Epidemiology and Control of Almond Scab and Alternaria Leaf Spot

Project No.: **17-PATH3-Adaskaveg**

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Objectives:

I. Etiology
   A. Determine if sexual reproduction occurs within or among orchard populations of *F. carpophilum* based on the frequency of mating-type genes using newly identified molecular primers.

II. Management
   A. Evaluate new and registered fungicides for their efficacy in managing scab and Alternaria leaf spot. New fungicides to be evaluated by themselves (e.g., Aproach, pyraziflumid, UC-1, pydiflumetofen) or as components of mixtures and pre-mixtures (e.g. EXP-AD, -AF, -13, IL-5412, UC-2) will be compared to dodine - Syllit, Luna Experience, Luna Sensation, or Merivon.
      i. Single-fungicide and pre-/mixture programs
      ii. Rotation programs of different fungicide chemistries
   B. For scab management, evaluate the effect of dormant and in-season applications
      i. Delayed dormant applications with Bravo-oil or Syllit as air applications to delay and reduce sporulation of twig lesions.
      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.
      i. Determine baseline sensitivities to UC-1 and new SDHI fungicides (isofetamid, pyraziflumid, pydiflumetofen) and evaluate cross resistance to other SDHIs (fluopyram, penthiopyrad, fluxapyroxad).

ii. Continue to characterize molecular mechanisms for SDHI resistance.
iii. Evaluate molecular mechanisms for DMI resistance in *F. carpophilum*.

Interpretive Summary (Note - This report is mainly based on our 2017 research because our 2018 project is ongoing at the time of preparing this report).

Scab (caused by *Fusicladium carpophilum;* formerly *Cladosporium carpophilum*) and Alternaria leaf spot (caused by *Alternaria alternata* and *A. arborescens*) have occurred at high incidence in many growing areas in California in recent years. Both are late spring/summer diseases that are found especially in locations with high humidity and poor air circulation (i.e., high-density plantings, orchards with inadequate soil drainage, or where trees require frequent and extended irrigations throughout the summer). Severity of both diseases was lower from 2014 to 2017 compared with previous seasons, likely because of reduced irrigation schedules due to a state-wide drought and subsequently less favorable disease conditions. No or very low levels of Alternaria spot developed at our study sites in 2017 (and in 2018 based on preliminary evaluations), but scab at both of our near-by trial sites with similar cultural practices was present at high incidence and severity. This may indicate that scab development is somewhat less dependent on high levels of relative humidity than is Alternaria leaf spot.

The sexual stage of *F. carpophilum* has never been observed on almond in California and its presence could have implications on disease and resistance management. This was confirmed using molecular genetic approaches. Thus, we found no evidence for sexual recombination, and thus, populations of the pathogen appear to only reproduce clonally by asexual reproduction (i.e., conidia). Still, resistance to QoI and SDHI and DMI fungicides has been documented (see below). The mechanism for the observed diversity in fungicide sensitivity is unknown.

In 2017, we continued to collaborate with growers, agrochemical companies, 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. Chlorothalonil-oil dormant treatments are being used widespread and effectively in northern California to delay scab inoculum production from overwintering twig lesions. Among in-season treatments (two applications starting at the onset of twig sporulation – generally in early April), Luna Experience, a mixture of Ph-D and Quash, as well as the experimental pre-mixtures UC-2 and EXP-AD (both containing a DMI fungicide) resulted in the lowest incidence. Spring-time treatments that were determined to be very effective in other years include chlorothalonil (proposed label change to 60 days PHI), other FRAC Code 3 fungicides such as Inspire Super, the FRAC Code U12 Syllit, and compounds containing FRAC Code 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 the less effective 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/environmentally-based between May and late June/early July in approximately three-week intervals with dew formation or rainfall. At one of our trial sites where Alternaria leaf spot developed at low incidence in 2017, experimental pre-mixtures (IL-

5412, IL-5413, IL-5414, and EXP-AD were similarly effective as several registered fungicides (e.g., Ph-D, Luna products, Quadris Top).

Our research on both diseases has demonstrated that effective management can be obtained with properly timed applications of currently registered fungicides belonging to three or four FRAC Codes. Rotations of these groups allows for resistance management with the goal to limit the further spread of QoI, SDHI, and DMI (scab only) resistance. For SDHI (FRAC Code 7) resistance in *Alternaria* species from almond, mutations have been identified in subunits B, C, and D of the succinate-dehydrogenase target gene that correspond with resistance to some of the SDHI subgroup fungicides. Other mutations still need to be identified. Currently, only moderate resistance with EC_{50} values of less than 0.5 ppm, but no high resistance, has been detected against fluopyram and pydiflumetofen that each belong to a different SDHI subgroup. For *F. carpophilum*, cross resistance patterns among SDHI sub-groups were different from *Alternaria* spp. Additionally, many isolates were highly insensitive against isofetamid, but as for *Alternaria* spp., only moderate resistance was identified against pydifumetofen. For both, *Fusicladium* and *Alternaria* spp., there is incomplete cross resistance among the sub-groups, but rotations among SDHI fungicides are still not recommended because new mutations may overcome sensitivity to all sub-groups. Furthermore, fungicides belonging to FRAC Code 7 should not be used by themselves, but only should be used in mixtures with FRAC Codes 3, 11, or 19. The new experimental DMI fungicide mefentrifluconazole was highly active against Alternaria isolates with EC_{50} values of less than 0.025 ppm, but many isolates of *F. carpophium* were found to be resistant.

Materials and Methods:

Determine if sexual reproduction occurs within or among orchard populations of *F. carpophilum*. Isolates of *Fusicladium* are collected each year from different locations to follow up our population structure of this pathogen. Fungal populations are evaluated by using microsatellite markers. Statistical analyses were used to describe genetic diversity of the fungus including Shannon's information index that is based on allele frequency data. Furthermore, genetic characteristics of the sampled isolates were evaluated by genetic indices such as the number of multi-locus genotypes, Stoddart and Taylor's index of multi-locus genetic diversity, and Simpson's index.

Fungicide evaluations for management of scab in 2017 and 2018. Dormant treatments were again evaluated in 2018. Air- applications with Bravo WeatherStik (4 pts/A)/oil to Monterey almond in Colusa Co. were done in January. Scab lesions on last fall's twigs growth were evaluated for sporulation in late spring of 2018.

In-season treatments were initiated after petal fall (after the onset of twig sporulation) in cv. Monterey and Carmel orchards in Colusa Co. Fungicides used in three-application programs (early May to mid-June) are indicated in **Table 1**. Disease was evaluated late August 2017 based on incidence of fruit with scab lesions and the number of lesions per fruit (disease severity).

Fungicide evaluations for management of Alternaria leaf spot of almond in 2017. The modified DSV model was used to determine initiation times of spray programs. Two trials with three applications each between mid-May and late June were established in Colusa Co. on cvs. Carmel and Monterey. Evaluations were done in mid-August 2017. For disease incidence, 8 shoots or 4 leaf clusters at mid- canopy height were rated using a severity scale from 0 = no disease to 4 = severe disease. Studies were also set up in 2018, and these are ongoing.

Establish and expand baseline sensitivities and monitor for shifts in sensitivity in populations of *Alternaria* and *Fusicladium* spp. to sub-groups of the SDHIs and to mefentrifluconazole. Sequencing of portions of the *Sdh*B, *Sdh*C, and *Sdh*D subunits of the target gene for isolates with different resistance phenotypes was continued. Baseline sensitivities for 40 isolates of *Alternaria* spp. and 22 isolates of *F. carpophilum* were determined using the spiral gradient dilution method as described previously.

Characterize molecular mechanisms for DMI resistance in *F. carpophilum* - 2017 and 2018 Research. Genetic characterization of the eburicol 14α-demethylase gene (*CYP51*) of *F. carpophilum* is being continued. The gene is very large and is being sequenced by chromosome walking where the region of interest is gradually expanded to both sides of a known sequence. Isolates with differential responses to difenoconazole, metconazole, propiconazole, and mefentrifluconazole will be sequenced to identify any mutations in the *CYP51* gene.

Statistical analysis of data. Experiments were designed with treatments in randomized complete blocks. All data were analyzed using analysis of variance and least significant difference (LSD) mean separation procedures ($P > 0.05$).

Results and Discussion:

Determine if sexual reproduction occurs within or among orchard populations of *F. carpophilum*. In collaboration with researchers studying *Fusicladium* spp. in the United States, microsatellite markers identified two putative sub-populations of *F. carpophilum* from almond. We have phenotypically characterized these with either fast or slow growth on standard cultural media. We also continued to study the population structure of *F. carpophilum* based on new collections of the pathogen. To date, no evidence for sexual recombination was found using molecular methods. The mechanism for the observed diversity among isolates of *F. carpophilum* in fungicide sensitivity to QoI, SDHI, and DMI fungicides appears to be from the selection of random mutations in target genes in the fungal populations on almond.

Scab management – 2017 and 2018 Research. Dormant treatments to reduce the production of primary inoculum in the springtime from overwintering twig lesions were applied by air in a trial on cv. Monterey in Jan. 2018. Sporulation was very low in all treatments and the control in the spring of 2018, and evaluations will be done again over the summer/fall of 2018. In previous studies, we identified chlorothalonil-oil as the most effective dormant anti-sporulation treatment with efficacy extending longer into the spring season than copper-oil. Full registration of Bravo is still pursued through the IR-4 program to change the PHI to 60 days and the rate from 4 pt/A to 6 pt/A. Dormant treatments are valuable components in integrated scab management. They should be applied when high disease levels were present in the previous season and a high level of twig infections is observed in orchard scoutings in the winter on previous year's shoot growth. 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).

Three spring applications of each treatment for the management of scab were evaluated in field trials on cvs. Carmel and Monterey almond in 2017 (data pending for 2018) and results are presented in **Table 1A, B**. Disease incidence in the controls was high (88.3% and 81.5%, respectively), and disease severity was moderate with an average of 8.3 or 6.5 lesions/fruit. On cv. Carmel where the presence of QoI resistance was confirmed, only the experimental pre-mixture UC-2 reduced the disease to low levels (i.e., 27.7% incidence, severity: 1.1 lesions/fruit) (**Table 1A**). Significant reductions in disease incidence and severity from the control were also obtained using mixtures of Quash and Ph-D (FRAC 3 + 19) or of Quash and Intuity (FRAC 3 +11), while some other treatments only resulted in a reduced severity (i.e., Merivon, IL-5412). Scab was more effectively managed in the cv. Monterey trial although a similar disease pressure existed, and timing of applications was the same as in the cv. Carmel trial. Thus, Luna Experience that was not effective on cv. Carmel was highly effective on cv. Monterey, and UC-2 and the Ph-D-Quash mixture reduced the disease to very low levels on cv. Monterey (**Table 1B**). Other very effective treatments on cv. Monterey included Rhyme, UC-1, and EXP-AD. The timing of the fungicide applications in our study may have been appropriate for Monterey but not for Carmel where twig lesion sporulation could have started earlier. Thus, infections may already have been established on Carmel when the first application was done.

These data indicate that very effective fungicides are currently available to manage almond scab, and others are in development. Under high-disease conditions, a three-spray program should include dormant applications with chlorothalonil-oil (or copper-oil) and two applications starting 2 to 3 weeks after petal fall (March or early April) or at the onset of twig lesion sporulation (we demonstrated previously that programs starting later in the season are not as effective). Under lower disease pressure, a dormant treatment or in-season treatments alone can be considered. If other summer diseases such as rust, Alternaria leaf spot, or hull rot also must be managed at an orchard site, 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). Single-site mode of action fungicides with good efficacy against scab (e.g., the DMIs Quash and Rhyme; Ph-D, UC-1, and possibly Fontelis and pyraziflumid) should always be used in mixtures or in pre-mixtures. Other effective treatments are Bravo, Syllit, and Inspire Super. Treatments containing a DMI compound are very effective, although the incidence of natural resistance against DMIs in *F. carpophilum* is high at some locations. Thus, overuse of DMIs could lead to practical or field resistance. Practical cross resistance occurs among compounds within FRAC Codes 7 and 11. Thus, resistance management should be strongly followed using rotations. None of these materials should ever be used under pre-existing, high disease levels. Because numerous products are available, a FRAC group should only be applied once per season after twig sporulation begins (this would still allow applications of these materials during bloom and petal fall when the scab pathogen is not actively growing). Under high disease levels, multi-site materials such as sulfur and captan can be used with short preharvest intervals.

Table 1. Efficacy of fungicides for the management of scab of almond - Colusa Co. 2017

A. cv. Carmel

				Applications			Dis. Incid. on fruit**		Dis. Sev. on fruit	
No.	Program	Treatment*	Rate (/A)	5-4	5-24	6-15	(%)	LSD^	Lesions	LSD
1	---	Control	---	---	---	---	88.3	a	8.3	a
2	Single	Aproach 2.08SC + NIS	8 fl oz	@	@	@	83.2	a	7.9	ab
3		Fontelis*	20 fl oz	@	@	@	83.5	a	8.1	ab
4	Mixtures	Ph-D + Quash*	6.2 + 2 oz	@	@	@	44.3	bc	3.2	cd
5		Aproach 2.08SC + Fontelis + NIS	8 + 14 fl oz	@	@	@	76.0	a	5.9	abc
6		Aproach 2.08SC + Fontelis + NIS	8 + 16 fl oz	@	@	@	71.8	a	6.5	abc
7		Quash + Intuity	2 oz + 2 fl oz	@	@	@	42.1	bc	2.9	cd
8	Pre-	Luna Sensation + NIS	7.8 + 8 fl oz	@	@	@	64.7	ab	4.7	abcd
9	mixtures	Luna Experience + NIS	8 + 8 fl oz	@	@	@	84.8	a	7.8	ab
10		Merivon + Sylcoat	6.5 + 3.84 fl oz	@	@	@	62.0	ab	3.2	cd
11		UC-2 + Sylcoat	6 + 3.84 fl oz	@	@	@	27.7	c	1.1	d
12		IL-5413	15.5 fl oz	@	@	@	69.6	ab	5.9	abc
13		IL-5414	15.5 fl oz	@	@	@	61.0	ab	4.9	abcd
14		IL-5412	15 fl oz	@	@	@	75.9	a	4.3	bcd

Pathogen population was determined to be QoI-resistant.

B. cv. Monterey

				Applications			Dis. Incid. on fruit**		Dis. Sev. on fruit	
No.	Program	Treatment*	Rate (/A)	5-4	5-24	6-15	(%)	LSD^	Lesions	LSD
1	---	Control	---	---	---	---	81.5	a	6.5	a
2	Single	Ph-D*	6.2 oz	@	@	@	24.9	bc	1.5	b
3		Rhyme*	7 fl oz	@	@	@	13.0	cdef	0.8	b
4		Fontelis*	20 fl oz	@	@	@	19.4	bcd	1.0	b
5		Pyraziflumid + NIS	3.38 + 4 fl oz	@	@	@	34.0	b	1.3	b
6		Pyraziflumid + NIS	5.08 + 4 fl oz	@	@	@	18.4	bcde	0.5	b
7		UC-1 + Sylcoat	4 + 3.84 fl oz	@	@	@	13.7	bcdef	0.5	b
8	Mixture	Ph-D + Quash*	6.2 + 3.5	@	@	@	4.0	def	0.1	b
9	Pre-	Luna Experience + NIS	8 + 8 fl oz	@	@	@	5.3	ef	0.2	b
10	mixtures	IL-5412	15 fl oz	@	@	@	23.9	bcde	1.2	b
11		UC-2 + Sylcoat	6 + 3.84 fl oz	@	@	@	7.7	cdef	0.4	b
12		EXP-AD	11 fl oz	@	@	@	7.1	def	0.3	b
13		EXP-AD	13.7 fl oz	@	@	@	3.0	f	0.1	b

* Treatments were applied using an air-blast sprayer at a rate of 100 gal/A. NIS =non-ionic surfactant (Breakthru). Treatments with an * received NIS (non-ionic surfactant) at the second and third applications only.

** For evaluation of scab on 8-23-17, 25-30 random fruit were collected from each tree and the number of lesions on each fruit was counted.

^ 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.

Fungicide evaluations for management of Alternaria leaf spot of almond in 2017. Our research in 2018 is ongoing, thus, information is presented here for our 2017 trials. No disease developed in a field trial on cv. Carmel in Colusa Co. where Fontelis, Rhyme, UC-1, pyraziflumid, Luna Sensation, Merivon, UC-2, as well as several mixture and rotation programs were evaluated. A low level of disease occurred in the cv. Monterey trial in Colusa Co. and severity on control trees was rated at 1.7 of a maximum rating of 4 (**Table 2**). Among treatments evaluated, experimental pre-mixtures (IL-5412, IL-5413, IL-5414, and EXP-AD were similarly effective as the registered Ph-D, Luna Sensation, Luna Experience, and Quadris Top.

Our data from previous years indicated that Alternaria leaf spot can be effectively managed when present at high levels with currently available (e.g., Luna Experience, Merivon, Quadris Top, Inspire Super, Fontelis + Aproach, Ph-D + Syllit, Ph-D + Quash) and new (e.g., UC-2) fungicides that are being developed for use on almond. Rotations with fungicide classes currently available should be used for resistance management. Additionally, fungicide applications should be used in an integrated program with cultural practices. For a summary on the management of scab and Alternaria leaf spot with currently registered fungicides we refer to the "Fungicide Efficacy Tables" for 2017 at http://www.ipm.ucdavis.edu).

Table 2. Efficacy of fungicide treatments for management of Alternaria leaf spot on cv. Monterey almond - Colusa Co. 2017

				Applications			Disease	
No.	Program	Treatment*	Rate (/A)	5-18	6-7	6-20	Rating	LSD^
1	---	Control	---	---	---	---	1.7	a
2	Single	Ph-D + NIS	6.2 oz + 8 fl oz	@	@	@	0.5	b
3	Mixture	Quash + Intuity + NIS	3.36 oz + 3.36 + 8 fl oz	@	@	@	0.7	b
4	Pre-mixtures	Luna Sensation + NIS	7.8 fl oz	@	@	@	0.6	b
5		Luna Experience + NIS	8 fl oz	@	@	@	0.6	b
6		Quadris Top + DynAmic	14 + 14 fl oz	@	@	@	0.4	b
7		EXP-AD + NIS	11 fl oz	@	@	@	0.6	b
8		EXP-AD + NIS	13.7 fl oz	@	@	@	0.6	b
9		IL-5412 + NIS	15 fl oz	@	@	@	0.5	b
10		IL-5413 + NIS	12.5 fl oz	@	@	@	0.5	b
11		IL-5413 + NIS	15.5 fl oz	@	@	@	0.5	b
12		IL-5414 + NIS	12.5 fl oz	@	@	@	0.5	b
13		IL-5414 + NIS	15.5 fl oz	@	@	@	0.4	b

* Treatments were applied using an air-blast sprayer at a rate of 100 gal/A. NIS = non-ionic surfactant.

** For disease evaluation (8-15-17), the amount of fallen leaves under each tree was rated on a scale from 0 to 4.

^ 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.

Establish and expand baseline sensitivities and monitor for shifts in sensitivity in populations of *Alternaria* and *Fusicladium* spp. to sub-groups of the SDHIs and to mefentrifluconazole. No fungicide failures were reported to us in 2017 (except for the QoI resistance in the Carmel scab study) and to date in 2018; thus, fungicides that we evaluated and are currently recommending to the almond industry are performing well, and our suggested resistance management strategies have been successful to date.

Resistance in *Alternaria* species from almond to certain SDHI subgroups was previously correlated by us with mutations in subunits B, C, and D of the succinate-dehydrogenase target gene. Other mutations still need to be identified. Currently, only moderate resistance (EC_{50} values <0.5 ppm), but no high resistance, has been detected against fluopyram and pydiflumetofen that each belong to a different SDHI subgroup. Pydiflumetofen overall showed the highest in vitro toxicity with EC_{50} values among 40 isolates between 0.001 and 0.045 ppm (except for one outlier with 0.215 ppm). Various levels of resistance have been found for boscalid (EC_{50} values up to >20 ppm), fluxapyroxad (EC_{50} values up to 1.6 ppm), penthiopyrad (EC_{50} values up to >20 ppm), isopyrazam (EC_{50} values up to 4.4 ppm), isofetamid (EC_{50} values up to 2.4 ppm), and pyraziflumid (EC_{50} values up to >40 ppm). For *F. carpophilum*, cross resistance patterns among SDHI sub-groups were different from *Alternaria* spp., but like *Alternaria* spp., only moderate resistance was identified in *F. carpophilum* against pydifumetofen (EC_{50} values up to >1.2 ppm). Many isolates of *F. carpophilum* were highly insensitive against boscalid, fluxapyroxad, penthiopyrad, isofetamid, and pyraziflumid (EC_{50} values up to >40 ppm), and some were resistant to fluopyram (EC_{50} values up to 34 ppm).

For both, *Fusicladium* and *Alternaria* spp., there is incomplete cross resistance among the sub-groups, but rotations among only SDHI fungicides are still not recommended because new mutations may overcome sensitivity to all sub-groups. Furthermore, fungicides belonging to FRAC Code 7 should not be used by themselves, only should be used in mixtures with FRAC Codes 3, 11, or 19. Management programs should rotate between the FRAC Codes with efficacy against these diseases.

Sequence analyses for isolates of *F. carpophilum* is much more difficult because the *Sdh* sequences are quite different from those of *Alternaria* spp. and of other published fungi. In comparing a 100-amino acid portion of *Sdh*B for eight isolates with different resistance phenotypes, no differences were detected that correlated with resistance level. Sequencing of other parts of this and of other subunits is ongoing.

Characterize molecular mechanisms for DMI resistance in *F. carpophilum* - 2017 and 2018 Research. The new experimental DMI fungicide mefentrifluconazole was highly active against Alternaria isolates with EC_{50} values for 40 isolates of $\geq$0.025 ppm, but many isolates of *F. carpophium* were found to be resistant. We compared in vitro growth and sporulation rates of DMI-sensitive and -resistant isolates. There were no consistent differences observed among the resistance phenotypes indicating no fitness penalty for resistance. Fungal resistance to DMIs is mediated either through mutations in the target gene *CYP51*, through increased expression of *CYP51*, or through increased expression of efflux pumps. To elucidate the mechanism in *F. carpophilum*, in last year's study, we did not find differences in gene expression among 22 isolates of *F. carpophilum* with different levels of DMI resistance. This year, we focused on sequencing part of the *CYP51* gene using chromosome walking. For an approximately 200-amino acid portion of the gene, we found no differences among six isolates with different levels of resistance. Currently, we are extending these sequence studies to obtain a larger portion of the gene. These latter results possibly eliminate the second mechanism listed above and thus, less sensitive isolates may be tolerating these fungicides by point mutations in regions of the gene that were not sequenced or by increased expression of efflux pumps. This latter type of resistance may be part of a constitutive mechanism that can be overcome with the use of higher rates of these types of fungicides since this mechanism usually conveys only low levels of fungicide resistance. In contrast, point mutations often lead to high levels of resistance.