Epidemiology and Control of Almond Scab and Alternaria Leaf Spot

Project No.: 15-PATH3-Adaskaveg

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Objectives:

I. Etiology
   A. Determine the *F. 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 SDHI fungicides: 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 2015 research because our 2016 project is ongoing). Scab (caused by *Fusicladium carpophilum;* formerly *Cladosporium carpophilum*) and Alternaria leaf spot (caused by *Alternaria alternata* and *A. arborescens* – several recent taxonomic studies indicate that *A. tenuissima* is con-specific – belonging to the same species -- with *A. alternata*) 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 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 in 2014 and 2015 compared to previous seasons likely because of reduced irrigation schedules due to a state-wide drought 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 continue to analyze populations of the pathogen from different growing areas in the state. To date, 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 in the spring of 2016. Twig lesion sporulation was very low in the untreated controls, likely because of low disease incidence in 2015. Among in-season treatments (two applications starting at the onset of twig sporulation), the experimental pre-mixtures EXP-2 and EXP-3, as well as Inspire, a rotation of Ph-D+ Quash with Fontelis+Tebucon, and the experimental EXP-1 resulted in the lowest scab incidence. Spring-time treatments that were determined to be very effective in other years include chlorothalonil (proposed label change to 60 days PHI), FRAC Group 3 fungicides such as Inspire Super, Syllit (FRAC Group U12), and compounds containing FRAC Group 11 (at locations where the pathogen population has not developed resistance). Fungicides belonging to FRAC Group 7 should not be used by themselves and only should be used in mixtures with FRAC Groups 3, 11, or 19. For scab management under high-disease conditions, a three-spray program should include dormant applications with chlorothalonil-oil (or copper-oil) and two in-season 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. 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 experimental pre-mixtures EXP-2 and EXP-3, as well as Ph-D, the mixtures Fontelis-Ph-D and Fontelis-Tebucon, Luna Sensation, and a rotation of Fontelis, Quash, and Ph-D were highly 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 with EC_{50} values of >2 ppm (as compared to <0.05 ppm for sensitive isolates) is common in some populations of *F. carpophilum*. Studies were continued to determine the molecular mechanism of resistance. Expression of the *CYP51* gene that is correlated with resistance in some other plant pathogens was not related to in vitro sensitivity values for metconazole or propiconazole of 20 isolates of *F. carpophilum* and additionally, no mutations were found in a 200-amino acid portion of the gene. Growth and sporulation rates of sensitive and resistant isolates were found to be similar, indicating no fitness penalty for the resistant phenotype.

Materials and Methods:

Etiology of scab and Alternaria leaf spot and population structure of scab. Additional isolates of *Fusicladium* are continuously being collected from different locations to follow-up our studies on the population structure of this pathogen. Fungal populations will be evaluated by AFLP analyses, fragment patterns will be scored on computer-generated gels, and the Parsimony Tree Length Permutation Test (PTLPT) method will be applied to determine if recombination occurs within or among populations.

Representative isolates of *Alternaria* spp. from almond that were previously identified morphologically as *A. alternata, A. tenuissima,* or *A. arborescens* were included as references in a taxonomic study on isolates of this genus. Genetic diversity was assessed by UPGMA analysis of AFLP data. ITS sequences of representative isolates of clusters from the UPGMA analysis were then obtained and analyzed together with reference sequences from Genbank.

Fungicide evaluations for management of scab in 2015 and 2016. Dormant treatments were again evaluated in 2016 and applications to Monterey almond in Colusa Co. were done in January. Treatments included Syllit or Bravo WeatherStik (4 pts/A), both mixed with 3.5% oil. Scab lesions on last fall's twigs growth were evaluated for sporulation in the spring of 2016.

In-season treatments were initiated after petal fall (after the onset of twig sporulation) in a cv. Monterey orchard in Colusa Co. Fungicides used in a two-application program are indicated in **Table 1**. Disease was evaluated late June 2015 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 2015. The modified DSV model was used to determine initiation times of spray programs. Two trials were established in Colusa Co. on cvs. Carmel (applications on 4-29, 5-20, 7-7-15) and Monterey (applications on 5-28 and 6-30-15). Treatments used are found in **Table 2A and 2B**). Evaluations were done in mid-August 2015. 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. Sequencing of a portion of the *Sdh*B subunit of the target gene for eight isolates with different resistance phenotypes was continued and sequences were compared.

Characterize molecular mechanisms for DMI resistance in *F. carpophilum* - 2015 and 2016 Research. Differences in fitness parameters for 22 isolates of the pathogen with different levels of DMI sensitivity were determined in in vitro studies. For this growth and sporulation rates on potato dextrose agar were determined using standardized protocols. Growth and sporulation rates were then regressed on EC_{50} values; statistical significance of the models were determined. The eburicol 14α-demethylase gene (*CYP51*) of *F. carpophilum* was partially sequenced by chromosome walking.

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. We will continue to study the population structure of *Fusicladium carpophilum* based on new collections of the pathogen. In a taxonomic study on isolates of *Alternaria*, many haplotypes with unique AFLP banding patterns were identified. Reference isolates of *A. alternata* and *A. ternuissima* from almond clustered together in a UPGMA dendrogram, whereas isolates of *A. arborescens* were found in another major cluster. Sequence analysis of the ITS region of representative isolates confirmed that *A. alternata* and *A. tenuissima* could not be separated, but *A. arborescens* was more distinct. Therefore, we agree with several recent taxonomic studies on isolates of the *Alternata* group from other hosts that *A. alternata* and *A. tenuissima* should be combined into a single species. *A. arborescens* is suggested by some authors to maintain its species status, whereas others propose that it should be assigned as a subspecies of *A. alternata*.

Scab management – 2015 and 2016 Research. Dormant treatments to reduce the production of primary inoculum in the springtime from overwintering twig lesions were applied in a trial on cv. Monterey in Jan. 2016. Sporulation was very low in all treatments in the spring of 2016 due to low disease pressure in 2015, and no efficacy data could be obtained. As of the writing of this report, disease levels at our trial locations are also low in 2016, although high rainfall and favorable infection conditions occurred in this year's spring. The drought conditions in the summer of 2015 also were unfavorable for twig lesions to develop in the fall 2015 and winter of 2016. This demonstrates the importance of primary inoculum availability for scab epidemics to develop and of treatments that can reduce sporulation. 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 and should be applied when high disease levels were present in the previous season and a high level of twig infections is present 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).

Table 1. Efficacy of fungicides for the management of scab of almond cv. Monterey - Colusa Co. 2015

				Applications		Dis. Incid. on fruit**		Dis. Sev. on fruit	
No.	Program	Treatment*	Rate (/A)	4/21	5/12	(%)	LSD^	Lesions	LSD
1	---	Control	-----	-----	-----	94.2	a	2.3	a
2	Single	Rhyme	7 fl oz	@	@	25.3	de	0.3	ef
3		Inspire	7 fl oz	@	@	13.8	e	0.1	f
4		Quash	3.36 oz	@	@	19.0	e	0.2	f
5		EXP-1	5.14 fl oz	@	@	16.6	e	0.2	f
6		RON	6 fl oz	---	@	69.5	b	1.2	b
7		RON	4 fl oz	---	@	52.0	bcd	0.9	bcd
8	Mixtures	Kenja + IB18121	8.6 fl oz + 12.9 fl oz	@	@	57.2	bc	0.9	bc
9		Ph-D + Tebucon 45 + NuFilm P	6.2 oz + 8 oz + 8 fl oz	@	@	18.6	e	0.2	f
10		Quash + S2200	3.36 oz + 3.36 oz	@	@	25.3	de	0.3	ef
11		Fontelis + Tebucon 45	20 fl oz + 8 oz	@	@	24.2	e	0.4	cdef
12	Pre-	Luna Experience	6 fl oz	@	@	50.0	bcd	0.8	bcde
13	mixtures	Luna Sensation	5 fl oz	@	@	34.1	cde	0.6	cdef
14		EXP-2	7 fl oz	@	@	14.8	e	0.1	f
15		EXP-3	7 fl oz	@	@	11.6	e	0.1	f
16		Merivon	6.5 fl oz	@	@	20.2	e	0.3	ef
17	Rotations	Ph-D + Quash + NuFilm P	6.2 oz + 3 oz + 8 fl oz	@	---	14.3	e	0.2	f
		Fontelis + Tebucon 45 + NuFilm P	20 fl oz + 8 oz + 8 fl oz	---	@				
18		Bravo WeatherStik	64 fl oz	@	---	27.7	de	0.3	def
		Quadris Top + DyneAmic	14 fl oz + 16 fl oz	---	@				
19		Catamaran	64 fl oz	@	---	34.7	cde	0.6	cdef
		Viathon	64 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 of scab on 6-30-15, 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.

Two applications of each treatment in the spring for the management of scab were evaluated in a field trial in 2015 (data not available yet for 2016) and results are presented in **Table 1.** Although disease incidence was high (94.2% in the control), disease severity was very low (2.4 lesions/fruit in the control). Several treatments resulted in very low disease levels. The lowest disease incidence was found with applications of the experimental pre-mixtures EXP-2 (14.8%) and EXP-3 (11.6%), as well as Inspire (13.8%), a rotation of Ph-D+ Quash with Fontelis+Tebucon (14.3%), and the experimental EXP-1 (16.6%); but several other treatments were also very effective. The experimental RON was not very effective, most likely because an application was only made at the second timing.

These data indicate that very effective fungicides and programs are currently available to manage almond scab. Additional new effective treatments are in development. 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. If other summer diseases such as rust, Alternaria leaf spot, or hull rot also have to 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).

In-season treatments should start at petal fall or at the onset of twig lesion sporulation; we demonstrated previously that programs starting later in the season are not as effective. Effective treatments are chlorothalonil, DMIs such as Quash, Inpire, 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. Thus, overuse of DMIs could lead to practical or field resistance. Practical cross resistance occurs among compounds within FRAC Groups 7 and 11. Thus, resistance management should be strongly followed with DMI (FRAC 3), as well as other modes of actions in FRAC Groups 7 and 11 should be used in rotations to prevent further selection of insensitivity. None of these materials should ever be used once an epidemic begins, and with many products available, only one treatment per season should be applied once twig sporulation begins (this would still allow applications of these materials during bloom and petal fall).

Fungicide evaluations for management of Alternaria leaf spot of almond in 2015. Our research in 2016 is ongoing, thus, information is presented here for our 2015 trials. Single-fungicides, mixtures, pre-mixtures, and rotation programs were used in two trials (**Tables 2A, B**). As for scab, the incidence of disease was high in both trials (82.3% and 73.7% in the controls), but Alternaria leaf spot severity was low. In the first trial on cv. Carmel, the first application on 4-29-15 was intended as a scab treatment (however, no scab developed in this trial), whereas the following two applications were targeting both, scab and Alternaria leaf spot. Several treatments reduced the incidence of disease to low levels (**Table 2A**). Numerically the lowest incidence was obtained using the experimental pre-mixtures EXP-2 (10.8%) and EXP-3 (13.8%), and a rotation of Fontelis, Quash, and Ph-D (18.7%). Still, several other treatments also performed very well, including the registered Ph-D (20.8%), Fontelis (26.2%), and a Fontelis-Tebucon mixture (23.9%), and most fungicides significantly reduced the incidence of disease from that of the control.

In the second trial on cv. Monterey, two-spray programs again mostly significantly reduced the disease from the control (**Table 2B**). The experimental pre-mixture EXP-3 was the most effective treatment (9.4% incidence). Among registered fungicides, the Fontelis-Ph-D and Fontelis-Tebucon mixtures (both 23.7%), as well as Luna Sensation (25.2%) performed very well.

Our data indicate that Alternaria leaf spot can be effectively managed with currently available fungicides in an integrated program with cultural practices. Additionally, we identified new highly effective fungicides and pre-mixtures that are being developed for use on almond. Rotation programs in our trials that included three FRAC groups 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 on almond. 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 a summary on the management of scab and Alternaria leaf spot with currently registered fungicides we refer to the "Fungicide Efficacy Tables" for 2015 at http://www.ipm.ucdavis.edu).

Table 2. Efficacy of fungicide treatments for management of Alternaria leaf spot of almond – Colusa County - 2015

A. cv. Carmel

				Applications		Dis. Incid.leaves		Dis. Sev. leaves	
No.	Program	Treatment	Rate (/A)	5/28	6/30	(%)	LSD^	Rating	LSD
1	---	Control	-----	---	---	73.7	a	1.3	a
2	Single	Fontelis + Kinetic	20 + 8 fl oz	@	@	42.0	bcd	0.5	bcd
3		EXP-1	5.14 fl oz	@	@	25.1	de	0.3	d
4		RON + Kinetic	3 + 8 fl oz	@	@	37.7	bcd	0.5	bcd
5		RON + Kinetic	4.5 + 8 fl oz	@	@	62.2	ab	0.8	bc
6		RON + Kinetic	6 fl oz + 8 fl oz	@	@	59.6	abc	0.9	ab
7	Mixtures	Ph-D + Fontelis + Kinetic	6.2 + 20 + 8 fl/oz	@	@	23.7	de	0.3	d
8		Fontelis + Tebucon 45 + Kin.	20 + 8 + 8 fl/oz	@	@	23.7	de	0.2	d
9		Fontelis + Abound + Kin.	20 + 12 + 8 fl oz	@	@	61.4	ab	0.8	bc
10	Pre-mixtures	Luna Sensation	5 fl oz	@	@	25.2	de	0.3	d
11		EXP-2	7 fl oz	@	@	20.4	de	0.2	d
12		EXP-3	7 fl oz	@	@	9.7	e	0.1	d
13		Merivon	6.5 fl oz	@	@	34.1	cd	0.4	cd
14	Rotation	Fontelis + Kinetic	20 + 8 fl oz	@	---	37.9	bcd	0.4	bcd
		Quash	3 oz	---	@				
		Ph-D	6.2 oz	---	---				

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

** For evaluation on 8-17-15, leaves (25-30 leaves total) from each replication 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.

Table 2. Efficacy of fungicide treatments for management of Alternaria leaf spot of almond - Colusa County – 2015 (Continued)

B. cv. Monterey

				Applications			Dis. Incid. leaves		Dis. Sev. leaves	
No.	Program	Treatment	Rate (/A)	4/29	5/20	7/7	(%)	LSD^	Rating	LSD
1	---	Control	-----	---	---	---	82.3	a	1.4	a
2	Single	Ph-D	6.2 oz	@	@	@	20.8	efg	0.2	def
3		Inspire + DyneAmic	7 fl oz + 16 fl oz	@	@	@	35.6	bcdefg	0.4	bcdef
4		Fontelis	20 fl oz	@	@	@	26.2	cdefg	0.3	bcdef
5		EXP-1	5.14 fl oz	@	@	@	21.2	efg	0.2	def
6	Mixtures	Fontelis + Tebucon 45	20 fl oz + 8 oz	@	@	@	23.9	defg	0.3	cdef
7		Fontelis + Abound	20 fl oz + 12 fl oz	@	@	@	49.7	bc	0.6	bc
8		Kenja + IB18121	8.6 fl oz + 12.9 fl oz	@	@	@	58.9	ab	0.7	b
9	Pre-mixtures	Luna Experience	6 fl oz	@	@	@	41.8	bcdef	0.5	bcdef
10		Luna Sensation	5 fl oz	@	@	@	33.1	bcdefg	0.3	bcdef
11		EXP-2	7 fl oz	@	@	@	10.8	g	0.1	f
12		EXP-3	7 fl oz	@	@	@	13.8	g	0.1	ef
13		Merivon	6.5 fl oz	@	@	@	44.3	bcde	0.6	bcd
14	Rotations	Fontelis	20 fl oz	@	---	---	18.7	fg	0.2	def
		Quash	3 oz	---	@	---				
		Ph-D	6.2 oz	---	---	@				
15		Bravo WeatherStik	64 fl oz	@	---	---	49.2	bcd	0.5	bcdef
		Quadris Top + DyneAmic	14 fl oz + 16 fl oz	---	@	@				

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

** For evaluation on 8-17-15, leaves (25-30 leaves total) from each replication 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.

Establish and expand baseline sensitivities and monitor for shifts in sensitivity in populations of *Alternaria* and *Fusicladium* spp. to sub-groups of the SDHI fungicide group. Additional fungicides will be included in our baseline sensitivity studies in 2016-2017. No fungicide failures were reported to us in 2015 and to date in 2016; 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.

For isolates of *Alternaria* spp. with different levels of SDHI resistance we previously conducted sequence analyses of sub-units of the target gene succinate dehydrogenase. 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*. Thus, resistance genotypes could be genetically defined in two of the four sub-units of the *Sdh* gene. As FRAC Group 7 materials are used additional selection will occur on the survivors. Thus, resistance management must be strictly practiced.

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 fungicide group resistance in *F. carpophilum* - 2015 and 2016 Research. 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. In regression analyses of spore production or mycelial growth rates on EC_{50} values, models were not significant. Still, other fitness parameters such as virulence would have to be evaluated to make more definite conclusions about persistence of resistant isolates within populations. Because inoculations with *F. carpophilum* are difficult to perform, this goal is not easily achieved.

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*, 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. 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.