Epidemiology and Control of Alternaria Leaf Spot

Project No.: **08-PATH3-Adaskaveg**

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Objectives:

I. Etiology
   A. Identify pathogenic species of *Alternaria* using molecular methods.
   B. Determine the pathogen species composition within and between selected orchards at the beginning and at the end of the log phase of the epidemic. This objective is contingent on the development of molecular methods for identification of the pathogens.

II. Management
   A. Evaluate new and registered fungicides for their efficacy in managing Alternaria leaf spot. Fungicides to be evaluated include non-strobilurin fungicides (e.g., dodine-Syllit, tebuconazole-Elite, difenoconazole-Inspire, Ph-D-polyoxin-D, iprodione-Rovral, as well as, other experimental materials) and efficacy will be compared to the strobilurin fungicides (Abound, Gem, Pristine).
      i. Small-scale trials – all materials listed above
      ii. Large-scale trials – approved materials (i.e., Inspire, Ph-D) pending the Section 18 request
   B. Use the DSV model with defined parameters for forecasting infection periods of the disease and timing of fungicide treatments as compared to calendar programs.
   C. Evaluate rotation programs of different fungicide chemistries in preventing resistance.
   D. Monitor for shifts in sensitivity in populations of *Alternaria* spp. to carboxamides and strobilurins
   E. Evaluate in-vitro sensitivity of *Alternaria* spp. against polyoxin, difenoconazole, and dodine (three fungicides with unique modes of action), as well as other potentially new fungicides.

Interpretive Summary:

In the last decade, with numerous changes in almond horticultural practices, Alternaria leaf spot has consistently caused tree defoliation during the summer growing season. It thus has become one of the major fungal foliar diseases of almond in California. Horticultural practices associated with this disease include high density plantings, shifts to micro-sprinkler irrigation and long irrigation sets, new varieties, and intense nutritional programs using both soil and foliar fertilizers for macro- and micro-nutrients.

With the introduction of the strobilurin- or QoI fungicides (with a single-site mode of action) in 1998, these materials were the only registered products available for managing the disease. Strobilurin-resistant isolates of *Alternaria* spp. were first detected in 2003 and 2004, and crop losses (practical or field resistance) have been common since 2005 in Kern, Glenn, Butte, and Tehama Co. Once resistance occurs within a class (same mode of action), all fungicides within the class are ineffective (i.e., azoxystrobin - Abound, trifloxystrobin - Gem, and pyraclostrobin - a component of Pristine). Still, Pristine was registered in 2006 for managing Alternaria leaf spot because the second fungicide in the product, boscalid (carboxamide class), was also active against the pathogen complex. In 2007, however, we detected carboxamide resistance for the first time in almond populations of *Alternaria* spp. in several counties including Kern, Glenn, and Butte Co. Resistant populations had EC_{50} values of 10-50 ppm, an over 100-fold increase.

Thus, in order to replace the strobilurins and carboxamides with new fungicides for managing Alternaria leaf spot and other foliar diseases of almond, our studies focused on generating data to identify, characterize, and register new effective materials of different modes of action against the pathogens. The two new compounds that showed most activity were the DMI (demethylation inhibitor – Group 3) fungicide difenoconazole and the chitin synthase inhibitor (Group 19) polyoxin-D (a biofungicide). Baseline sensitivity data were developed for difenoconazole with an EC_{50} value range of 0.01-0.05 ppm and is ongoing for polyoxin-D. In our 2008 trials in Kern and Colusa Co., three calendar-based applications of polyoxin-D (Endorse, PhD), difenoconazole (Inspire), metconazole (Quash), or a SBI-strobilurin pre-mixture (Adament) were highly effective in reducing the incidence of disease from the control.

In the spring of 2008 and 2009, difenoconazole received a Section 18 registration for management of Alternaria leaf spot of almond in Butte, Glenn, Kern, and Tehama Co. In 2009, polyoxin-D received a federal registration at a low rate for multiple crops; while the California registration is pending. Additional efforts are needed to establish higher rates that are much more effective against *Alternaria* spp. Field trials were again conducted in 2008 and 2009 with an emphasis on the evaluation of difenoconazole, polyoxin-D, USF-2015 (fluopyram), Omega (fluazinam), and several new fungicide pre-mixtures (each component being a different mode of action) that are still under development including A13703, A13909, A8122B, USF2016, and USF2017. Integrated approaches and effective fungicide-use programs that implement resistance management strategies hold the most promise for successfully managing the disease under the horticultural practices of almond in California.

Introduction:

Alternaria leaf spot of almond is a disease that is caused by three species in the *Alternaria alternata* complex: *A. arborescens*, *A. alternata*, and *A*. *tenuissima*. Under favorable conditions for disease development, trees can be completely defoliated by early to mid-summer. The disease occurs mostly in the southern and northern production regions of California but continues to spread as horticultural practices including high density plantings, micro-sprinkler irrigation and long irrigation sets, new varieties, and intense nutritional programs using both soil and foliar fertilizers for macro- and micro-nutrients are implemented. It is most severe in areas where dew forms, the air is stagnant, and temperatures are high during the summer months. The disease can be severe in almond orchards that have been planted in high density or in orchards with poor soils where trees require frequent and extended irrigations into the summer.

Properly timed fungicide treatments have been very effective for a number of years; however, strobilurin (QoI) and carboxamide fungicide-resistant populations of *Alternaria* spp. have developed over the last 5 to 6 years because of the over-use of one group of fungicides with a single-site mode of action. QoI-resistant isolates were first detected in 2003 and 2004 and crop losses were common by 2005 and 2006 in Kern Co. and by 2007 in several almond-producing counties in northern California including Glenn, Butte, and Tehama Co. Once resistance occurs within a class, all fungicides within the class are ineffective (i.e., azoxystrobin - Abound, trifloxystrobin - Gem, and pyraclostrobin - a component of Pristine) because they have the same mode of action.

Similarly, carboxamide (boscalid – the second fungicide in Pristine) resistance was detected in 2007 (one year after introduction of the fungicide with a 25-day PHI) and again in 2008. Resistant isolates of *Alternaria* spp. were found in the last two years in several counties including Kern, Glenn, and Butte Co. The baseline sensitivity of isolates of the pathogen complex from almond that were never exposed to the fungicide boscalid consisted of a range of EC_{50} values from 0.015-0.058 ppm. Carboxamide resistant isolates had a 100-fold increase (EC_{50} values 10 - 50 ppm) from the baseline sensitivity. In order to replace the strobilurins and carboxamides with new fungicides for managing Alternaria leaf spot and other foliar diseases of almond, our studies focused on generating data to identify, characterize, and register new effective materials of different modes of action against the pathogens.

Materials and Methods:

Evaluation of DSV model for predicting infection periods and timing fungicide applications

Data from the Western Farms weather monitoring system in Kern Co. was used to summarize environmental data collected from almond orchards including the site where one of our fungicide efficacy trials was conducted. Temperature-leaf wetness data was evaluated using the DSV model (previously described in our Annual Reports for the Almond Board of California).

Development of baseline sensitivity data and resistance assessment studies

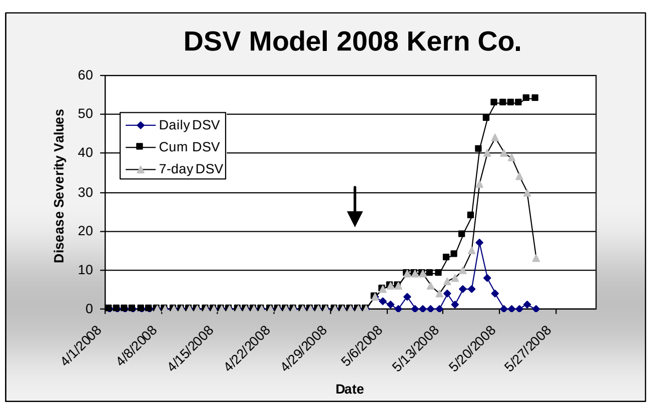
In laboratory assays, isolates of the three species of Alternaria that were collected over the last several years were exposed to a continuous range of concentrations of metconazole, difenoconazole, polyoxin-D, and fluazinam using the spiral gradient dilution technique. The isolates were collected from almond orchards for selected counties in northern and southern California. Inhibition of growth was recorded at the effective concentration to inhibit 50% growth (EC_{50} value). Values were compared for populations never exposed to the fungicide (baseline) and to populations that have been exposed to selected registered fungicides (i.e., azoxystrobin, boscalid, etc.).

<u>Fungicide evaluations for management of Alternaria leaf spot of almond in 2008</u>
Three trials were established: one in Kern Co. and two trials in Glenn Co. (near Tehama Co. border). The first trial was on cvs. Carmel and Monterey, whereas the second and third trials were on cv. Carmel. In the Kern Co. plot, the main plot received five-week-after petal-fall applications (April 15) of Ziram and Rovral, or received no application (control treatment). Trees were then treated with a subsequent DSV model-based application program and three sprays were applied (May 14, June 4, and June 20). In Glenn Co., timings included three applications: May 13, June 6, and June 23. Treatments in both plots included strobilurins in pre-mixtures with a carboxamide (i.e., Pristine) or a SBI fungicide (i.e., Adament), SBI fungicides (i.e., Inspire, Orbit, Quash), the chitin inhibitor polyoxin-D (i.e., Ph.D. or Endorse), and mixtures of Orbit or Inspire with Vangard (Inspire Super) or with a strobilurin (A13703,A13909), as well as Syllit, USF-2015, -2016, -2017, and A8122B . Evaluations of all trials were done in mid-August. For disease evaluation in the field, trees were rated for defoliation based on a scale from 0 (= no defoliation) to 4 (= more than 75% of the leaves had fallen). For incidence, ca. 100 leaves from each of four single-tree replications were evaluated (% leaves infected of the total number of leaves).

Results and Discussion:

<u>Evaluation of the DSV model for predicting infection periods and timing fungicide applications</u>
Data from environmental monitoring studies were used to develop disease severity predictions. Specifically, we used temperature-leaf wetness data in the DSV model to predict pathogen infection periods and fungicide-application timings on almond **(Figure 1)**. The arrow in the figure indicates timing of the first fungicide application.

Figure 1. First Alternaria Leaf Spot infection period determined by the modified DSV model. (Arrow indicates timing of first fungicide application when DSV reached 10)



Alternaria leaf spot of almond is greatly influenced by microclimatic conditions such as temperature and wetness within orchards. The Disease Severity Value (DSV) model has been modified and evaluated on almond in our previous trials over several years and we have been successful in forecasting Alternaria leaf spot of almond. There is a close correlation between the increase in actual disease and increases in DSV that are determined by the number of hours of wetness within an adjusted threshold temperature of $\geq$62 F. In addition, we demonstrated that the actual development of disease incidence correlated with environmental conditions that occurred 25 days ($\pm$ 7 days) before the onset of disease symptoms (latency period of the disease progress curve).

Evaluation of materials in toxicity studies and development of baseline sensitivity data. New compounds that show the best promise for managing the disease are the DMI (demethylation inhibitor – Group 3) fungicides difenoconazole and metconazole, as well as the chitin synthase inhibitor (Group 19) polyoxin-D (a biofungicide). Baseline sensitivity data were developed for difenoconazole and polyoxin-D with EC_{50} values ranging from 0.01-0.05 ppm and 0.080-0.325, respectively. This indicates that these materials are highly toxic to the pathogen complex. A forth fungicide, fluazinam (a dinitroaniline fungicide or Group 29) was highly inhibitory in lab studies with EC_{50} values ranging from 0.008-0.013 ppm. Thus, new materials are being identified for potential registration on almond.

Fungicide evaluations for management of Alternaria leaf spot of almond. In our 2008 trials in Kern Co., three calendar-based applications of polyoxin-D (Endorse or PhD), difenoconazole/cyprodinil (Inspire Super), metconazole (Quash), or several numbered fungicides (e.g., USF2015A, USF2017A, etc.) were highly effective in reducing the incidence of disease on almond cultivars Monterey and Carmel from that of the control and significantly decreased defoliation on cv. Monterey (**Table 1**).

Table 1. Efficacy of fungicide treatments for management of Alternaria Leaf Spot on almond cvs. Monterey and Carmel - Kern Co. 2008

				Cv. Monterey				Cv. Carmel			
				Leaf Spot**		Tree Defoliation**		Leaf Spot**		Tree Defoliation	
No.	Program	Treatments*	Rate	Incid. (%)	LSD	Rating**	LSD	Incid. (%)	LSD	Rating**	LSD
1	---	Control	-----	59.9	a	2.8	a	53.6	a	3	a
2	Single	Syllit 400SC	32 fl oz	19	bc	1.3	b	14.7	bcd	2.3	ab
3	fungicides	Orbit 3.6EC	8 fl oz	23.7	b	1.8	ab	27.8	ab	2.2	ab
4		Quash 50WDG	2.5 oz	13.8	bcd	1.7	b	14.2	bcd	2.4	ab
5		USF 2015A SC	6 fl oz	7.5	cd	1.2	b	7	bcd	1.8	ab
6		Polyoxin D 11.3%	16 oz	4.2	c	1.3	b	0.8	d	2.3	ab
7		Polyoxin D 11.3%	32 oz	3.5	c	1.7	b	1.5	cd	1.8	ab
8	Pre-mixtures	Adament 50WG	8 oz	21.3	bc	1.6	b	12.2	bcd	2.3	ab
9		USF2016A SC	6 fl oz	Not done				8.2	bcd	1.9	ab
10		USF2017A SC	8 fl oz	12.5	bcd	1.5	b	8.8	bcd	1.9	ab
11		Inspire Super SC	16 fl oz	Not done				19.2	bc	2.3	ab
12		Inspire Super SC	20 fl oz	7.7	cd	1.9	ab	9	bcd	1.8	ab
13		A8122B-IO SC	7 fl oz	13.7	bcd	1.8	ab	Not done			
14		A13703G-AI SC	14 fl oz	Not done				9	bcd	2.8	ab
15		A15909A-AO SC	21 fl oz	Not done				11.5	bcd	2	ab
16		Pristine 38WG	14.5 oz	9.5	bcd	1.8	ab	10.7	bcd	1.6	b

* - The plot was of a split-plot design with replicated blocks of either untreated trees or trees treated by the grower on 4-15-08 with 8 lb Ziram 76DF/A and 1 pt Rovral 4F/A at 5-wk-after petal fall. The sub-plot consisted of spring applications of selected fungicides of different single-site mode of action. Treatments were applied on 5/14, 6/4, and 6/20 using an air-blast sprayer at a rate of 100 gal/A and there were 3 single-tree replications for each sub-plot treatment (see text for details).

** - Evaluations for disease incidence were done on 8-8-08 and for tree defoliation on 9-17-08. For disease incidence on leaves, 200 leaves from each of the 4 single-tree replications were evaluated for the presence of disease.

*** - For evaluation of tree defoliation, trees were rated based on a scale from 0 (= full canopy) to 4 (100% defoliation).
In the statistical analysis the petal fall treatments did not statistically affect the efficacy of the following
Thus, for each almond cultivar, data were combined for the subplots with or without the petal fall treatment.
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.

Pristine also significantly reduced the disease on both cultivars although a high level of strobilurin resistance was detected in the *Alternaria* spp. population in this orchard with over 80% of the isolates being resistant. The efficacy of Pristine can be explained by the relatively low incidence of boscalid resistance in pathogen populations of this orchard (ca. 30%) and therefore, rendering this fungicide still effective in reducing the disease. Treatments at 5 weeks after petal fall with iprodione (Rovral) and ziram did not significantly reduce disease. This was due to the late first infection period that occurred approximately one month after the iprodione/ziram application (**Figure 1**).

In our 2008 trials in Glenn/Tehama Co., three calendar-based applications of polyoxin-D (Endorse, PhD), difenoconazole or difenconazole/cyprodinil (Inspire and Inspire Super), metconazole (Quash), or several numbered fungicides (e.g., USF2015A, USF2017A, etc.) were highly effective in reducing the incidence of disease and significantly decreased defoliation on almond cultivar Carmel from that of the control (**Table 2**). In both of these trials, Pristine was mostly ineffective, only reducing disease from 79% and 94% in the controls to 65% and 63%, respectively. The inactivity of Pristine can be explained by the high incidence of pyraclostrobin and boscalid resistance in pathogen populations of this orchard (ca. 80%). Dodine (Syllit) was moderately effective (**Table 1**) or mostly suppressive **(Table 2)** in 2008 trials. Studies with these and other fungicides such as Omega are ongoing in the 2009 season.

Table 2. Efficacy of fungicide treatments for management of Alternaria Leaf Spot on almond cv. Carmel – Glenn/Tehama Co. 2008

A. Orchard 1

				Application Dates			Dis. Incid. on leaves**		Tree Defoliation**	
No.	Program	Treatments*	Rate	5-13	6-6	6-23	(%)	LSD***	Rating	LSD***
1	---	Control	-----	-----	-----	-----	79.0	a	2.41	a
2	Single	Syllit 400SC	32 fl oz	@	@	@	50.8	c	1.75	a
3	fungicides	Orbit 3.6EC	8 fl oz	@	@	@	35.3	d	0.91	b
4		Quash 50WDG	2.5 oz	@	@	@	27.8	de	0.56	b
5		Inspire 2.08SC	7 fl oz	@	@	@	26.3	e	0.19	c
6		Polyoxin D 11.3%	32 oz	@	@	@	18.0	f	0.22	c
7		Polyoxin D 11.3%	16 oz	@	@	@	17.8	f	0.16	c
8	Pre-mixtures	Adament 50WG	8 oz	@	@	@	45.5	c	1.84	a
9		Inspire Super SC	16 fl oz	@	@	@	20.8	ef	0.68	c
10		A8122B-IO SC	7 fl oz	@	@	@	21.0	ef	0.28	c
11		Pristine 38WG	14.5 oz	@	@	@	65.3	b	2.25	a

A. Orchard 2

				Application Dates			Dis. Incid. on leaves**		Tree Defoliation**	
No.	Program	Treatments*	Rate	5-13	6-6	6-23	(%)	LSD***	Rating**	LSD***
1	---	Control	-----	-----	-----	-----	94.0	a	2.5	a
2	Single	Syllit 400SC	48 fl oz	@	@	@	73.0	b	2.1	a
3	fungicides	Orbit 3.6EC	8 fl oz	@	@	@	38.0	c	1.6	ab
4		Quash 50WDG	2.5 oz	@	@	@	32.0	c	1.5	ab
5		USF 2015A SC	6 fl oz	@	@	@	18.0	d	0	d
6		Polyoxin D 11.3%	32 oz	@	@	@	15.0	d	0.5	cd
7		Polyoxin D 11.3%	16 oz	@	@	@	15.0	d	0.75	cd
8	Pre-mixtures	Adament 50WG	8 oz	@	@	@	42.0	c	1.7	ab
9		USF 2017A SC	8 fl oz	@	@	@	37.0	c	1.1	bc
10		Inspire Super SC	16 fl oz	@	@	@	34.0	c	1.1	bc
11		A8122B-IO SC	7 fl oz	@	@	@	47.0	c	1.1	bc
12		Pristine 38WG	14.5 oz	@	@	@	63.0	b	1.7	ab

* - Treatments were applied using an air-blast sprayer at a rate of 100 gal/A.

** - Evaluations were done on 8-12-08. For disease incidence on leaves, 100 leaves from each of the 4 single-tree replications were evaluated for the presence of disease.
For evaluation of tree defoliation, trees were rated based on a scale from 0 (= full canopy), 1 (<25% defoliation) to 4 (100% 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.

Progress on new and amended fungicide registrations

In 2008 trials, the Group 3 fungicides difenoconazole and metconazole, as well as the Group 19 polyoxin-D continued to be the most effective treatments. Metconazole is being directly registered by Valent USA Corp. and polyoxin-D by Arysta Life Science; whereas, difenoconazole (Syngenta Crop Protection) is being registered through the IR-4 specialty crop pesticide registration program. We have also requested a registration of polyoxin-D from 6.2 oz/A to a higher rate such as 12 to 16 oz/A on almond pending EPA review. The current registration of polyoxin-D will suppress disease but higher rates or new application strategies will need to be evaluated. Difenoconazole was also recommended for emergency registration in 2008 and 2009. This was approved in May (State Crisis Exemption) and tolerances were established in August 2008 (Federal Section 18). In May of 2009, the Section 18 for difenoconazole was successfully renewed for the management of Alternaria leaf spot of almond in Butte, Glenn, Kern, and Tehama Co. with a 30-day preharvest interval. A summary of registered or nearly registered fungicides that have been evaluated for managing Alternaria leaf spot is shown in **Table 3**.

Conclusion:

Management of Alternaria leaf spot with fungicides as part of an integrated strategy should start with petal fall applications that include Rovral followed by late spring applications with materials having different modes of action. Currently, no highly effective fungicides are registered in the late spring season (May-June) when the disease is initiated because resistance has developed in pathogen populations to the QoI and carboxamide fungicides. Thus, registration of Inspire (difenoconazole-Group 3), Quash (metconazole – Group 3), and the biofungicide Endorse or PhD (Polyoxin-D-Group 19) is critical in the management of Alternaria leaf spot of almond. Each fungicide group has a different single-site mode of action and thus, these materials will have to be used strictly in rotations or mixtures to delay the development of resistance and maintain their efficacy. Also, the pre-mixture Adament was moderately effective, but concerns exist because one of the components of this product is a QoI fungicide (i.e., trifloxystrobin). Additional materials, such as fluopyram (USF 2015) and various premixtures (USF 2016, USF 2017, A15703, A15909, etc.) with activity against *Alternaria* spp. need to be thoroughly tested in field trials (**Table 3**).

Because of the current limited arsenal of chemical treatments available, other components of an integrated approach in disease management are even more critical. These include insect and mite control, as well as cultural practices that lead to a decreased humidity in the micro-environment in the orchard such as hedging, improvement of water penetration into the soil by adding gypsum, and changing the watering or irrigation schedule to less frequent irrigation using soil moisture probes. If all components of the disease triangle (host, environment, and pathogen) are considered, fungicide treatments will be the most beneficial.

Table 3. Relative efficacy and registration status of selected fungicides for management of Alternaria Leaf Spot of almond.

Fungicide	Class	Mode of action	Efficacy`	Remarks
Bravo, Echo	Aromatic nitrile	Multiple	+*	Label limits on PHI
Ziram	Dithiocarbamate	Multiple	+*	Label limits on PHI
Captan	Pthalimide	Multiple	+	2*ee* amendment***
Vangard/Scala	Anilinopyrimidine	Single	+	2*ee* amendment (Scala)***
Rovral	Dicarboximide	Multiple	+++/+*	Label limits/Sect.18 disallowed
Abound/Gem	Strobilurin or QoI	Single	+++**	Res. Populations
Pristine	QoI./carboxamide	Multiple	++++**	Res. Populations
Adament	QoI/SBI	Multiple	++++**	Registration requested
Quash	SBI	Single	++++	Registration requested
Inspire/ Inspire Super	SBI/SBI+AP	Single/ Multiple	++++	Sect. 18-2008^/IR-4 studies
Polyoxin-D	Biofungicide	Single	++++	IR-4 studies
Fluazinam	Dinitroaniline	Single	?	Under evaluation

` - Rating: ++++ = most effective, - = not effective, ? = unknown.
*- Restricted to applications until 5 weeks after petal fall
** - Widespread resistance against strobilurin and carboxamide fungicides.
*** - These fungicides provide suppressive activity only.
^ - Renewal of Section 18 recommended for 2009 season.