Epidemiology and Control of Alternaria Leaf Spot

Project No.: **09-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., fluopyram or Luna Privilege (USF2015), metconazole-Quash, difenoconazole-Inspire, polyoxin-D or Ph-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) pending the Section 18 request and polyoxin-D (Ph-D) at registered rates
   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-D, difenoconazole, and fluopyram (three fungicides with unique modes of action).

Interpretive Summary:
(Note-This report is based on our 2009 data because our 2010 project is ongoing. Goals and plans for the 2010 season are discussed).

Alternaria leaf spot of almond is a disease that is caused by three very closely related species in the *Alternaria alternata* complex, *A. arborescens*, *A. alternata*, and *A*. *tenuissima*. All three species are often found together in the same orchard. 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 but continues to spread into new areas throughout California. Severe outbreaks occur in areas where dew forms, the air is stagnant, and temperatures are high during the summer months. Additionally, the disease can be severe in almond orchards that have been planted at high density or in orchards with poor soils with inadequate drainage and where trees require frequent and extended irrigations into the summer.

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 for the disease on almond in our previous reports. Properly timed fungicide treatments at DSV infection periods have been very effective in managing the disease for a number of years. Due to the widespread occurrence of populations of *Alternaria* spp. resistant to the single-site mode of action strobilurin (QoI) and carboxamide fungicides over the last 6 to 7 years, some of the most effective treatments such as azoxystrobin (Abound), trifloxystrobin (Gem), and pyraclostrobin-boscalid (Pristine) have become ineffective at many locations. Thus, our studies focused on the evaluation, best usage pattern, and registration of new effective materials of different modes of action against the pathogens. Compounds that we identified in the last few years include the DMI (demethylation inhibitor – Group 3) fungicides difenoconazole (Inspire) and metconazole (Quash), as well as the chitin synthase inhibitor (Group 19) polyoxin-D (Ph-D; a biofungicide). These treatments were compared to Pristine. In addition, we continued to evaluate fluopyram (Luna Privilege), a Group 7 fungicide in the pyridylethylamide (SDHI) class that is related to boscalid, but represents a different sub-group of this class, dodine (Syllit), as well as several new pre-mixtures including fluopyram-trifloxystrobin (Luna Sensation), difenoconazole-cyprodonil (Inspire Super), azoxystrobin-difenoconazole (Quadris Top), azoxystrobin-propiconazole (Quilt Xcel), and difenoconazole-propiconazole (Inspire XT). We are also evaluating chlorothalonil (Bravo, Echo, Equus, Chlorothalonil) as a multi-site material for managing Alternaria and we have proposed a label change to reduce the preharvest interval to 60 days from the current 150-day interval.

Materials and Methods:

Re-evaluation of the DSV model for predicting infection periods and timing fungicide applications. The original DSV model was developed for predicting black mold of tomato caused by *Alternaria alternata*. In our studies, we used data from the Western Farms weather monitoring system (a network of environmental weather stations) in Kern County to summarize data collected from almond orchards including different sites where fungicide efficacy trials were conducted over four years (2003 to 2008). Temperature-leaf wetness data were summarized daily and plotted as daily values, a 7-day index (incremental addition and removal of newest and oldest daily values in a 7-day increment), and a seasonal accumulation.

Forecasting of the disease was based on these parameters and actual disease progress was evaluated and compared in each year of the study.

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, chlorothalonil, and fluopyram using the spiral gradient dilution technique. The isolates were collected from almond orchards from selected counties in northern and southern California. Inhibition of growth was recorded at the effective concentration to inhibit growth by 50% (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-QoI, boscalid-SDHI, etc.).

Fungicide evaluations for management of Alternaria leaf spot of almond in 2009. Three trials were established: one in Kern County and two trials in Glenn County (near Tehama County border). The first trial was on cvs. Carmel and Monterey, the second and third trials were on cv. Carmel. In the Kern County 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 County, timings included three applications: May 13, June 6, and June 23. Treatments in both plots included strobilurins in pre-mixtures with a SDHI (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, as well as Syllit, Luna Privilage, Luna Sensation, Luna Experience, and Inspire XT. 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:

Re-evaluation of the DSV model for predicting infection periods and timing fungicide applications. 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 >62 F (**Figure 1**). In addition, we demonstrated that the actual development of disease correlated with environmental conditions that occurred 25 days ($\pm$ 7 days) before the onset of disease symptoms (latency period of the disease progress curve) (**Figure 2**).

Disease severity values (DVS) as a function of leaf wetness duration and average air temperature during the wetness period.

Mean temperature (C) during wetness	Leaf wetness duration (hours)				
15-17*	0-6	15-17	16-20	21	---
17.1-20	0-3	4-8	9-15	16-22	23+
20.1-25	0-2	3-5	6-12	13-20	21+
25.1-29	0-3	4-8	9-15	16-20	23+
DSV	0	1	2	3	4

* - Original model was 11-17C

Figure 1. The DSV model for forecasting diseases caused by *Alternaria alternata*

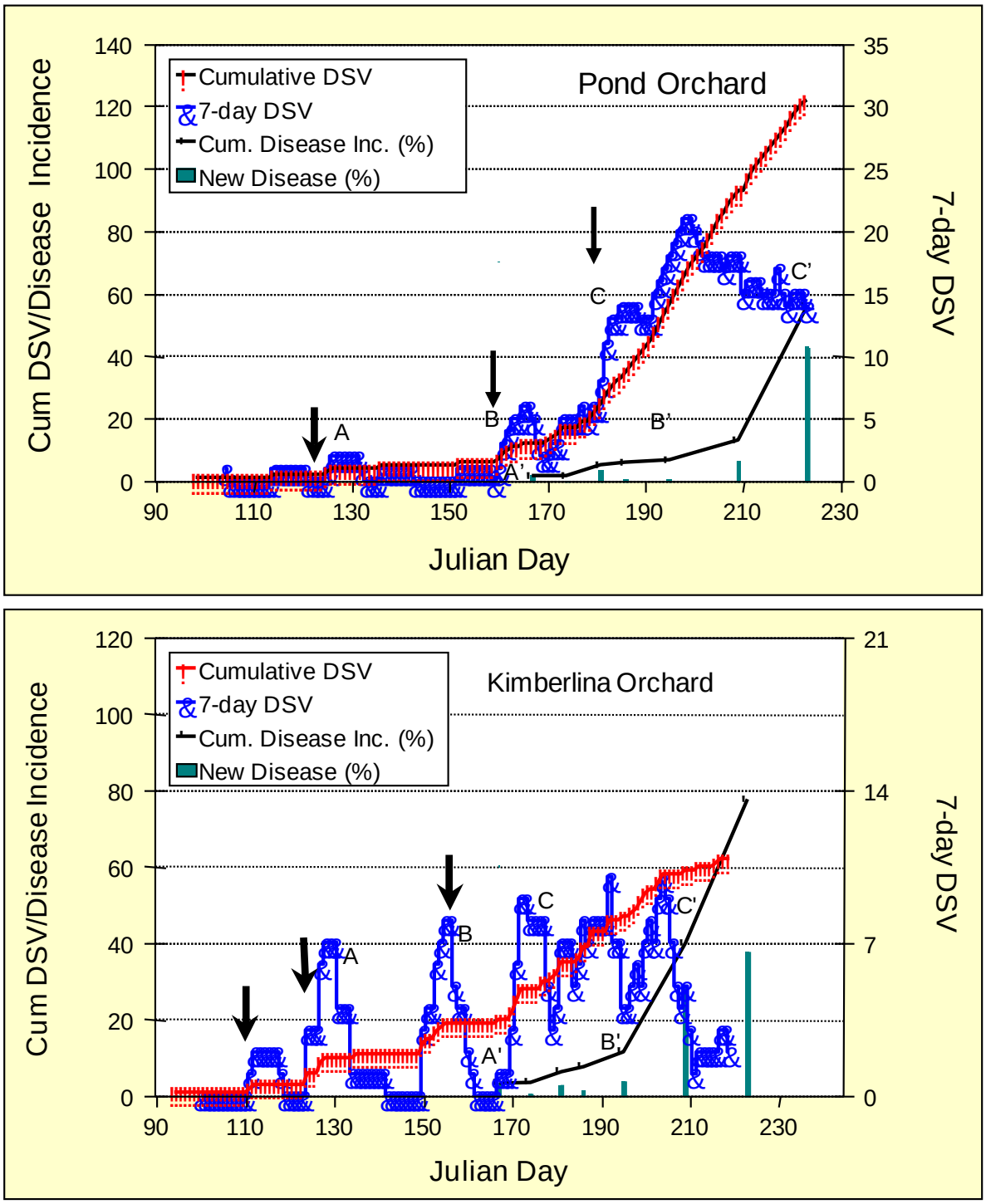

Figure 2. Alternaria leaf spot infection periods determined by the modified DSV model. (Arrow indicates timing of fungicide application when DSV reach 10). Julian days are consecutive days determined on an annual basis. For example Day 90 is April 1.

Fungicide evaluations for management of Alternaria leaf spot of almond in 2009. Our research in 2010 is ongoing we are planning to evaluate our plots in Aug. Thus, information is presented for 2009. In our trials in Kern County on cultivars Carmel and Monterey, results were similar for both cultivars and thus, data were combined. Three calendar-based treatments significantly reduced the incidence of disease for most materials and significantly reduced the number of lesions per leaf (disease severity) and tree defoliation for all materials evaluated as compared to the control (**Table 1**). Treatments containing Ph-D (polyoxin-D) were most effective in reducing the disease. There was no significant difference in disease incidence and severity between the two rates of this fungicide (i.e., 6.2 or 12.4 oz) used, but tree defoliation was rated significantly lower for the higher rate. Three applications of Ph-D at the low rate done at 10-day intervals resulted in a similar level of disease incidence and severity as compared to low-rate treatments that were timed at longer intervals. Tree defoliation for this treatment was low and statistically similar to the high-rate treatment of Ph-D. These low-rate treatments at shorter intervals were done because the fungicide is currently only registered at the low rate. Due to the high persistence of Ph-D, we hoped to obtain an accumulated high residue on the trees equivalent to a high-rate application.

Most effective in this trial was a mixture of Ph-D (used at the 6.2-oz rate) with Inspire XT (a mixture of propiconazole and difenoconazole) or with Inspire Super (a mixture of cyprodinil and difenoconazole). Still, other treatments such as Inspire and Inspire Super by themselves, as well as USF2015 (fluopyram, Luna Privilege), USF2016 (Luna Sensation), and Quash also performed well. Inspire and Inspire Super were similarly effective, indicating that the addition of cyprodinil to difenoconazole did not result in improved efficacy. Fluazinam (Omega), dodine (Syllit), Adament, Pristine, and Prophyt were among the less effective treatments. Laboratory fungicide sensitivity tests indicated a high incidence of QoI (>90% of the isolates) and boscalid (>60%) insensitivity in the *Alternaria* spp. population in this orchard, explaining the low efficacy of Pristine. Interestingly, most isolates that were insensitive to boscalid were still sensitive to Luna Privilege (USF2015), a second-generation fungicide in the same class as boscalid.

Similar results as in the Kern County trial were obtained in the trials in Glenn and Butte Counties. Thus, Ph-D at the 12-oz and higher rates and mixtures of Ph-D and Inspire Super or Inspire XT reduced disease to the lowest levels (**Tables 2, 3**). Tree defoliation for these treatments at the August evaluation dates was very low. At a rating in October of the Glenn County trial, still less than 20% of the leaves had dropped (rating of 0.75) in the Ph-D 16-oz and Ph-D – Inspire Super treatments, as compared to >75% of the leaves on control trees (**Table 2**). Again, application of a low rate of this fungicide at shorter intervals beginning in mid-May resulted in numerically higher but statistically similar levels of disease as compared to the same low rate applied at longer intervals, possibly indicating that degradation of the fungicide may be occurring and that treatments applied into early summer may help to longer protect trees from infection (**Table 2**). Quash, Luna Privilege, and Luna Sensation were again among the better treatments and rotations of Ziram with Quash or of Dithane with Luna Privilege also performed well. Pristine was intermediately effective in the Glenn County trial. More than 90% of the isolates of *Alternaria* spp. sampled from this orchard were insensitive to QoI fungicides, but only 7% were also insensitive against the carboxamide boscalid.

Thus, our three field trials in 2009 confirmed the high efficacy and long residual activity of the biofungicide Ph-D. Several other fungicides including Inspire and Quash (both group 3), Luna

Privilege, all belonging to classes other than the QoIs, as well as a mixture of Luna Privilege with the QoI trifloxystrobin (Luna Sensation) provided very good protection for most of the summer season. Metconazole (Quash) was registered directly by Valent USA Corp. Agricultural Products; whereas difenoconazole (Inspire, Syngenta Crop Protection) was registered through the IR-4 specialty crop pesticide registration program. Ph-D (Arysta Life Science) has received federal registration as a biopesticide, was exempted from tolerance in 2009 for a number of crops including almond, and is registered as of April 2010.

Table 1: Efficacy of fungicide treatments for management of Alternaria leaf spot on almond cvs. Monterey and Carmel, Kern County, 2009.

				Dis. Incid. leaves***		Dis. Severity leaves		Tree Defoliation	
No.	Program	Treatments*	Rate (/A)	(%)	LSD****	lesions/leaf	LSD	Rating**	LSD
1	---	Control	---	92.3	a	2.46	a	2.46	a
2	Single	Omega 500F	16 fl oz	81.0	bcd	1.61	b	1.29	bcd
3	fungicides	Syllit 3.4FL	48 fl oz	87.2	ab	1.64	b	1.48	b
4		Prophyt	64 fl oz	83.6	abc	1.75	b	1.40	bc
5		Quash 50WG	4 oz - 3.5 oz	67.4	ef	1.01	efg	0.81	efg
6		Quash 2DC	7 fl oz - 5 fl oz	73.2	cde	1.17	cde	0.88	defg
7		Inspire 2EC	7 fl oz	70.9	de	0.96	efgh	0.56	fghi
8		Luna Privilege (USF2015) SC	4 oz	64.4	efg	0.90	efgh	0.54	ghi
9		Ph-D (Polyoxin-D) 11.2DF	6.2 oz	56.5	fgh	0.77	fghi	0.75	efgh
10		Ph-D (Polyoxin-D) 11.2DF	12.4 oz	51.1	gh	0.64	ghi	0.29	ij
11		Ph-D (Polyoxin-D) 11.2DF**	6.2 oz	66.1	ef	1.00	efg	0.33	hij
12	Mixtures	Ph-D + Inspire Super	6.2 oz - 14 fl oz	45.7	h	0.58	hi	0.13	ij
13	and	Ph-D + Inspire XT	6.2 oz - 7 fl oz	43.2	h	0.50	i	0.08	j
14	Pre-	Ph-D + Quash 2DC	6.2 oz - 3.75 fl oz	49.8	h	0.60	hi	0.48	ghij
15	mixtures	Inspire Super SC	14 fl oz	79.9	bcd	1.43	bcd	1.00	cdef
16		Inspire XT (A8122B)	7 fl oz	70.1	def	1.09	def	0.56	fghi
17		Quilt Xcel (A15909C)	26 fl oz	84.9	ab	1.57	b	1.08	bcde
18		Adament 50WG	6 oz	84.6	ab	1.64	b	1.17	bcde
19		Luna Sensation (USF2016 SC)	4 oz	66.2	ef	0.94	efgh	0.50	ghij
20		Pristine 38WG	14.5 oz	80.1	bcd	1.54	bc	1.14	bcde

* Treatments were applied using an air-blast sprayer at a rate of 100 gal/A on 5-12, 6-4, and 6-24-09. For Quash, the first application was done at the higher rate and the following applications at the lower rate.

** Three applications of Polyoxin-D were done at 10-day intervals starting on 5-12-09, 5-22-09, and 6-1- 09.

*** Evaluations for disease were done on 8-11-09. For disease incidence on leaves, 60 leaves from each of the 3 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 (>90% defoliation).
In the split-plot statistical analysis, there was interaction between the two cultivars and any of the three disease measures, thus, data were combined for cvs. Carmel and Monterey.

**** 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 on almond cv. Carmel - Glenn Co. 2009

				Evaluation on 8-1-09***						Eval. on 10-8-09	
				Dis. Incid. Leaves		Dis. Sev. Leaves		Tree Defoliation		Tree Defoliation	
No.	Program	Treatments*	Rate (/A)	(%)	LSD****	lesions/leaf	LSD	Rating	LSD	Rating	LSD
1	---	Control	----	99.9	a	2.92	a	2.35	a	3.50	a
2	Single fungicides	Omega 500F	16 fl oz	91.1	b	1.78	b	1.22	b	2.19	bcd
3		Luna Privilege (USF2015)	4 fl oz	79.5	cd	1.17	de	0.47	def	1.19	fgh
4		Quash 50WG	3.5 oz	73.6	d	1.17	de	0.69	cde	1.69	cdefg
5		Ph-D (Polyoxin-D) 11.2DF	6.2 oz	59.9	ef	0.78	fg	0.25	ef	1.38	efgh
6		Ph-D (Polyoxin-D) 11.2DF	12.4 oz	39.9	g	0.46	g	0.22	ef	1.06	gh
7		Ph-D (Polyoxin-D) 11.2DF	16 oz	51.9	fg	0.66	g	0.06	f	0.75	h
8		Ph-D (Polyoxin-D) 11.2DF**	6.2 oz	72.7	de	1.02	ef	0.59	cde	1.94	bcdef
9	Mixtures and Pre-mixtures	Ph-D + InspireSuper	6.2 oz - 14 fl oz	50.7	fg	0.60	g	0.06	f	0.75	h
10		Luna Sensation (USF2016)	4 fl oz	79.2	cd	1.26	de	0.72	bcde	1.63	defg
11		Inspire Super SC	20 fl oz	73.1	de	1.10	def	0.56	cdef	2.63	b
12		Inspire Super SC	14 fl oz	86.7	bc	1.68	bc	0.94	bcde	2.38	bcd
13		Quilt Xcel (A15909C)	26 fl oz	78.0	cd	1.29	de	1.03	bc	2.44	bc
14		Pristine 38WG	14.5 oz	79.6	cd	1.39	cd	0.63	cde	2.13	bcdef

* Treatments were applied using an air-blast sprayer at a rate of 100 gal/A on 5-20, 6-9, and 6-30-09.

** Polyoxin-D was applied on 5-20, 5-31, and 6-9.

*** For disease incidence, 60 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%) to 4 (>90% 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.

Table 3: Efficacy of fungicide treatments for management of Alternaria leaf spot on almond cv. Carmel - Butte Co. 2009

No.	Program	Treatments*	Rate (/A)	Applications 4-21	5-20	6-16	Dis. Incid. leaves** (%)	LSD***	Dis. Severity leaves** lesions/leaf	LSD***
1	---	Control	---	---	---	---	99.2	a	2.52	a
2	Single Fungicides	Ph-D 11.2DF	6 oz	@	@	@	39.9	b	0.48	bc
3		Ph-D 11.2DF	12 oz	@	@	@	25.8	b	0.29	c
4	Mixtures and Premixtures	InspireXT + Ph-D 11.2 DF	7 - 6 fl oz	@	@	@	20.4	b	0.23	b
5		Inspire Super SC	20 fl oz	@	@	@	39.1	b	0.48	bc
6		Ziram 76WDG	8 lbs	@	-	-	39.0	b	0.46	b
		Quash 50WG	3.5 oz	-	@	@				
7	Rotations	Dithane M45	8 lbs	@	-	-	39.6	b	0.43	b
		Luna Privilege (USF2015) SC	4 fl oz	-	@	@				

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

** Evaluations for disease were done on 8-13-09. For disease incidence on leaves, 60 leaves from each of the 11 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 (>90% 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.

Table 4. Relative efficacy and registration status of selected fungicides for management of Alternaria leaf spot.

Fungicide	Class	Mode of action	Efficacy`	Remarks
Bravo, Echo, Equus	Aromatic nitrile	Multiple	+	IR-4 program - planned 2013
Ziram	Dithiocarbamate	Multiple	+*	2 ee amendment***
Captan	Pthalimide	Multiple	+	
Vangard/Scala	Anilinopyrimidine	Single	+	2*ee* amendment (Scala)***
Rovral	Dicarboximide	Multiple	+*	Label limits on PHI
Abound/Gem	QoI	Single	+++**	Res. Populations
Pristine	QoI/SDHI	Multiple	++++**	Res. Populations
Adament	QoI/SBI	Multiple	++++**	Registration requested
Quash	SBI	Single	++++	Registered as of 2010
Inspire/ Inspire Super	SBI/SBI+AP	Single/ Multiple	++++	Registered as of 2010
Polyoxin-D	Biofungicide	Single	++++	Registered as of 2010 Registration Planned

` - Rating: ++++ = most effective, - = not effective, ? = unknown.

*- Restricted to applications until 5 weeks after petal fall

** - Known resistant populations against QoI (strobilurin) and SDHI fungicides .

The usage rate of Ph-D is 6.2 oz/A on almond. We will continue to support the almond industry of California in evaluating new materials for the 2010 growing season (**Table 4**). Chlorothalonil (Bravo, Echo, Equus) was recommended and accepted into the IR-4 program for extended usage into the spring season. Expected registration with a new label is 2013.

Conclusion. Based on our research to date, management of Alternaria leaf spot with fungicides as part of an integrated strategy should start with 5-wk-after petal fall applications that include Rovral followed by three late-spring applications with materials having different modes of action. The effectiveness of Rovral at this timing will depend on the occurrence of favorable environmental conditions for the disease in early spring. With cool late-spring temperatures, the 5-wk-after petal fall treatment could be ziram or captan alone (mainly for scab) but with warm late-spring temperatures, Rovral could be included. Following the emergency registration of Inspire (difenoconazole) in 2008-09, several highly effective fungicides (Inspire, Quash, and Ph-D) were registered for usage in spring 2010; whereas other materials such as Luna Privilege and pre-mixtures such as Luna Sensation are planned for registration for summer (2010) with concurrent federal and statewide registration. Research is ongoing and the latest information will be presented at the ABC in December 2010.

Because resistance has developed in pathogen populations to the QoI and carboxamide fungicides, rotations and mixtures are strongly suggested. Thus, registration of Inspire (difenoconazole-Group 3), Quash (metconazole – Group 3), the biofungicide Ph-D (polyoxin-D-Group 19) and eventually other products like Luna Sensation are critical in the management of Alternaria leaf spot of almond. Each fungicide group has a different 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. Because pre-mixtures such as Adament and Luna Sensation have components that include Groups 7 (carboxamides) and 11 (QoIs) fungicides, these materials have to be used in areas where resistance has not developed. Moreover, they should be used only in rotations with the other materials and limited to one application per season for managing this disease. Additional materials with activity against *Alternaria* spp. need to be identified and tested in field trials **(Table 4**). To this end, chlorothalonil was submitted to the IR-4 program for residue trials and ultimately label changes to allow extended usage into the late spring season.

The development of other components for an integrated Alternaria leaf spot management program is critical because of the current limited arsenal of chemical treatments available. 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 and integrated into a management program, then the performance of fungicide treatments will also be enhanced.

Suggested management programs that can be followed next season and that are planned with future registrations of fungicides in our guidelines are shown in **Table 5**.

Table 5. Suggested fungicides and timings for management of Alternaria leaf spot

Year	Program	5-wk-after PF	DSV IP1* Mid-May	DSV IP2* Early June	DSV IP3* Late June
2010	1	Ziram+Rovral	Inspire or Quash	Ph-D	SBI**/Ph-D
2010	2	Ziram	Ph-D	Inspire or Quash	SBI/Ph-D
2010	3	Ziram+Rovral	Inspire or Quash	QoI/SDHI**	Ph-D
Future	4	Chlorothalonil	Ph-D, Quilt Xcel, or Quash	QoI/SDHI, QoI/SBI or SBI,**	Ph-D, Inspire XT

* DSV IP1-3 - The model Disease Severity Value (DSV) is successfully being used to predict one to three infection periods (IP1-IP3) from May to late June (early July) as described in the text

** SBI (FRAC 3) or sterol biosynthesis inhibitor such as Inspire or Quash. SDHI/QoI (FRAC 7/11) fungicides include Pristine or Luna Sensation. These fungicides should not be used where known FRAC 7/11 resistance occurs.