Epidemiology and Control of Alternaria Leaf Spot

Project No.: **07-PATH3-Adaskaveg**

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Interpretive Summary:

Strobilurin- or Q_oI-resistant isolates of *Alternaria* spp. were first detected in 2003 and 2004, and crop losses (practical resistance), were common in 2006 in Kern Co. and again in 2007 in several almond-producing counties in California including (Kern, Glenn, Butte, and Tehama Co.). Once resistance occurs within a class (same mode of action), all fungicides within the class are also ineffective (i.e., azoxystrobin - Abound, trifloxystrobin - Gem, and pyraclostrobin - a component of Pristine), once resistance develops. Still, Pristine was registered and recommended against Alternaria leaf spot with a 25-day PHI interval because the second fungicide, boscalid (carboxamide class), in the premixture was also active against the pathogen complex. The baseline sensitivity was determined for boscalid with EC_{50} values of 0.015-0.058 ppm. In 2007, 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. Practical resistance is expected to be common next year if resistance spreads within the pathogen population similar as for the Q_oI fungicides. Thus, in order to replace the strobilurins and carboxamides with new fungicides for managing Alternaria leaf spot and other foliar diseases of almonds, 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 show the best promise are 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. Difenoconazole is being registered through the IR-4 program and

polyoxin-D has been submitted into the program. In our 2007 trials in Kern and Colusa Co., three calendar-based applications of polyoxin-D (Endorse), difenoconazole (Inspire), or a SBI-strobilurin pre-mixture (Adament), were highly effective in reducing the incidence of disease from the control and they performed similar to, or significantly better than multiple applications of Pristine, Captan, or Scala. Rovral was also highly effective in these trials; however, the United States Enviromental Protection Agency (EPA) has indicated they will not change the current registration of this fungicide on almonds. In 2008, difenoconazole received a Crisis Exemption and pending Section 18 registration for management of Alternaria leaf spot of almonds in Butte, Glenn, Kern, and Tehama Co. A full registration (Section 3) was also obtained for propiconazole on almonds in California. Field trials were again conducted in 2008 with an emphasis on the evaluation of difenoconazole, propiconazole, metconazole, polyoxin-D, fluazinam, and several new fungicides and pre-mixtures (one component in each being an SBI fungicide), that are still under development. These trials are still ongoing and no results are available at this time. Growth chamber experiments under controlled temperature-wetness conditions were repeated, demonstrating that only plants with mite injuries developed disease symptoms in these studies. Thus, injuries from mites and potentially other insects are an important focus in an integrated management strategy for Alternaria leaf spot.

Objectives:

Etiology

1. Identify pathogenic species of *Alternaria* using molecular methods.
2. 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.

Epidemiology

1. Growth chamber studies to evaluate disease development under defined environmental conditions (wetness, relative humidity, temperature).
2. Develop disease progress curves in relation to microclimates in commercial orchards.
    a) Continue to compare environmental parameters (wetness, relative humidity, temperature), occurring at the edges and within an orchard and relate these to disease development.
    b) Continue to compare environmental parameters between different orchards and relate to disease development. Dataloggers will be placed in these different locations.
    c) Continue to evaluate the DSV model as a method of forecasting the disease with the goal of improved timing of fungicide treatments.
        i) Evaluate the minimum temperature modified-DSV model, as well as 10- and 20-point thresholds of the 7-day index of the DSV model for predicting disease and timing of fungicides.

ii) Determine optimal fungicide application timings based on different methods for calculating thresholds of the DSV model.

Management:

1) Evaluate new fungicides for their efficacy in managing Alternaria leaf spot. Fungicides to be evaluated include Pristine (pyraclostrobin + boscalid), and non-strobilurin fungicides (e.g., Elite, difenconazole – Inspire, polyoxin-D - Endorse, and other experimentals e.g., V-10116), and compare efficacy to strobilurin fungicides (Abound, Flint, Pristine).
2) Evaluate rotation programs that use calendar-based fungicide applications in comparison with applications based on the modified DSV model.
3) Monitor for strobilurin field-resistance and shifts in sensitivity in populations of *Alternaria* spp.

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 throughout the almond production areas in the central valleys of California. It is most serious in the lower San Joaquin valley where dew forms, the air is stagnant, and temperatures are high. In other areas including the northern Sacramento valley, almonds sometimes have been planted in poorer soils and irrigation practices have changed and include more frequent and extended watering into the summer. This situation has increased the occurrence of the disease and there continues to be reports of serious disease outbreaks in northern and southern growing areas.

Because the occurrence of Alternaria leaf spot of almond is greatly influenced by microclimatic conditions such as temperature and wetness within orchards, the Disease Severity Value (DSV) model that is used for forecasting of Alternaria diseases on other crops was evaluated on almond in our previous years' studies. We demonstrated a close correlation between the increase in actual disease and increases in DSV that are determined by the number of hours of wetness within defined temperature ranges. In subsequent studies, we adjusted threshold temperatures to $\geq$62 F to refine the accuracy of the model for almonds in California. In addition, we demonstrated that the actual development of disease incidence correlated with environmental conditions that occurred 25 days ($\pm$ 3 days), before the onset of disease symptoms (the disease progress curve latency period). Thus, temperature-leaf wetness conditions used for calculation of the DSVs reflect the actual disease development. The DSV model is a tool for predicting infection periods for the Alternaria pathogen on almonds and for timing of fungicide applications (This model was used for fungicide applications in 2007 and 2008).

Materials and Methods:

Progress on New and Amended Fungicide Registrations

For management of Alternaria leaf spot, currently the strobilurin fungicides (e.g., azoxystrobin - Abound, trifloxystrobin - Gem, pyraclostrobin/boscalid - Pristine), are registered for late spring applications when the disease develops. Other fungicides such as Rovral and Bravo have label restrictions limiting them to the petal fall period. Thus, pathogen resistance (see below), or label restrictions of these fungicides emphasize that new materials need to be identified, developed, and registered. With the strobilurins, resistance in pathogen populations was documented four years ago on almonds and subsequent crop losses ensued. Pristine was registered only up to 5 weeks after petal fall but in 2006 following our request the PHI interval was changed for a shorter interval that now allows usage up to 24 days before harvest. In 2007, we pursued late spring season usage of Rovral under an emergency registration for highly effective control, as well as Captan and Scala 2*ee* registrations as suppressive treatments for Alternaria leaf spot control of almonds. Follow-ups on the registration progress of difenoconazole, metconazole, and polyoxin-D were also made a priority. In May of 2008, difenoconazole received a Crisis Exemption and pending Section 18 registration (due In July), for management of Alternaria leaf spot of almond in Butte, Glenn, Kern, and Tehama Co. with a 30-day preharvest interval. Use of propiconazole was also pursued for managing the disease.

Growth Chamber Studies to Evaluate Disease Development under Defined Environmental Conditions

Inoculations of potted cv. Carmel and Sonora almond plants were conducted in growth chamber experiments under defined temperature-humidity conditions. Two kinds of plants were used: healthy plants and plants that were previously infested with mites and that were treated with a miticide before the experiment. After inoculation with conidia of representatives of the three *Alternaria* species affecting almonds (*A. alternata, A. tenuissima, A. arborescens*), plants were incubated at >95% relative humidity and at day/night temperatures of 30 C/25 C.

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 azoxystrobin, boscalid, difenoconazole, and polyoxin-D 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.).

Fungicide Evaluations for Management of Alternaria Leaf Spot of Almond in 2007

Two split-plot trials were established in Kern Co. and two trials were done in Colusa Co. The first trial was on cvs. Carmel and Monterey, whereas the second trial was only on cv. Monterey. In both plots, the main plots received five-week-after petal-fall applications (16 April), of Captan, or received no application (control treatment). Trees in the first plots were then treated with a subsequent DSV model-based application program and three sprays were applied (May 8, May 31, June 25). Treatments 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 or V10116), the chitin inhibitor polyoxin-D (i.e., Endorse), iprodione (i.e., Rovral), and mixtures of Orbit or Inspire with Captan, Inspire with Vangard (Inspire Super) or with a strobilurin (A13703(I-V). In the second plot, polyoxin-D, Scala, or Vangard were mixed with Captan. In the third and forth plots, Bravo was applied at 5 weeks after petal fall. Subsequent treatments included three applications on 25 April, 18 May, and 6 June, of Inspire, Orbit, V-10116 (metconazole), polyoxin-D, Rovral, Adament, Pristine, and Inspire/Vangard, Rovral/Captan, and Pristine/Captan. In the forth plot, Scala, Captan, Scala/Captan, and polyoxin-D/Captan mixtures were applied on the 6th and 21st June. 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). In 2008, similar trials were repeated with additional active ingredients.

Results and Discussion:

Progress on New and Amended Fungicide Registrations:

In 2007, Captan and Scala were registered under 2ee amendments for Alternaria leaf spot, but both only have suppressive action based on our evaluations. The label for Rovral, however, still only includes up to 5 weeks after petal fall applications and EPA is unwilling to change the PHI based on our Section 18 submission in 2007. Pristine was labeled with a 25-day PHI in 2006. With the widespread occurrence of strobilurin (Q_oI) resistance and recent identification of boscalid resistance (see below), in populations of *Alternaria* spp. on almond, practical (field) resistance and crop loss has subsequently occurred.

First evaluated by us in 2005, the two new fungicides Inspire (difenoconazole), and Endorse (polyoxin-D), continued to be the most effective treatments in our 2007 trials. Difenoconazole is a sterol biosynthesis inhibiting (SBI) fungicide that is registered in other countries for the control of several diseases including those caused by *Alternaria* spp., whereas polyoxin is a chitin inhibiting biofungicide that is registered in the US on turf and in other countries for the control Alternaria diseases on several crops. IR-4 residue studies were recommended and the field and lab portions of the registration process were completed in 2006 and 2007, respectively. Submission to EPA is planned in 2007/08, and a registration on almonds is expected in 2009/10. Arysta Life Science has also requested a biofungicide registration of polyoxin-D at 50-100 g ai/A. This registration request is pending EPA review. Difenoconazole and polyoxin-D were also recommended for emergency registration in 2008.

Growth Chamber Studies to Evaluate Disease Development under Defined Environmental Conditions:

After 10-14 days of incubation, Alternaria leaf spot symptoms and sporulation of the fungus only developed on mite-injured plants, but not on healthy plants, and all three species of the pathogen caused disease in these growth chamber studies. Thus, for a second year, we demonstrated that mite-injured almond leaves are much more susceptible to Alternaria leaf spot. Field observations in 2007 also indicated that with less mite injury, the disease was less severe. Thus, mite or other types of leaf injury may increase the severity of Alternaria leaf spot outbreaks on almonds.

Development of Baseline Sensitivity Data and Resistance Assessment Studies:

The baseline sensitivity of *Alternaria* spp. from almond that was never exposed to the fungicide boscalid consisted of a range of EC_{50} values from 0.015-0.058 ppm. In 2007, 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. Practical or field resistance is expected to be common in the next year if resistance spreads within the pathogen population similar to Q_oI fungicides. Thus, in order to replace the strobilurins and carboxamides with new highly effective fungicides for managing Alternaria and other foliar diseases of almond, we identified two new effective materials of different modes of action: the DMI (demethylation inhibitor – Group 3), fungicide difenoconazole and the chitin synthase inhibitor (Group 19), polyoxin-D (a biofungicide). All isolates evaluated were sensitive to difenoconazole and EC_{50} values for inhibition of mycelial growth ranged from 0.01 to 0.05 ppm. Research is ongoing for polyoxin-D.

Fungicide Evaluations for Management of Alternaria Leaf Spot of Almond in 2007:

In the first trial, the five-week-after-petal-fall application of Captan did not significantly improve disease management as compared to no petal fall fungicide treatment and there was no interaction with the late spring applications of fungicides. Thus, data from the two main plots were combined. The late spring applications, however, were significant treatments in reducing Alternaria leaf spot.

All of the treatments significantly reduced disease from 78.9% in the untreated controls to less than 30% and less than 50% incidence on cvs. Carmel and Monterey, respectively (Table 1A, B). Defoliation ratings were reduced from 2.7 and 3.2 to less than 1.3 and 2.1 on cvs. Carmel and Monterey, respectively. The best treatments in reducing the incidence of disease and defoliation were polyoxin-D, Rovral, and Inspire on both cultivars (Table 1A, B). 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. With the further spread of boscalid resistance due to over-use of Pristine, however, efficacy of this fungicide will most likely continue to decline. Our data also indicate that the addition of captan to selected fungicides was not beneficial for disease control and sometimes decreased the performance when used in tank mixtures. The addition of other fungicides also did not improve the performance of Inspire (Table 1A,B). Late spring, early summer applications of Polyoxin-D, Scala, or Vangard that were all mixed with Captan significantly reduced the disease, but these treatments were not as effective as the earlier timings (Table 1C). Thus, we identified the most effective treatment timing for management of Alternaria leaf spot to be late spring applications that are best done following the temperature-wetness-based DSV model. The petal fall application with Captan did not reduce the disease; however, when we used a mixture of Captan with Rovral at this timing in last year's trials, there was a significant reduction in disease. This implies that Rovral is a more effective fungicide against Alternaria leaf spot than Captan. Rovral was not used as a petal fall treatment in 2007 because we had submitted this fungicide to be re-labeled to allow late spring treatments, and we successfully used Rovral this year at these timings. With EPA not approving this re-labeling, Rovral should best be used in petal fall treatments that are followed by late-spring applications with other fungicides to obtain a high level of disease control. Although Captan is not very effective against Alternaria spot, a mixture of Rovral with Captan will have the benefit to be effective against scab as well.

In a field trial in Colusa Co., Bravo was applied at petal fall to reduce scab. With a lower level of Alternaria leaf spot in the untreated control as compared to the Kern Co. trial, all treatments including the DMI fungicides (Orbit, Inspire, and Quash or V-10116), and plolyoxin-D as well as premixtures of strobilurins with other fungicides when applied between late April and early June were very effective in managing the disease (Table 2A). Pristine was also effective at this location where boscalid resistance was not found. Late spring applications with Scala, Captan, or mixtures of Captan with Scala or Polyoxin were less effective and subsequently resulted in high levels of tree defoliation (Table 2B).

In summary, 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 other materials. Because no effective materials are currently registered, registration of Inspire (difenoconazole), Quash (metcanazole-V-10116), and the biofungicide polyoxin-D will be instrumental in the management of Alternaria leaf spot of almonds. These materials will have to be strictly used in rotations or mixtures to delay the development of resistance and maintain their efficacy. Also, the pre-mixture Adament should be pursued for registration, but concerns exist because one of the components of this product is a strobilurin fungicide. Still, other single active ingredient fungicides of different modes of action need to be identified so that rotations can be developed to prevent resistance in the target pathogen populations.

Due to 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 including 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 are considered, fungicide treatments will be the most beneficial.

Table 1. Efficacy of fungicide treatments for management of Alternaria leaf spot on almond cvs. Carmel and Monterey - Kern Co. 2007

A. Trial 1 - Carmel

Product	Rate	Incid. (leaves) (%)	LSD	Defoliation Rating**	LSD
Control	--	78.9	a	2.7	a
Inspire 250EC	5 fl oz	18.0	bcd	1.1	bcd
Inspire 250EC	7 fl oz	16.6	bcd	0.5	e
Orbit 3.6EC	6 fl oz	27.4	bc	0.9	bcde
Polyoxin D 11.2WG	50 g ai	14.1	cd	0.5	e
Polyoxin D 11.2WG	100 g ai	11.9	d	0.6	de
Rovral 4F	16 fl oz	21.8	bcd	1.3	bc
Rovral 4F	32 fl oz	12.8	cd	0.9	bcde
Inspire + Captan 80WDG	7 fl oz - 5 lb	23.7	bcd	1.4	b
Rovral 4F + Captan	32 fl oz - 5 lb	19.5	bcd	0.7	bd
Pristine 38 WG + Captan	14.5 oz - 5 lb	31.4	b	1.3	bc
Inspire Super	14 fl oz	28.1	b	0.8	cde
A13703(I-V)	10 fl oz	22.4	bcd	1.0	bcde
Pristine 38WG	14.5 oz lb	18.2	bcd	0.9	bcde

B. Trial 1 - Monterey

Product	Rate	Incid. (leaves) (%)	LSD	Defoliation Rating**	LSD
Control	---	78.9	a	3.2	a
Inspire 250EC	7 fl oz	21.4	de	0.7	fg
Orbit 3.6EC	6 fl oz	17.6	e	1.7	bcd
Polyoxin D 11.2WG	50 g ai	16.6	e	1.6	bcd
Polyoxin D 11.2WG	100 g ai	15.7	e	0.9	efg
Rovral 4F	32 fl oz	23.6	de	1.1	def
Inspire + Captan	7 fl oz - 5 lb	49.2	b	1.9	bc
Orbit 3.6EC + Captan	6 fl oz- 5 lb	36.7	bc	2.1	b
Inspire Super	11.5 fl oz	26.3	cde	1.3	cdef
Inspire Super	14 fl oz	16.8	e	1.7	bcd
Inspire Super	20 fl oz	25.6	cde	1.4	cde
A13703(I-V)	10 fl oz	27.6	cde	0.5	g
Adament 50WG	8 oz	31.8	cd	1.4	cde
Pristine 38WG	14.5 oz lb	21.2	de	1.8	bc

C. Trial 2 - Monterey

Product	Rate	Incid. (leaves) (%)	LSD	Defoliation Rating**	LSD
Control	---	75.2	a	3.3	a
Polyoxin D 11.2WG + Captan	6.3 oz - 5 lb	44.8	b	2.7	b
Scala 600SC + Captan	18 fl oz - 5 lb	50.4	b	2.5	b
Vangard 75WG + Captan	10 oz - 5 lb	41.2	b	2.6	b

* - Trees were treated with or without Captan at 5-wk-after petal fall (grower applied). Treatments were applied using an air-blast sprayer at 100 gal/A and there were 4 single -tree replications. In Tables A & B, applications were on 5/8, 5/31, and 6/25/07. In Table C, applications were on 5/31 and 6/25/07.

** - Evaluations were done on 8-9-07. For disease incidence on leaves, 200 leaves from each of the 4 single-tree reps were evaluated for the presence of disease. For evaluation of tree defoliation on 9-12-07, 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 ANOVA and LSD mean separation ($P > 0.05$).

Table 2. Efficacy of fungicide treatments for management of Alternaria leaf spot on almond cv. Carmel - Colusa Co. 2007

A. Trial 3 - Carmel

		Incid. (leaves)		Defoliation	
Product	Rate	(%)	LSD	Rating**	LSD
Control	---	31.6	a	2.50	ab
Inspire 250EC	7 fl oz	3.9	bc	0.88	d
Orbit 3.6EC	6 fl oz	3.5	c	1.63	bcd
V-10116	4 fl oz	5.0	bc	1.13	dc
Polyoxin D 11.2WG	50 g ai	9.4	b	1.13	dc
Polyoxin D 11.2WG	100 g ai	2.6	c	1.88	bcd
Rovral 4F	32 fl oz	6.1	bc	1.50	bcd
Inspire + Vangard 75WG	7 fl oz - 5 lb	5.6	bc	1.25	bd
Rovral 4F + Captan	32 fl oz - 5 lb	3.0	bc	2.88	a
Pristine + Captan	6 fl oz- 5 lb	4.8	bc	2.63	ab
Adament 50WG	8 oz	5.3	bc	2.50	ab
Pristine 38WG	14.5 oz lb	2.6	c	1.38	bcd

B. Trial 4 - Carmel

		Incid. (leaves)		Defoliation	
Product	Rate	(%)	LSD	Rating**	LSD
Control	---	46.6	a	3.63	a
Scala 600SC	18 fl oz	16.9	b	2.88	ab
Captan 80WDG	5 lb	16.3	b	2.63	b
Scala 600SC + Captan	18 fl oz - 5 lb	21.6	b	2.88	ab
Polyoxin D + Captan	10 oz - 5 lb	21.0	b	3.13	ab

* - Trees were treated with Bravo at 5-wk-after petal fall (applied by the grower). Treatments were applied using an air-blast sprayer at a rate of 100 gal/A and there were 4 single-tree reps for each sub-plot treatment. In Table A, treatments were applied on 4/25, 5/18, and 6/6/07. In Table B, treatments were applied on 6/6 and 6/21/07.

** - Evaluations were done on 8-15-07. 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 on 9-12-07, trees were rated based on a scale from 0 (= full canopy),1 (<25% defoliation) to 4 (100% defoliation). Values followed by thesame letter are not significantly different based on an ANOVA and LSD mean separation (P > 0.05).