Epidemiology and Control of Almond Scab and Alternaria Leaf Spot

Project No.: 14-PATH3-Adaskaveg

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Objectives:

I. Etiology
  A. Determine the *Fusarium carpophilum* population composition within selected orchards and determine if sexual reproduction occurs within or among orchard populations using molecular methods.

II. Management
  A. Evaluate new and registered fungicides for their efficacy in managing scab and Alternaria leaf spot. Fungicides to be evaluated by themselves or as components of mixtures and 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 and in-season applications
    i. Dormant applications with Bravo-oil to delay and reduce sporulation of twig lesions in a large-scale field plot.
    ii. In-season applications with registered (Bravo, Manzate/Dithane, Ziram) and new fungicides (see above). (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 succinate dehydrogenase inhibitor (SDHI) fungicide class: pyridine-carboxamides (boscalid), pyrazole-carboxamides (fluxapyroxad), and pyridinyl-ethyl-benzamides (fluxopyroxad), DMIs, polyoxin-D, and QoIs.
    i. Continue to characterize molecular mechanisms for SDHI resistance.
    ii. Characterize molecular mechanisms for DMI resistance in *F. carpophilum*.

Interpretive Summary (Note - This report is mainly based on our 2014 research because our 2015 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 occurred at high incidence in many growing areas in California in recent years. Both are summer diseases that occur especially in locations with high humidity and where air circulation is poor (i.e., high-density plantings, orchards with soils with inadequate drainage, or where trees require frequent and extended irrigations throughout the summer). Severity of both diseases was lower in 2014 compared to previous seasons likely due to reduced irrigation schedules and subsequently less favorable disease conditions.

Because the sexual stage of *F. carpophilum* has never been observed on almond in California and the presence of the sexual stage could have implications on disease management, we analyzed three populations of the pathogen from different growing areas in the state. Using molecular population genetical approaches, we found no evidence for sexual recombination, and thus, populations of the pathogen appear to only reproduce clonally by asexual reproduction (i.e., conidia).

In the management of scab, dormant treatments with copper-oil, chlorothalonil (e.g., Bravo)/oil, or Syllit-oil could not be evaluated due to very low sporulation in the untreated controls, likely because of the dry weather. Among in-season treatments (two applications starting at the onset of twig sporulation), rotations of Catamaran (FRAC M5/33) with Viathon (FRAC 3/33) and of Bravo (FRAC M5) with Quadris Top (FRAC 3/11) resulted in the lowest scab incidence in the first trial; and Luna Experience (FRAC 3/7), Fontelis-Tebucon (FRAC 7+3), and the experimental EXP1 were most effective in the second trial. Spring-time treatments that were determined to be very effective in other years include chlorothalonil (proposed label change to 60 days PHI), FRAC 3 fungicides such as Quash or Inspire Super, Ph-D (FRAC 19), Syllit (FRAC U12), and compounds containing FRAC 7 and FRAC 11 (at locations where the pathogen population has not developed resistance). 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.

Fungicides applications for Alternaria leaf spot are best timed using the DSV model, or alternatively, are done calendar-based between May and late June/early July in approximately three-week intervals. The DSV model is based on daily temperature and leaf wetness parameters. Accurate modeling of the disease using daily temperatures, incidence of dew periods, and precipitation has not been successful to date. 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. In our trials on Alternaria leaf spot, the new EXP1, as well as mixtures of EXP1 with EXP2 or EXP3 were highly effective. Isofetamid by itself or mixed with the experimental IB18111 also performed well. Among registered treatments, Merivon, Luna Sensation, Luna Experience, and rotations of the two Luna products as well as those of Inspire Super with Quadris Top, Ph-D with Tebucon, Ph-D/Tebucon with Inspire Super and Ph-D, Syllit with Ph-D/Tebucon, and of Bravo with Quadris Top, were the most effective.

Thus, our research on both diseases demonstrates that effective disease management can be obtained with properly timed applications of currently registered fungicides belonging to three or four FRAC groups. Rotations of these groups allows for resistance management with the goal to limit the further spread of QoI, SDHI, and DMI (scab only) resistance.

DMI resistance is common in some populations of *F. carpophilum*. Studies were conducted to determine the molecular mechanism of resistance. Expression of the *CYP51* gene that is correlated with resistance in some other plant pathogens, however, was not related to in vitro sensitivity values for metconazole or propiconazole of 20 isolates of *F. carpophilum*. Therefore, a different mechanism appears to be present in this organism.

Materials and Methods:

Etiology of scab and Alternaria leaf spot and population structure of scab. The population structure of *Fusicladium carpophilum* was 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). 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 2014 and 2015. Data for evaluation of dormant treatments are presented for 2015 and for in-season treatments for 2014. Dormant treatments with copper, Syllit, or Bravo WeatherStik (4 pts/A) all mixed with 3.5% oil were applied in a commercial cv. Monterey orchard in Colusa Co. in January 2015. Scab lesions on last fall's twigs growth were evaluated periodically for sporulation in the spring of 2015.

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 Quash (FRAC 3), the experimental EXP, the pre-mixtures Luna Sensation (FRAC 7/11), Luna Experience (FRAC 3/7), and Merivon (FRAC 7/11), the mixtures Fontelis + Tebucon (FRAC 7+3), Ph-D + Tebucon (FRAC 19+3), Quash + Protexio (FRAC 3+17), Quash + the experimental S2200, EXP1 + EXP2, EXP1 + EXP3, isofetamid + IB18121, as well as several rotation programs: Syllit + Tebucon (FRAC U12+3), Catamaran + Viathon (FRAC M5/33+3/33), Bravo + Quadris Top (FRAC M5+3/11), and Ph-D and Tebucon (FRAC 19+3). Disease was evaluated late July 2014, 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 2014. The modified Disease Severity (DSV) model was used to determine initiation times of spray programs. One trial was established in Kern Co. (cv. Monterey) and two trials in Colusa Co.

(cvs. Carmel and Monterey). Each site received three applications between May 14 and June 25 (Kern Co.) or between May 13 and July 9 (Colusa Co.). Treatments included Rhyme (FRAC 3), isofetamid (FRAC 7), EXP1, mixtures of isofetamic with IB18111 or IB18121, Fontelis + Tebucon, EXP1 + EXP2, EXP1 + EXP3, Ph-D + Tebucon, the premixtures Luna Sensation, Luna Experience, and Merivon, as well as rotations of Ph-D/Tebucon + Inspire Super + Ph-D, Syllit + Ph-D/Tebucon, Inspire Super + Quadris Top, Ph-D + Tebucon or Fontelis, Luna Sensation + Luna Experience, and Bravo + Quadris Top. Evaluations were done in late August 2014. For disease incidence 8 shoots or four leaf clusters at mid canopy height were rated using a severity scale from 0 = no disease to 4 = severe disease.

Establish and expand baseline sensitivities and monitor for shifts in sensitivity in populations of *Alternaria* and *Fusicladium* spp. to sub-groups of the SDHIs - 2014 Research. Samples of scab-infected fruit were obtained from an orchard in San Joaquin Co. where applications with Fontelis were not very effective. Thirteen isolates each were evaluated from treated and untreated trees for their sensitivity to penthiopyrad using the spiral gradient dilution method as described previously.

Characterize molecular mechanisms for DMI resistance in *F. carpophilum* - 2014 and 2015 Research. The eburicol 14α-demethylase gene (*CYP51*) of *F. carpophilum* was partially sequenced and primers were designed to study gene expression of twenty isolates of the pathogen with different fungicide sensitivity levels in the absence or presence of propiconazole or metconazole. The relative copy number of *CYP51* was determined in comparisons with expression of beta-tubulin.

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) means separation procedures ($P > 0.05$).

Results and Discussion:

Etiology of scab and Alternaria leaf spot and population structure of scab. The AFLP data of three populations of *F. carpophilum* were used in a Parsimony Tree Length Permutation Test (PTLPT) to determine the possible occurrence of sexual recombination. Analyses of each population and of the combined data set for the three populations did not support the hypothesis that sexual recombination is occurring. This indicates that *F. carpophilum* populations from almond in California are only reproducing asexually.

Based on analyses of DNA banding patterns following RAPD and UP-PCR, *Fusicladium* isolates from almond, peach, and pecan clustered by host. Pecan isolates were the most divergent, and isolates from almond and peach formed their own clusters. Peach isolates showed low genetic variability; whereas almond isolates were more diverse. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) bootstrap analysis and DNA sequence data suggested divergence among isolates from the two stone fruit hosts that may indicate distinctive taxa. Cultural, morphological, and genetic studies are ongoing.

Scab management – 2014 and 2015 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. Monterey in Colusa Co. Sporulation was low in all treatments due to the dry spring. In previous years, copper-oil and chlorothalonil-oil dormant and delayed dormant treatments significantly delayed and reduced sporulation. Bravo-oil consistently extended longer into the spring season than copper-oil treatments and therefore, was more effective. 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 and the rate from 4 pt/A to 6 pt/A.

When these dormant treatments are applied, spring-time fungicide applications may not be needed under less favorable disease conditions. Additional benefits of effective dormant treatments are that 1) They are an anti-resistance strategy because with reduction of primary inoculum, a smaller pathogen population is exposed to subsequent selection by in-season treatments, 2) A reduced amount of inoculum in an orchard will maximize the efficacy of subsequent fungicide treatments, and 3) The delay in sporulation and inoculum availability aligns the application of in-season treatments for scab with those of other summer diseases (e.g., Alternaria leaf spot).

Table 1. Efficacy of fungicide treatments for management of scab of almond cv. Monterey - Colusa Co. 2014

A. Plot 1

				Applications		Dis. Incid. on fruit**		Dis. Sev. on fruit	
No.	Program	Treatment*	Rate (/A)	4/30	5/28	(%)	LSD^	Lesions	LSD
1	---	Control	---	---	---	83.9	a	1.6	a
2	Single	Quash	3.36 oz	@	@	38.0	b	0.5	b
3	Mixtures	Quash + S2200	3.36 + 3.36 oz	@	@	37.2	b	0.4	b
4		Quash + V10135 (Protexio)	3.36 oz + 14.4 fl oz	@	@	50.5	ab	0.7	b
5		Ph-D + Tebucon 45 + NuFilm P	6.2 + 8 oz + 8 fl oz	@	@	61.7	ab	0.9	ab
6	Rotations	Syllit 65WG	32 oz	---	@	52.4	ab	0.7	b
		Tebucon 45 + NuFilm P	8 oz + 8 fl oz	@	---				
7		Catamaran	64 fl oz	@	---	26.1	b	0.3	b
		Viathon	64 fl oz	---	@				
8		Bravo WeatherStik	64 fl oz	@	---	33.1	b	0.4	b
		Quadris Top + DyneAmic	14 + 16 fl oz	---	@				
9		Ph-D + NuFilm P	6.2 oz + 8 fl oz	@	---	56.9	ab	0.9	b
		Tebucon 45 + NuFilm P	8 oz + 8 fl oz	---	@				

B. Plot 2

				Applications		Dis. Incid. on fruit**		Dis. Sev. on fruit	
No.	Program	Treatment*	Rate (/A)	4/30	5/28	(%)	LSD^	Lesions	LSD
1	---	Control	---	---	---	97.7	a	2.4	a
2	Single	EXP1	5.14 fl oz	@	@	24.8	cd	0.3	cd
3	Mixtures	EXP1 + EXP2	3.43 + 4.57 fl oz	@	@	30.5	cd	0.4	bcd
4		EXP1 + EXP3	3.43 + 5.48 fl oz	@	@	54.3	bc	0.7	bc
5		Isofetamid + IB18121	8.6 + 13.7 fl oz	@	@	58.8	b	0.9	b
6		Fontelis + Tebucon 45	14 fl oz + 8 oz	@	@	22.4	cd	0.3	cd
7	Pre-	Luna Experience	6 fl oz	@	@	14.4	d	0.1	d
8	mixtures	Luna Sensation	5 fl oz	@	@	36.0	bcd	0.4	bcd
9		Merivon	6.5 fl oz	@	@	37.4	bcd	0.5	bcd

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

** For evaluation of scab on 7-24-14, 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.

^ 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.

Spring-time applications (two applications of each treatment) for the management of scab were evaluated in field trials in 2014 (data not available for 2015) and results are presented in **Table 1.** In the first trial, rotations of Catamaran (FRAC M5/33) and Viathon (FRAC 3/33) and of Bravo (FRAC M5) with Quadris Top (FRAC 3/11) resulted in the lowest incidence of disease but Quash (FRAC 3) and Quash mixed with S2200 were also effective (**Table 1A**). Highly effective treatments were identified in the second plot and Luna Experience, that was not included in the first plot, was the most effective treatment, reducing the incidence from 97.7%

in the control to 14.4% (**Table 1 B**). All other treatments including the experimental EXP1 and several mixtures (e.g., Fontelis-Tebucon) and pre-mixtures were also very effective.

These data indicate that very effective fungicides and programs are currently available to manage almond scab. 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 at petal fall or at the onset of twig sporulation; we demonstrated previously that programs starting later in the season are not as effective for fungicides that do not have post-infection activity. Effective petal fall treatments are chlorothalonil, DMI fungicides such as Quash or Inspire Super, Syllit, Ph-D, and compounds containing SDHIs or QoIs (at locations where the pathogen population has not developed resistance). 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 as well as other classes of chemicals should be used in rotations to prevent further selection of insensitivity.

Fungicide evaluations for management of Alternaria leaf spot of almond in 2014. Our research in 2015 is ongoing; we will evaluate our Alternaria field plots in Aug./Sept. 2015. Thus, information is presented here for 2014 trials using three-spray programs in Kern and Colusa Co. Single-fungicides, mixtures, pre-mixtures, and rotation programs were used. In Kern Co., disease severity on the highly susceptible cv. Monterey was lower than in 2013 (likely due to reduced irrigation schedules and subsequently less favorable disease conditions), and no disease was found on the less susceptible cv. Fritz. On cv. Monterey, the new EXP1 was a highly effective single-treatment that reduced the incidence from 97.5% in the control to 12.5% (**Table 2**). Mixtures of EXP1 with EXP2 or EXP3 were also very effective. Isofetamid by itself or mixed with the experimental IB18111 also performed well. Among registered treatments, Merivon, Luna Sensation, Luna Experience, and rotations of the two Luna products as well as those of Inspire Super with Quadris Top, Ph-D with Tebucon, and Bravo with Quadris Top gave very good disease control with an incidence of 25% or lower.

Disease incidence was also lower than in 2013 in two field plots in Colusa Co. on cvs. Carmel and Monterey, and a rating scale was used to evaluate disease. Among the most effective treatments were Luna Sensation and Luna Experience (**Table 3 A,B**), Merivon (**Table 3 B**), EXP1 by itself and mixed with EXP2 or EXP3 (**Table 3 B**), and rotations of Ph-D/Tebucon,

Table 2. Efficacy of fungicide treatments for management of Alternaria leaf spot on almond cv. Monterey - Kern Co. 2014

				Application timings			Dis. Incid. on leaves		Dis. Sev. on leaves	
No.	Program	Treatment*	Rate	5/14	6/4	6/25	(%)	LSD^	Rating	LSD
1	---	Control	---	---	---	---	97.5	a	1.4	a
2	Single	Rhyme	7 fl oz	@	@	@	100.0	a	1.2	b
3		Isofetamid	17 fl oz	@	@	@	25.0	cdef	0.3	def
4		EXP1	5.14 fl oz	@	@	@	12.5	ef	0.1	ef
5	Mixtures	EXP1 + EXP2	3.43 + 4.57 fl oz	@	@	@	8.3	f	0.1	f
6		EXP1 + EXP3	3.43 + 5.48 fl oz	@	@	@	20.8	cdef	0.2	def
7		Isofetamid + IB18111	8.6 + 4.63 fl oz	@	@	@	25.0	cdef	0.3	def
8		Isofetamid + IB18111	10.3 + 5.57 fl oz	@	@	@	16.7	def	0.2	def
9		Isofetamid + IB18121	8.6 + 13.7 fl oz	@	@	@	37.5	bcde	0.4	cde
10		Isofetamid + IB18121	10.3 + 11 fl oz	@	@	@	55.1	b	0.6	c
11		Ph-D 11.3DF + Tebucon	6.2 + 8 oz	@	@	@	27.3	cdef	0.3	cdef
12		Fontelis + Tebucon	14 + 8 fl oz	@	@	@	16.7	def	0.2	def
13	Pre-	Luna Experience	6 fl oz	@	@	@	20.8	cdef	0.2	def
14	mixtures	Luna Sensation	5 fl oz	@	@	@	20.8	cdef	0.2	def
15		Merivon	6.5 fl oz	@	@	@	11.6	ef	0.1	f
16	Rotations	Inspire Super + DyneAmic	20 + 16 fl oz	@	---	@	12.5	ef	0.1	ef
		Quadris Top + DyneAmic	14 + 16 fl oz	---	@	---				
17		Bravo Weather-Stick	4 pt	@	---	---	25.0	cdef	0.3	def
		Quadris Top + DyneAmic	14 + 16 fl oz	---	@	@				
18		Ph-D 11.3DF + NuFilm P	6 oz	@	---	@	16.7	def	0.2	def
		Tebucon	8 oz	---	@	---				
19		Quadris Top + DyneAmic	14 + 16 fl oz	---	@	---	45.8	bc	0.5	cd
		Inspire Super + DyneAmic	20 + 16 fl oz	---	---	@				
20		Luna Sensation	5 oz	---	@	---	25.0	cdef	0.3	def
		Luna Experience	6 oz	---	---	@				

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

** Evaluations for disease were done on 8-28-14. Four leaf clusters from each of the 3 single-tree replications were evaluated for the presence and severity of disease using a rating scale from 0 = healthy to 4 = heavily diseased with 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.

Inspire Super and Ph-D or of Syllit and Ph-D/Tebucon (**Table 3 A**). The FRAC 3 compound Rhyme was not effective in any of the three trials conducted on the management of Alternaria leaf spot.

Our data indicate that Alternaria leaf spot can be effectively managed with currently available fungicides in an integrated program with cultural practices. Several rotation programs in our trials included three or four different FRAC groups (e.g., **Table 2** - Treatments 16, 17, 20; **Table 3 A** - Treatments 11 and 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. 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). For an overview on the management of scab and Alternaria leaf spot with currently registered fungicides we refer to the guidelines presented in our 2012 report or to the "Fungicide Efficacy Tables" for 2013 at http://www.ipm.ucdavis.edu (these tables are currently being updated for a 2015 version).

Establish and expand baseline sensitivities and monitor for shifts in sensitivity in populations of *Alternaria* and *Fusicladium* spp. to sub-groups of the SDHIs - 2014 Research. Samples of scab-infected fruit were obtained from an orchard in San Joaquin Co. where applications with Fontelis were not very effective. Among a total of 26 isolates, EC_{50} values for penthiopyrad varied widely from 0.2 ppm to 54.7 ppm. Six of thirteen isolates of untreated control trees had EC_{50} values <2 ppm, whereas for isolates from Fontelis-treated trees, all values were >2 ppm. This indicates a response to selection for resistance after repeated Fontelis applications and stresses the high resistance risk to this FRAC sub-group.

For isolates of Alternaria spp. with different levels of SDHI resistance we previously conducted sequence analyses of three succinate dehydrogenase sub-units. We found that differential resistance to fungicides (e.g., boscalid – bos, fluxapyroxad – fluxa, penthiopyrad – penthio, fluopyram – fluop) belonging to three SDHI sub-groups could be genetically defined: the Bos-MR/HR (moderate resistance/high resistance) + 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*. These mutations were described previously for *A. alternata,* but were not correlated with moderate or high resistance.

Isolates of *F. carpophilum* also could be placed into distinct SDHI resistance sub-groups (see our 2013 Annual Report). Sequence analyses for this species has been more difficult because the *Sdh* genes are quite different from those of *Alternaria* spp. and of other published fungi. Partial sequences of *Sdh*B and *Sdh*C from *F. carpophilium* were obtained using degenerate primers in touchdown PCR and a general gene walking approach. Approximately 1,000 bp of sequence of *Sdh*B and 450 bp of *Sdh*C were obtained and were confirmed as parts of the corresponding genes when comparing with other fungal species using the Basic Local Alignment Search Tool (BLAST) for gene sequencing. This information will be used in comparative sequence analyses of isolates with different SDHI resistance phenotypes to determine if phenotypes are also correlated with distinct mutations in the target gene for this organism.

Characterize molecular mechanisms for DMI resistance in *F. carpophilum* - 2014 and 2015 Research. Fungal resistance to DMIs is mediated either through alterations in the structure of the target enzyme *CYP51*, through increased expression of *CYP51*, or through increased expression of efflux pumps. To elucidate the mechanism in *F. carpophilum*, the *CYP51* gene was partially sequenced and primers were designed. Gene expression of twenty isolates of the pathogen with different fungicide sensitivities was quantified in the absence or presence of propiconazole or metconazole. Isolates were placed into three sensitivity groups for the two fungicides and expression levels (calculated as copy number) of *CYP51* were compared (**Table 4**). For both fungicides there was no correlation between fungicide sensitivity and *CYP51* expression. Values of copy number in the presence/in the absence of the fungicide were similar for the three propiconazole sensitivity groups (**Table 4**). For metconazole, one outlier among the seven sensitive strains showed a 16.6-fold increase in expression in the presence of metconazole, and this contributed to the higher average copy number of the sensitive strains. Based on these results, the mechanism of DMI resistance in *F. carpophilum* is not based on expression of this gene and needs to be further elucidated.

Table 3. Efficacy of fungicide treatments for management of Alternaria leaf spot of almond - Colusa Co. 2014

A. Cv. Carmel

No.	Program	Treatment*	Rate (/A)	Applications 5/13	6/3	7/1	Disease rating of fruit	LSD^
1	---	Control	---	---	---	---	2.4	a
2	Single	Rhyme	5 fl oz	@	@	@	2.4	a
3		Rhyme	7 fl oz	@	@	@	2.2	a
4		Isofetamid	17 fl oz	@	@	@	1.3	bc
5	Mixtures	Isofetamid + IB18111	8.6 + 4.63 fl oz	@	@	@	1.2	bcd
6		Isofetamid + IB18111	10.3 + 5.57 fl oz	@	@	@	1.2	bcd
7		Isofetamid + IB18121	8.6 + 13.7 fl oz	@	@	@	1.0	bcd
8		Isofetamid + IB18121	10.3 + 11 fl oz	@	@	@	0.8	cd
9	Pre-	Luna Experience	6 fl oz	@	@	@	1.1	bcd
10	mixtures	Luna Sensation	5 fl oz	@	@	@	0.9	cd
11	Rotations	Ph-D 11.3DF + Tebucon 45DF	6.2 + 8 oz	@	---	---	0.7	d
		Inspire Super + DyneAmic	20 + 16 fl oz	---	@	---		
		Ph-D 11.3DF	6.2 oz	---	---	@		
12		Syllit 65WG	32 oz	@	---	---	1.0	bcd
		Ph-D 11.3DF + Tebucon 45DF	6.2 + 8 oz	---	@	@		
13		Inspire Super + DyneAmic	20 + 16 fl oz	---	@	---	1.6	b
		Quadris Top + DyneAmic	14 + 16 fl oz	@	---	@		

B. Cv. Monterey

No.	Program	Treatment*	Rate (/A)	Applications 5/20	6/10	7/9	Disease rating of fruit	LSD^
1	---	Control		-----	-----	-----	2.3	a
2	Single	Rhyme	5 fl oz	@	@	@	2.1	a
3		Rhyme	7 fl oz	@	@	@	2.3	a
4		EXP1	5.14 fl oz	@	@	@	0.7	cd
5	Mixtures	EXP1 + EXP2	3.43 + 4.57 fl oz	@	@	@	0.7	cd
6		EXP1 + EXP3	3.43 + 5.48 fl oz	@	@	@	0.6	d
7		Isofetamid + IB18111	10.3 + 5.57 fl oz	@	@	@	1.4	b
8	Pre-	Luna Experience	6 fl oz	@	@	@	0.9	cd
9	mixtures	Luna Sensation	5 fl oz	@	@	@	1.1	bcd
10		Merivon	6.5 oz	@	@	@	0.7	cd
11	Rotations	Ph-D 11.3DF + Tebucon 45DF	6.2 + 8 oz	@	---	---	1.1	bcd
		Inspire Super + DyneAmic	20 + 16 fl oz	---	@	---		
		Ph-D 11.3DF + Nufilm P	6.2 oz + 8 fl oz	---	---	@		
12		Fontelis	20 fl oz	---	@	---	0.9	bcd
		Ph-D 11.3WDG	6.2 oz	@	---	@		
13		Inspire Super + DyneAmic	20 + 16 fl oz	---	@	---	1.2	bc
		Quadris Top + DyneAmic	14 + 16 fl oz	@	---	@		

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

** For evaluation, 8 shoots at mid canopy height were rated for the presence of disease on 8-20-14 using a severity scale from 0 = no disease to 4 = severe disease.

^ 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.

Table 4. Expression of *CYP51* by *F. carpophilum* isolates with different sensitivities to DMIs in absence or presence of metconazole or propiconazole.

			Range of copy number in presence / copy number in absence of fungicide	Average copy number in presence / copy number in absence of fungicide
Metconazole	7	0.013 - 0.100	0.6 – 16.6	4.7
	6	0.12 - 0.23	0.9 – 3.7	2.4
	8	1.04 – 2.33	0.8 – 5.4	2.5
Propiconazole	6	0.038 -0.079	0.8 – 2.2	1.2
	6	0.17 – 0.78	0.3 – 3.1	1.4
	7	1.77 – 6.70	0.9 – 2.1	1.4

* Expression of *CYP51* was calculated as copy number of the gene as compared to a standard single-copy household gene (i.e., beta-tubulin).