

Project Name: Epidemiology and Control of *Alternaria* Leaf Spot

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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. Epidemiology
 - A. Growth chamber studies to evaluate disease development under defined environmental conditions (wetness, relative humidity, temperature).
 - B. Develop disease progress curves in relation to microclimates in commercial orchards
 - i. Continue to compare environmental parameters (wetness, relative humidity, temperature) occurring at the edges and within an orchard and relate these to disease development.
 - ii. 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 different lower thresholds using the 1-day, 7-day or cumulative indices of the DSV model.
 - ii. Determine optimal fungicide application timings based on different methods for calculating thresholds of the DSV model.
- III. Management
 - A. 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., difenoconazole - Score, polyoxin, and kasugamycin, as well as other experimentals) and compare efficacy to strobilurin fungicides (Abound, Flint).
 - B. Evaluate rotation programs that use a minimal number of fungicide applications in comparison with applications based on the DSV model.
 - C. Monitor for strobilurin field-resistance in addition to shifts in sensitivity in populations of *Alternaria* spp.

SUMMARY

The development of additional classes of fungicides that are highly effective for managing *Alternaria* leaf spot on almond is essential because the most effective fungicides that are currently registered with an appropriate use pattern all belong to the strobilurin class of fungicides. Strobilurin-resistant isolates of *Alternaria* spp. have been commonly found in orchards in Kern Co. Thus, in order to maintain the strobilurins as highly effective

fungicides for managing foliar diseases of almond, our studies focused on generating efficacy data to identify new effective fungicides of different chemical classes for potential registration and use in rotation with the strobilurins. To avoid the occurrence of field resistance, rotational programs of different classes of effective fungicides are our best strategy. Pristine and the other registered product (e.g., Rovral) are not labeled for late-spring to early-summer use patterns. At best, these products are suppressive as currently registered. No efforts are underway to re-label Rovral and re-labeling of Pristine (another strobilurin fungicide) may several years. In our 2005 trials that were conducted in Kern Co., two calendar-based applications of the antibiotic polyoxin or an experimental SBI material (CGA-169374) were highly effective in reducing the incidence of disease from the control and they performed similar to or significantly better than Abound, Pristine, Vanguard, or Elite. With support from the Almond Board of California, IR-4 residue studies have been recommended to accelerate registration of the new SBI material CGA-169374 for late spring and early summer usage. In this year's trials, we also continued evaluating the DSV epidemiological model for forecasting and managing *Alternaria* leaf spot of almond. In this model, disease severity values (DSVs) are calculated based on leaf wetness duration and temperature during the leaf wetness period. DSVs are accumulated over time and rapid increases in this value are indicative of infection periods. In two orchard locations in Kern Co., we evaluated the model using different thresholds and start dates for severity value accumulation. A critical observation this year was that increases in 7-day DSV values were followed by increases in disease (following a latent period of 21 to 27 days as described in previous reports). Application strategies for fungicides based on a 10- to 20-point increase in 7-day DSV values before mid-June resulted in a significantly lower incidence of disease than in the control. Thus, we are fine-tuning a forecasting model for predicting the disease.

Introduction. *Alternaria* leaf spot of almond is a disease that is caused by three species in the *A. 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 but 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 where almonds sometimes have been planted in poorer soils, irrigation practices have changed and include more frequent and extended watering into the summer. This situation has increased the occurrence of the disease.

Biology of *Alternaria* species. Conidial germination tests that were conducted on different agar media indicated that conidia of *Alternaria* spp. germinate well under low- and high-nutrient conditions. Thus, the fungus is not a fastidious organism and is able to grow under a variety of nutritional regimens. Conidial survival was tested at different relative humidities (23-85% RH) and viability was evaluated in spore germination assays. For all humidity conditions tested, survival was high after incubation for one month. This indicates that the pigmented conidia of the fungus are quite resistant to desiccation and nutrient reserves within the conidia are not used up readily.

Epidemiology. The occurrence of *Alternaria* leaf spot of almond is greatly influenced by microclimatic conditions within orchards. Prediction models for *Alternaria* diseases that are based on wetness and temperature parameters have been developed for other crops where they are being used successfully. To optimize the timing of fungicide applications on almond and to minimize costs, the Disease Severity Value (DSV) model for forecasting *Alternaria* diseases on other crops was evaluated on almond. For the last several years we have investigated correlations between the actual disease increase and increases in DSV. The disease severity in this model is determined based on the number of hours of wetness within defined temperature ranges. For our field trials, dataloggers were placed in each orchard with sensors recording leaf wetness, temperature, rainfall, and relative humidity. This was done in cooperation with Jeff Diebert at Western Farms. Disease incidence and severity were measured periodically from May to mid-August. Two to three terminal branches from opposite sides of each of 7 unsprayed trees were tagged and the number of infected leaves and the number of lesions per leaf larger than 2 mm in diameter were counted. From the evaluation data, disease incidence (number of infected per total leaves counted at each evaluation date) and disease severity (number of lesions per leaf) were calculated. Based on our previous studies, increases in the DSV value were followed by an increase in disease 21 to 27 days later. Using this unit of measure, disease progress was followed in a cv. Carmel orchard (Fig. 1) and in a cv. Sonora orchard (Fig. 2) in Kern Co.

Cumulative DSVs followed closely the actual disease progress at both locations, but as in our previous reports with a 20- to 25-day latency. Thus, temperature-leaf wetness conditions used for calculation of the DSVs reflect the actual disease development. The 7-day DSV values exhibited peaks that indicated conducive periods for infection. These peaks were followed by rapid increases in disease, again after a 20- to 25-day latency period as

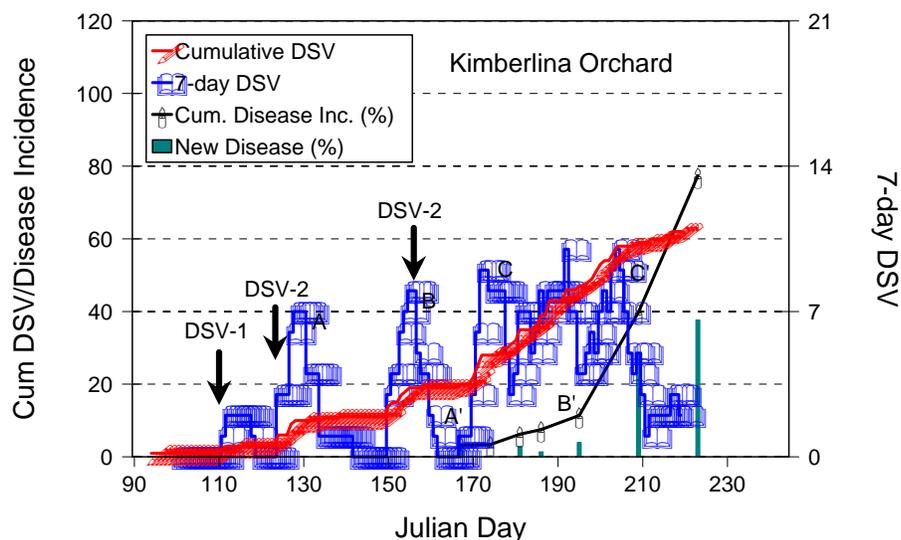


Fig. 1. Disease progress curves for *Alternaria* leaf spot and an evaluation of the DSV model in the Kimberlina almond orchard. Cumulative DSV (Cum DSV) and disease incidence (%) are shown on the left y-axis, whereas the 7-day DSV and severity values are on the right y-axis. Disease incidence values were based on leaves remaining on the tree and were not corrected for defoliation. Using a 20 to 25-day latency, increases in DSV and disease incidence were: A-A', B-B', and C-C'. Arrows indicate application dates based on increases in DSV-1 above 10 or DSV-2 above 20 until mid-June.

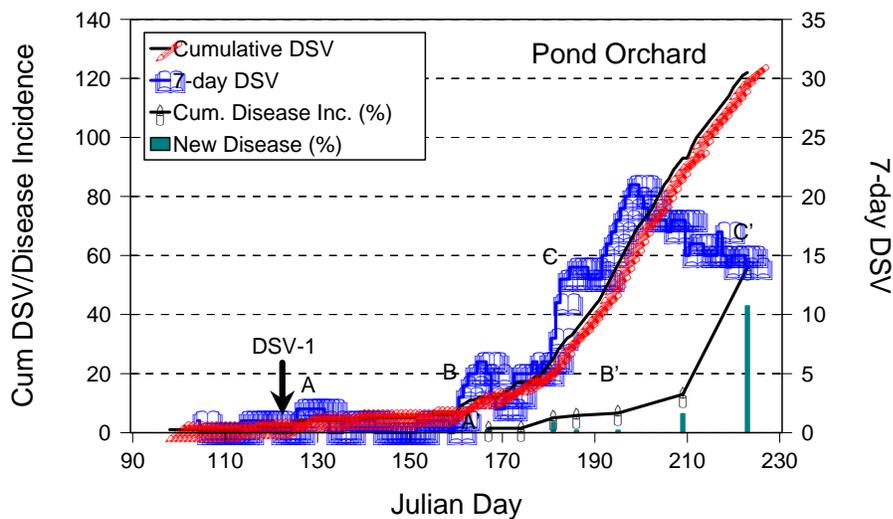


Fig. 2. Disease progress curves for *Alternaria* leaf spot and an evaluation of the DSV model in the Pond Ave. almond orchard. Cumulative DSV (Cum DSV) and disease incidence (%) are shown on the left y-axis, whereas the 7-day DSV and severity values are on the right y-axis. Disease incidence values were based on leaves remaining on the tree and were not corrected for defoliation. Using a 20-25-day latency, increases in DSV and disease incidence were at: A-A' and B-B'. Arrows indicate the application date based on increases in DSV-1 above 10 until mid-June.

illustrated in Figs. 1 and 2. Thus, the DSV model looks very promising as a tool for predicting infection periods for the *Alternaria* pathogen on almond and probably can be used to time fungicide applications. We are currently doing additional analyses on our disease progress data to determine adjustments that could be used in our 2006 modeling trials.

Fungicide evaluations for management of *Alternaria* leaf spot of almond. Currently, the strobilurins Abound, Flint, Pristine (the more effective component in this mixture is a strobilurin) and Rovral are registered for management of *Alternaria* leaf spot on almond. There are limitations with the use of these fungicides that emphasize the identification and development of new materials. With the strobilurins, a major concern is the documented resistance in pathogen populations on almond and pistachio. The strobilurins are still effective in managing diseases of almond and field resistance and crop losses have not yet occurred. In pistachio, however, resistant populations have already caused crop losses. Thus, the strobilurin fungicides are still effective fungicides for reducing the incidence of *Alternaria* leaf spot of almond. To keep the strobilurins available as effective rotational materials it is important to implement resistance management strategies that include limiting strobilurin-containing fungicides to two applications per season, development of new classes of fungicides, and fungicide rotations and mixtures between different classes of fungicides. On another aspect, Pristine and Rovral are registered only up to 5 weeks after petal fall and thus, are not available when the disease occurs in late spring and early summer. Furthermore, the labels of these latter two fungicides are limited to disease suppression.

Two field trials were established again in 2005 in Kern Co. in orchards with a history of the disease. Fungicides evaluated as single-fungicide applications or as mixtures and

rotations included: Abound (azoxystrobin), Flint (trifloxystrobin), Pristine (mixture of pyraclostrobin and boscalid), Echo (chlorothalonil), Elite (tebuconazole), Rovral (iprodione), Scala (pyrimethanil), Vanguard (cyprodinil), CGA-169374 (difenoconazole), and the antibiotic Polyoxin Z. Two or three calendar-based applications (April 13, April 26, and May 26) were done in each orchard using an air-blast sprayer. In addition, applications with Abound or Pristine were done in one orchard each based on the DSV model. DSV-1 model sprays in the Pond orchard followed increases in the 7-day DSV values of 10 points starting in April and one fungicide application was done on April 26. DSV-1 model sprays in the Kimberlina orchard followed increases in the 7-day DSV values of 10 points starting in April and one fungicide application was done on April 19. DSV-2 model sprays in the Kimberlina orchard used 7-day DSV value increases of 20 points and applications were done on May 5 and June 10. For disease evaluation 10-15 terminal branches were collected from each tree and were scored in the laboratory for incidence and severity (number of lesions/leaf) of disease.

Disease incidence (on the July and early August evaluation dates) based on the number of infected leaves of the total number of leaves on untreated control trees was 32.7% in the Pond orchard and 29.6% in the Kimberlina orchard. In the Pond orchard, two calendar-based applications of the antibiotic polyoxin numerically reduced the incidence to the lowest values as compared to the control, similar to last year (Table 1). The other experimental material, CGA-169374, was similarly effective. Other treatments that significantly reduced the incidence of disease from the control included Abound, Pristine, Vanguard, and Elite. In the Kimberlina orchard all treatments were effective in reducing the incidence of *Alternaria* leaf spot (Table 2). Numerically, Pristine had the lowest disease. Additional early-season (April 13) applications with either Rovral or Echo that were used as rotational materials did not further improve the performance of Pristine. All other chemicals tested, including polyoxin and CGA-169374, performed statistically similar.

Based on our field trials in 2004 and 2005, we identified potential alternatives for managing *Alternaria* leaf spot that include the antibiotic Polyoxin D, as well as the SBI fungicides Elite and CGA-169374. With financial support from the Almond Board of California IR-4 residue studies have been recommended to accelerate registration of this material that originally was only rated as a 'B' priority. We are also requesting that the PHI interval for the recently federally registered Pristine be changed for a shorter preharvest interval that would allow usage through April and May. Once new alternative fungicides are available, more effective resistance management strategies can be implemented that include fungicide rotations between different classes of fungicides and mixtures.

Fungicide applications with either Abound or Pristine that were done using modified DSV models performed similarly to the calendar-based treatments. Results from the Pond Ave. trial indicated that the single late-April application for the DSV-1 model was important and that the additional calendar-based spray late-May did not result in further reduction in disease. In the Kimberlina trial two DSV-2 applications using a threshold of 20 numerically performed better than the single DSV-1 application that was based on a threshold of 10.

Table 1. Evaluation of fungicides for management of Alternaria leaf spot - 2005

A. Pond Ave - Kern Co.

Treatment*	Product Rate/A	Incidence**		Severity**	
		(%)	LSD	Lesions/leaf	LSD
Control	---	32.7	a	1.3	a
Scala 600F	18 fl oz	16.3	ab	1.0	abc
Vanguard 75WG	10 oz	10.4	bc	0.5	cd
USF2010 500SC	6 fl oz	16.3	ab	0.7	bcd
Flint 50WG	3 oz	19.7	ab	1.1	ab
Abound 2F	12 fl oz	6.6	bc	0.4	d
Abound 2F DSV1	12 fl oz	6.5	bc	0.4	d
Pristine 38WG	0.92 lb	5.6	bc	0.5	cd
Elite 45WP	8 oz	6.7	bc	0.5	cd
Polyoxin D 11.3DF	2.2 lb	2.9	c	0.4	d
CGA-169374 250EC	6 fl oz	3.6	c	0.3	d

* - Fungicides were applied on April 26 and May 26, 2005 at 100 gal/A. Abound was also applied based on the DSV model for 1 application on April 26, 2005.

** - Disease was evaluated on 8-1-05. Incidence is the percentage of leaves infected per total evaluated. Disease severity is the number of lesions per leaf per total leaves evaluated.

B. Kimberlina Ave - Kern Co.

Treatment	Product Rate/A	Incidence		Severity	
		(%)	LSD	Lesions/leaf	LSD
Control	---	29.6	a	2.0	a
USF2010 500SC	6 fl oz	10.1	bc	0.7	b
Rovral 4F - Pristine 38WG	1 pt - 0.92 lb	5.4	bc	0.3	b
Echo 720 - Pristine 38WG	4 pt - 0.92 lb	8.2	bc	0.5	b
Pristine 38WG	0.92 lb	4.4	c	0.3	b
Elite 45WP	8 oz	11.4	bc	0.7	b
Polyoxin D 11.3DF	2.2 lb	12.3	bc	0.8	b
CGA-169374 250EC	6 fl oz	12.7	b	0.9	b
Pristine 38WG using DSV1	0.92 lb	12.9	b	0.8	b
Pristine 38WG using DSV2	0.92 lb	7.7	bc	0.5	b

* - Rovral and Pristine were applied on April 13, 2005.

** - All other fungicides were applied on April 26 and May 26, 2005 at 100 gal/A. Pristine was also applied based on the DSV model using two different thresholds.

*** - Disease was evaluated on 7-21-05. Incidence is the percentage of leaves infected per total evaluated. Disease severity is the number of lesions per leaf per total leaves evaluated.

Incidence of disease in the Pond orchard over the last two years was much lower and disease developed later than in previous years. Cultural practices were changed in this orchard that led to a decreased humidity in the micro-environment and that most likely is responsible for the reduced incidence of Alternaria spot. These practices included hedging, improvement of water penetration into the soil by adding gypsum, and changing the watering schedule to less frequent irrigation using soil moisture probes. Thus, Alternaria spot management, like for other diseases, has to be done in an integrated approach if conditions allow. If all components of the disease triangle are considered, fungicide treatments will be the most beneficial.