislaus Co. that were evaluated for their sensitivity against several SDHI fungicides previously were grouped based on their inhibition by boscalid, fluopyram, fluxapyroxad, and penthiopyrad. For *Alternaria* spp., regions of the succinate dehydrogenase gene that were described to contain mutations determining resistance were sequenced and sequences were aligned to identify mutations.

Development of a modified DSV model for initiating Alternaria leaf spot management practices based on precipitation, dew point (instead of leaf wetness) and threshold temperatures during the spring season. In the last several years, wide ranges of environmental factors have been observed in our Alternaria management plots in southern and northern California that resulted in different levels of observed disease. Optimal timing for the initiation of fungicide applications and of subsequent applications is critical for obtaining high levels of disease management. Over the course of this project we adapted the DSV model to almond and it has been effective for forecasting Alternaria leaf spot of almond. To refine the usefulness of microclimate data, we continued to evaluate temperatures (mean, min/max), daily incidence of dew periods (based on temperatures below dew point), and daily precipitation as indicators of wetness periods at temperatures conducive for disease based on the DSV model. Regional and site-specific environmental data sets are being downloaded from the CIMIS database for parameters described above. Numbers of days with dew periods, total precipitation, and minimum temperatures were graphed over time (Julian days) from April through June. Timing of fungicides and disease incidence, severity, and intensity (incidence*severity) were determined for the two locations in northern and southern California production areas for 2012 to 2014. Data is being evaluated and disease will be correlated to accumulated days with dew, temperature thresholds and total precipitation.

Statistical analysis of data. Experiments were designed with treatments in randomized blocks. Data for the large scab field trial were analyzed using split-plot procedures. All data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures ($P > 0.05$).

Results and Discussion:

Etiology of scab and Alternaria leaf spot and population structure of scab. The AFLP dataset from analyses of *F. carpophilum* populations was used in a “Parsimony Tree Length Permutation Test” or PTLPT to determine the possible occurrence of sexual recombination in each of the three field populations. Analysis included genetic and genotypic diversities, genetic differentiation among three populations, analysis of molecular variance (AMOVA), and cluster analysis using different approaches, such as a Bayesian approach and a Principal Component Analysis (PCA). Final results of these analyses are pending.

In comparative studies of *Fusicladium* isolates from almond and peach, DNA banding patterns based on RAPD and UP-PCR indicated a low genetic variability among peach isolates (4.2%

of markers were polymorphic) and higher variability among almond isolates (42.0% of markers were polymorphic). Dice coefficients of similarity ranged from 0.932 to 1.000 for isolates of *F. carpophilum* from peach and from 0.214 to 0.976 for almond isolates. UPGMA bootstrap analysis suggested some divergence among isolates of the species from the two hosts.

Scab management – 2013 and 2014 Research. Dormant treatments with multi-site inhibitor fungicides to reduce the production of primary inoculum in the springtime from overwintering twig lesions were evaluated in a trial on cv. Carmel in Butte Co. A high efficacy in sporulation inhibition was demonstrated again for the Bravo-Oil treatment where 6.2% of the twig lesions were found to be sporulating in late April as compared to 91.9% in the control (**Table 1**). Captan-Oil was only weakly effective (68.2% incidence of sporulation), whereas for Manzate-Oil there was no significant difference in incidence from the control. In previous years, copper-oil also significantly reduced sporulation and can be an alternative to Bravo-oil.

Table 1. Efficacy of dormant treatments on sporulation of overwintering scab lesions on cv. Carmel almond - Butte Co. 2014

Treatment	Twig sporulation**			
	Inc. (%)	LSD^	Rating	LSD
Control	91.9	a	2.4	a
Manzate ProStick 6 lb + Oil 3.5 gal	79.2	ab	1.8	b
Captan 80WDG 5 lb+ Oil 3.5 gal	68.2	b	1.6	b
Bravo 6 pts + Oil 3.5 gal	6.2	c	0.1	c

* Treatments were applied using an air-blast sprayer at 100 gal/A in January 2014.
** Lesions were evaluated April 23, 2014, using a rating scale: 0=no sporulation, 1 = very sporulation, 2 = lesion partially covered with dark sporulation, 3 = lesion completely covered with sporulation or full concentric ring sporulating.
^ Values followed by the same letter are not significantly different based on an analysis of variance and LSD mean separation ($P > 0.05$) procedures.

Thus, as in previous years, Bravo/oil performed exceptionally well and had a long-lasting effect on the suppression of twig sporulation. When these dormant treatments are applied, spring-time fungicide applications may not be needed under less favorable disease conditions. We previously established that this performance is only obtained when Bravo is tank-mixed with oil and oil should not be used with in-season applications. Full registration is being pursued through the IR-4 program to change the PHI to 60 days.

Spring-time applications (two applications each treatment) for the management of scab were evaluated in field trials in 2013 (data not available for 2014) and results are presented in **Table 2 A, B**. In the first trial, the pre-mixtures Luna Sensation, Luna Experience, and Merivon, as well as mixtures of Syllit and Tebucon/Propimax or of Isopyrazam and Propimax were most effective (**Table 2A**). The incidence of scab was reduced from 94.7% in the control to 37.9% or less. Isopyrazam and Syllit by themselves were only moderately effective, indicating that Propimax (propiconazole) and Tebucon (tebuconazole) significantly contributed to the efficacy of the two mixtures. In the second plot, Merivon, Luna Sensation, Custodia, Ph-D+Quash, and the rotation of Catamaran+Viathon were among the best treatments, reducing the incidence of

Table 2. Efficacy of Fungicide treatments for management of scab of almond cv. Monterey – Colusa Co. 2013

A. Plot 1

				Applications		Dis. Incid. on fruit**		Dis. Sev. on fruit	
No.	Program	Treatment	Rate (/A)	4/23	5/15	(%)	LSD^	Lesions	LSD
1	---	Control	---	---	---	94.7	a	2.1	a
2	Single	Ph-D 11.3DF + NuFilm P	6 oz	@	@	59.4	bc	0.8	bc
3	Fungicides	Ph-D 11.3DF + Nufilm-P	12 oz	@	@	65.6	b	0.8	bc
4		Isopyrazam	8.2 fl oz	@	@	67.1	b	0.9	b
5		Syllit 65WG	2 lb	@	@	56.1	bcd	0.7	bcd
6	Mixtures	Isopyrazam + Propimax	8 fl oz + 8 fl oz	@	@	32.9	de	0.4	de
7		Isopyrazam + Propimax	11 fl oz + 8 fl oz	@	@	24.2	e	0.2	e
8		Syllit + Tebucon***	2 lb + 6 oz	@	@	24.3	e	0.3	e
9		Syllit + Tebucon***	1.5 lb + 6 oz	@	@	31.5	e	0.4	de
10	Pre-	Luna Experience	6 fl oz	@	@	29.7	e	0.3	de
11	mixtures	Luna Sensation	5 fl oz	@	@	37.9	cde	0.5	cde
12		Merivon	5 fl oz	@	@	21.1	e	0.2	e
13		Merivon	6 fl oz	@	@	24.0	e	0.3	e

B. Plot 2

				Applications		Dis. Incid. on fruit**		Dis. Sev. on fruit	
No.	Program	Treatment	Rate (/A)	5/1	5/22	(%)	LSD^	Lesions	LSD
1	---	Control		-----	-----	96.1	a	2.1	a
2	Single	CHA-1323	3.5 fl oz	@	@	49.3	bcde	0.6	cdef
3	Fungicides	CHA-1323	7 fl oz	@	@	64.2	bc	0.8	bc
4		Isopyrazam	11 fl oz	@	@	62.5	bcd	0.8	bc
5	Mixtures	Ph-D 11.3DF + Quash 50WG	6.2 oz + 2.5 oz	@	@	21.8	f	0.2	ef
6		Captan Gold + Custodia	2.5 lb + 17.25 fl oz	@	@	49.4	bcde	0.6	cdef
7		Isopyrazam + Abound	11 fl oz + 12 fl oz	@	@	75.2	b	1.1	b
8	Pre-	Custodia	17.25 fl oz	@	@	38.5	cdef	0.4	cdef
9	mixtures	Luna Sensation	5 fl oz	@	@	34.7	def	0.4	cdef
10		Merivon	6 fl oz	@	@	19.7	f	0.2	f
11	Rotations	Bravo Weather-Stick	4 pt	@	----	49.7	bcd	0.6	cdef
		Quadris Top + Dyne-Amic	14 fl oz + 16 fl oz		@				
12		Bravo Weather-Stick	4 pt	@	----	52.1	bcd	0.7	bcde
		A13703N + Dyne-Amic	14 fl oz + 16 fl oz		@				
13		Bravo Weather-Stick	4 pt	@	----	56.3	bcd	0.7	bcd
		Inspire Super + Dyne-Amic	20 fl oz + 16 fl oz		@				
14		Catamaran	4 pt	@	----	22.5	ef	0.2	cdef
		Viathon	32 fl oz	----	@				

* Treatments were applied using an air-blast sprayer at a rate of 100 gal/A and there were 4 single-tree replications for each treatment.

** For evaluation of scab on 7-16-13, 25-30 fruit were scored and a scale was used from 0=no disease, 1 = <25%, 2 = 26-50%, 3 = 51-75%, 4 = >75% of fruit surface covered with lesions.

*** For the second application, Propimax at 6 fl oz/A was used instead of tebuconazole.

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

disease from 96.1% in the control to 38.5% or less (**Table 2B**). Isopyrazam was again moderately effective, and was similar to the 7-oz rate of Rhyme or the mixture of isopyrazam and Abound. Thus, Abound did not increase the efficacy, most likely due to a high incidence of QoI resistance at this location. Rotations from Bravo to Quadris Top or Inspire Super were also moderately effective.

Table 3. Efficacy of fungicide treatments for management of Alternaria Leaf Spot on almond cvs. Monterey and Fritz – Kern County 2013

							cv. Monterey				**cv. Fritz**			
				Applications			Dis. Inc. leaves		Dis. Sev. leaves		Dis. Inc. leaves		Dis. Sev. leaves	
No.	Program	Treatment*	Rate	5-8	5-30	6-20	(%)	LSD^	Rating	LSD	(%)	LSD^	Rating	LSD
1	---	Control	---	---	---	---	95.6	a	2.24	a	73.9	a	1.05	a
2	Single	Ph-D + NF-P	6 oz	@	@	@	46.0	cdef	0.67	def	14.2	cd	0.14	cd
3	Fungicides	Ph-D + NF-P	12 oz	@	@	@	55.4	cdef	0.69	cdef	9.2	de	0.09	cd
4		CHA-1323 (Rhyme)	5 fl oz	@	@	@	69.6	abcd	0.91	cde	35.8	bc	0.36	bc
5		CHA-1323 (Rhyme)	7 fl oz	@	@	@	91.1	ab	1.33	bc	36.2	bc	0.42	bc
6		Isopyrazam	11 fl oz	@	@	@	65.0	bcde	0.84	cdef	19.7	bcd	0.20	bcd
7	Mixtures	Isopyrazam - Abound 2F	7 + 12 fl oz	@	@	@	53.6	cdef	0.58	def	9.9	de	0.10	cd
8		Isopyrazam - Abound 2F	11 + 12 fl oz	@	@	@	52.4	cdef	0.59	def	33.2	bcd	0.36	bc
9		Ph-D + Quash 50WG + NF-P	6.2 + 2.5 oz + 8 fl oz	---	@	@	32.9	def	0.33	ef	25.3	bcd	0.25	bcd
10	Pre-	Pristine 38WG	14.5 oz	@	@	@	18.7	f	0.20	f	13.4	cd	0.13	cd
11	mixtures	Merivon	6.5 oz	@	@	@	47.0	cdef	0.54	def	20.8	bcd	0.21	bcd
12		Merivon	6.5 oz	---	@	@	48.0	cdef	0.55	def	0.0	e	0.00	d
13		Q8Y78	24 fl oz	@	@	@	46.1	cdef	0.54	def	18.5	bcd	0.18	cd
14	Rotations	Bravo Weather-Stick	4 pts	@	---	---	77.9	abc	1.04	cd	26.0	bcd	0.26	bcd
		Quadris Top - Dyne-Amic	14-16 fl oz	@	@	@								
15		Bravo Weather-Stick	4 pts	@	---	---	36.3	cdef	0.40	def	21.9	bcd	0.22	bcd
		A13703N - Dyne-Amic	14-16 fl oz	@	@	@								
16		Bravo Weather-Stick	4 pts	@	---	---	49.9	cdef	0.63	def	21.8	bcd	0.22	bcd
		Inspire Super - Dyne-Amic	20 fl oz	@	@	@								
17		Bravo + A13703N - Dyne-Amic	64 + 14 + 16 fl oz	---	@	---	95.6	ab	1.84	ab	47.6	ab	0.54	b
		Inspire Super - DyneAmic	20 + 16 fl oz	---	---	@								
18		Inspire Super - Dyne-Amic	20 oz + 16 oz	@	---	@	44.1	cdef	0.50	def	16.3	cd	0.16	cd
		Ph-D + NF-P	6.2 oz + 8 oz	---	@	---								
		Quash 50WG	3.5 oz	---	---	@								
19		Luna Experience	6 oz	@	---	@	46.5	cdef	0.52	ef	14.7	cd	0.15	cd
		Luna Sensation	5 oz	---	@	---								
20		Luna Sensation	5 oz	@	---	@	29.1	ef	0.31	def	34.8	bc	0.37	bc
		Luna Experience	6 oz	---	@	---								
21		Luna Sensation	5 oz	---	@	---	46.7	cdef	0.55	def	18.5	cd	0.19	cd
		Luna Experience	6 oz	---	---	@								

* Treatments were applied using an air-blast sprayer at a rate of 100 gal/A and there were 3 single-tree replications for each treatment. NF-P = NuFilm P.

** Evaluations for disease were done on 8-21-13. For Alternaria disease incidence on leaves, 30-40 leaves from each of the 3 single-tree replications were evaluated for the presence of disease. For evaluation of disease severity, a rating was used with 0=healthy, 1 = 1 lesion/leaf, 2 = <50% leaf area diseased, no sporulation, 3 = 75% of leaf area diseased, 4 = >75% of leaf area diseased, sporulation.

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

Our data on scab management demonstrate that very effective fungicides and programs are currently available to manage the disease. Under high-disease conditions, a three-spray program should include dormant applications with chlorothalonil-oil (or copper-oil) and two petal-fall applications. Under lower disease pressure, a dormant treatment or in-season treatments alone can be considered. Large-scale trials could establish if dormant treatments with Bravo-oil alone can manage the disease. If a grower has other summer diseases such as rust, Alternaria leaf spot, or hull rot to manage, late spring and early summer applications with selected fungicides could also manage scab (because the onset of scab epidemics is delayed by the dormant application).

In season treatments should start with petal fall applications or at the onset of twig sporulation; we demonstrated previously that programs starting later in the season are not as effective for most fungicides (compounds that do not have post-infection activity). Effective petal fall treatments are chlorothalonil, DMI fungicides such as Quash or Inspire Super, Syllit, compounds containing SDHIs, QoIs (at locations where the pathogen population has not developed resistance), or Ph-D. Treatments containing a DMI compound are very effective, although the incidence of natural resistance against DMIs in *F. carpophilum* is high at some locations (see 2011 Annual Report). Thus, this class of chemicals, as well as others, can be effectively used, but they should be rotated with other classes or FRAC Groups (representing different modes of action) to prevent further selection of insensitivity.

Fungicide evaluations for management of Alternaria leaf spot of almond in 2013. Our research in 2014 is ongoing and we will evaluate our Alternaria field plots in Aug./Sept. 2014. Thus, information is presented here for 2013 trials using three-spray programs in Kern and Colusa Co. Single-fungicides, mixtures, pre-mixtures, and rotation programs were used. In Kern Co., the same treatments were evaluated on the highly susceptible cv. Monterey and on the less susceptible cv. Fritz. Therefore, reductions in disease by many of the treatments were generally higher on cv. Fritz. The lowest levels of disease were observed using Pristine on cv. Monterey, indicating that this older pre-mixture still has its value when SDHI and QoI resistance is at a low level (**Table 3**). Ph-D, Merivon, the coded pre-mixture Q8Y78, Ph-D/Quash, as well as several rotations also performed well, reducing the incidence of leaf spot from 95.6% in the control to 48% or less and the severity rating from 2.24 in the control to 0.69 or less. On cv. Fritz, incidence by most treatments was reduced from 73.9% in the control to 36.2% or less and the severity rating from 1.05 in the control to 0.42 or less (**Table 3**).

In both field plots in Colusa Co., the incidence of Alternaria leaf spot was high, but disease severity was moderate. Again all treatments, with the exception of the natural product ARY 0951-001 and the 7 fl-oz rate of the DMI Rhyme, significantly reduced incidence and severity of disease (**Table 4 A, B**). Ph-D alone or in rotations, Luna Experience, and Merivon continued to perform very well.

Our data indicate that Alternaria leaf spot can be effectively managed with currently available fungicides in an integrated program with cultural practices. Rotation programs used included three or four different FRAC groups (e.g., **Table 4A** - Treatment 10; **Table 4B** - Treatment 12) and thus, are excellent examples for resistance management programs using fungicide classes currently available. Resistance to boscalid, a first generation SDHI and a component in Pristine, can be managed using newer SDHI sub-groups contained in recently registered pre-mixtures such as Luna Sensation and Merivon. No high-resistance and only moderate-resistance has been found to fluopyram to date. In resistant isolates, EC_{50} values for fluxapyroxad (one component of Merivon) are at lower levels as compared to EC_{50} values for boscalid or penthiopyrad. Due to widespread resistance to QoIs, these SDHI pre-mixtures should be used in rotation with other FRAC classes such as DMIs (FRAC 3) or Ph-D (FRAC 19).

Table 4. Efficacy of fungicide treatments for management of Alternaria Leaf Spot of almond cvs. Monterey and Carmel – Colusa County 2013

A. cv. Monterey

				Applications			Dis. Inc. leaves		Dis. Sev. leaves		Tree Defoliation	
No.	Program	Treatment	Rate (/A)	5-29	6-18	7-16	(%)	LSD^	Rating	LSD	Rating	LSD
1	---	Control	---	---	---	---	99.0	a	2.8	ab	1.5	a
2	Single	ARY 0951-001	25 fl oz	@	@	@	100.0	a	2.9	a	1.4	b
3		ARY 0951-001	54 fl oz	@	@	@	98.3	a	2.3	b	1.1	b
4	Mixtures,	Custodia	17.25 fl oz	@	@	@	46.7	bc	0.7	c	0.3	c
5	pre-mixtures	Captan Gold + Custodia	2.5 lb + 17.25 fl oz	@	@	@	39.9	bc	0.6	c	0.2	c
6		Catamaran + Viathon	4 pt + 32 fl oz	@	@	@	53.3	b	0.8	c	0.1	c
7	Rotations	Bravo Weather-Stick	4 pt	@	@	---	49.5	bc	0.7	c	0.2	c
		Quadris Top + Dyne-Amic	14 + 16 fl oz	@	@	@						
8		Bravo Weather-Stick	4 pt	@	@	---	42.8	bc	0.5	c	0.1	c
		A13703N + Dyne-Amic	14 + 16 fl oz	@	@	@						
9		Bravo Weather-Stick	4 pt	@	@	---	32.9	bc	0.4	c	0.0	c
		Inspire Super + Dyne-Amic	20 + 16 fl oz	@	@	@						
10		Ph-D 11.3DF	6.2 oz	@	---	---	30.2	c	0.4	c	0.0	c
		Quash 50WG	3.5 oz	---	@	---						
		Ph-D + Inspire Super + Dyne-Amic	6.2 oz + 20 + 16 fl oz	---	---	@						

B. cv. Carmel

				Applications			Dis. Incid. leaves		Dis. Sev. leaves		Tree Defoliation	
No.	Program	Treatment	Rate (/A)	5-22	6-11	7-12	(%)	LSD^	Rating	LSD	Rating	LSD
1	---	Control	---	---	---	---	100.0	a	2.5	a	1.8	a
2	Single	Ph-D 11.3DF	6.2 oz	@	@	@	33.0	f	0.4	f	0.3	d
3		Isopyrazam	8.2 fl oz	@	@	@	64.9	cde	0.9	cde	0.5	bcd
4		Isopyrazam	11 fl oz	@	@	@	71.2	bcd	1.0	cd	0.3	d
5		Rhyme	3.5 fl oz	@	@	@	74.7	bcd	1.1	cd	1.1	b
6		Rhyme	7 fl oz	@	@	@	91.4	ab	1.7	b	0.9	bc
7	Mixtures	Isopyrazam + Abound	11 + 12 fl oz	@	@	@	71.8	bc	1.2	c	0.4	cd
8		Fontelis + Tebucon	14 + 6 fl oz	@	@	@	70.9	bcd	1.0	cd	0.5	bcd
9	Pre-mixtures	Luna Sensation	5 fl oz	@	@	@	49.0	def	0.6	def	0.2	d
10		Luna Experience	6 fl oz	@	@	@	32.4	f	0.4	f	0.3	d
11		Merivon	6 fl oz	@	@	@	41.3	ef	0.5	ef	0.3	d
12	Rotation	Ph-D 11.3DF	6.2 oz	@	---	---	40.1	ef	0.5	ef	0.4	d
		Inspire Super + Dyne-Amic	20 + 16 fl oz	---	@	---						
		Ph-D + Quash 50WG	6.2 + 2.5 oz	---	---	@						

* Treatments were applied using an air-blast sprayer at a rate of 100 gal/A and there were 3 single-tree replications for each treatment.

** For evaluations of Alternaria disease incidence on 7-31-13, 30-40 leaves from each of the 4 single-tree replications were evaluated for the presence of disease. For evaluation of disease severity, a rating was used with 0 = healthy, 1 = 1 lesion/leaf, 2 = <50% leaf area diseased, no sporulation, 3 = 75% of leaf area diseased, 4 = >75% of leaf area diseased, sporulation. For evaluation of tree defoliation on 8-16-13, trees were rated based on a scale from 0 = full canopy, 1 = <10%, 2 = 10-25%, 3 = 25-50%, and 4 = >50% defoliation.

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

We evaluated the in vitro sensitivity of twenty *Alternaria* spp. isolates from almond (collected from locations in three counties over three years) to chlorothalonil. EC_{50} values for mycelial growth varied widely and ranged from 0.29 ppm to 1.54 ppm with an average of 0.91 ppm, and thus, were quite high as compared to the newer generation of fungicides. Still, this multi-site mode of action fungicide, that is effective against scab and Alternaria leaf spot, potentially will have a critical role in preventing the over-use of SDHI and DMI fungicides, thus, reducing the potential for selecting resistant pathogen populations. For an overview on the management of scab and Alternaria leaf spot with currently registered fungicides we refer to the guidelines presented our 2012 report or to the "Fungicide Efficacy Tables" for 2013 at http://www.ipm.ucdavis.edu.

In vitro sensitivity of *Fusicladium carpophilum* and *Alternaria* spp. to selected SDHI fungicides – 2013 and 2014 Research. In 2013 screenings, isolates of *Alternaria* spp. were rated as sensitive, moderately resistant, or highly resistant against representatives (i.e., the pyridine carboxamide boscalid, the pyrazole-carboxamides fluxapyroxad and penthiopyrad, and the pyridinyl-ethyl-benzamide fluopyram) of three SDHI sub-groups. Most isolates could be placed into one of two groupings with no strict cross-resistance among the sub-groups or even within a subgroup (i.e., fluxapyroxad and penthiopyrad). The first group contained isolates moderately or highly resistant to boscalid, highly resistant to fluxapyroxad and penthiopyrad, and moderately resistant to fluopyram (Bos-MR/HR + fluxa-HR + penthio-HR + fluop-MR). The second group contained isolates moderately resistant to boscalid, fluxapyroxad, and penthiopyrad, and sensitive to fluopyram (Bos-MR + fluxa-MR + penthio-MR + fluop-S). Six additional isolates showed various sensitivity levels against the four fungicides, did not always show strict cross-resistance within sub-groups, and could not be placed into distinct groups.

Table 5. Molecular mechanisms of SDHI resistance in *Alternaria* spp. from almond - Sequencing of three *Sdh* subunits of the succinate dehydrogenase gene of sensitive isolates and isolates from two SDHI cross-resistance groups (see text for details).

						SDH sequence analysis										
		Sensitivity of isolates to:				*SdhB*		*SdhC*			*SdhD*					
Isolate ID	Cross resis. Group	Bosca-lid	Fluxa-pyroxad	Penthio-pyrad	Fluo-pyram	277	286	79	134	135	28	31	48	123	133
3286	---	S	S	S	S	H	C	G	H	R	A	I	T	D	H
3723	---	S	S	S	S	H	C	G	H	S	T	M	A	D	H
4288	---	S	S	S	S	H	C	G	H	S	T	M	A	D	H
4260	---	S	S	S	S	H	C	G	H	S	A	I	T	D	H
4773	---	S	S	S	S	H	C	G	H	S	A	M	T	D	H
4775	---	S	S	S	S	H	C	G	H	S	T	M	A	D	H
4767	1	MR	HR	HR	MR	H	C	G	R	S	A	I	T	D	H
3772	1	MR	HR	HR	MR	H	C	G	R	S	T	M	A	D	H
4776	1	HR	HR	HR	MR	H	C	G	R	S	T	M	A	D	H
4780	1	HR	HR	HR	MR	H	G	G	R	S	T	M	A	D	H
4779	1	HR	HR	HR	MR	H	S	G	R	S	T	M	A	D	H
4772	1	HR	HR	HR	MR	H	C	G	R	S	A	I	T	D	H
3734	2	MR	MR	MR	S	Y	C	G	H	S	T	M	A	D	H
3779	2	MR	MR	MR	S	Y	C	G	H	S	T	M	A	D	H
3785	2	MR	MR	MR	S	Y	C	G	H	S	T	M	A	D	H
3790	2	MR	MR	MR	S	Y	C	G	H	S	T	M	A	D	H
3775	2	MR	MR	MR	S	Y	C	G	H	S	T	M	A	D	H

Mutation

Partial sequencing of three succinate dehydrogenase sub-units revealed that the Bos-MR/HR + fluxa-HR + penthio-HR + fluop-MR phenotype correlated with a H134R mutation in *SdhC,* and the Bos-MR + fluxa-MR + penthio-MR + fluop-S phenotype correlated with a H277Y mutation in *SdhB* (**Table 5**; the first, green-highlighted group of isolates is sensitive to all SDHI fungicides). These mutations were described previously for *A. alternata,* but were not correlated with moderate or high resistance. Mutations that we identified in the *sdhD* sub-unit were not correlated with resistance. Thus, differential resistance to SDHI fungicides could be genetically defined.

Isolates of *F. carpophilum* also could be placed into distinct SDHI resistance sub-groups (see our 2013 Annual Report). Sequence analyses for this species is more difficult and currently ongoing because the *Sdh* sequences are quite different from those of *Alternaria* spp., published primers cannot be used, and new primers have to be designed based on more extensive sequence analyses.

These studies indicate that differential resistance to SDHI fungicides is based on distinct mutations in the target gene. Cross-resistance among SDHI fungicides is not complete and that in field use, rotations between certain sub-groups in some cases potentially could provide a resistance management strategy. Because genotypes of pathogen populations at specific locations, however, are generally not known and genotype composition may change over time, rotation between SDHI subgroups is not recommended.

Development of a modified DSV model for initiating Alternaria leaf spot management practices based on precipitation, dew point (instead of leaf wetness), and threshold temperatures during the spring season. We continued to evaluate daily temperatures (mean, min/max), daily incidence of dew periods (based on temperatures below dew point), and daily precipitation as indicators of wetness periods at temperatures conducive for disease based on the DSV model. Measures of disease were correlated to accumulated days with dew, temperature thresholds, and total precipitation in the two locations in Kern and Colusa Co. in 2012 and 2013 and we are currently evaluating 2014 data. Total disease was related to the number of dew periods. Locations with fewer days with wetness from dew had lower disease levels at the end of the season. The distribution of days with dew over the spring season, however, varied widely. As reported previously, precipitation levels also varied widely in Delano from 15.8 mm (2013) to 59 mm (2012) and in Colusa from 23.7 mm (2013) to 41 mm (2012) and were associated with both higher and lower disease levels. Minimum temperatures above 16 C also related to total disease. Again, we are summarizing 2014 data.

Optimal timing of the initiation of fungicide treatments and of subsequent applications is critical for obtaining high disease management. The data sets for determining initiation times of management programs based on dew periods and rainfall from regional weather station databases are being evaluated so that an area-wide forecast can be made. This would alleviate site-specific data collection and still allow for timely applications of fungicides.

Still, selected timings of fungicides during May and June provided very good disease control in both locations in 2013 (**Tables 3, 4**), as well as in 2014 (data summary pending). In the South, we start earlier in late April, early May; whereas in the North we start in mid- to late May. Fungicide applications are subsequently applied into late June (early July) in approximately three-week intervals and continue to provide a high level of disease control. Thus, the DSV model is still functioning to describe disease progress and infection periods when data is collected from individual orchards.