Annual Report Almond Research Board **2003-04**

Correct Project Number: 03-JA-02

Project No. 02-JA3: Epidemiology and Control of Alternaria Leaf Spot

Principal Investigator (s): J. E. Adaskaveg
University of California
Dept. of Plant Pathology
Riverside, CA 92521

Cooperating: B. L. Teviotdale (UCCE, Kearney Ag Center), H. Förster (UC Riverside), and M. Viveros and P. Schrader (UCCE, Kern Co.).

Objectives

I. 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. Compare environmental parameters (wetness, relative humidity, temperature) occurring at the edges and within an orchard and relate these to disease development.

   ii. Compare environmental parameters between different orchards and relate to disease development. Dataloggers will be placed in these different locations.

C. Determine the pathogen species composition within and between selected orchards at the beginning and at the end of the log phase of the epidemic.

   i. Identify species by morphological characters and molecular methods.

D. Evaluate the DSV model as a method of forecasting the disease with the goal of improved timing of fungicide treatments.

II. Management

A. Evaluate new fungicides for their efficacy in managing Alternaria leaf spot. Fungicides to be evaluated include strobilurins (Abound, Cabrio), anilinopyrimidines (Vangard, Switch), and numbered compounds (Pristine).

B. Evaluate rotation programs that use minimal number of fungicide applications in comparison with applications based on the DSV model.

SUMMARY

In our 2003 trials, we focused on evaluating the DSV epidemiological model for forecasting and managing Alternaria leaf spot of almond and on evaluation of new fungicides. The DSV model was developed for predicting black mold of tomatoes caused by *A. alternata*. This pathogen is one of the three species of *Alternaria* identified in causing Alternaria leaf spot of almond. In this model, disease severity values or DSVs are a function of 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., field dataloggers were placed in each orchard with sensors recording leaf wetness, temperature, rainfall, and relative humidity. Additionally, disease incidence and severity were measured periodically from May through the end of August. A critical conclusion made previously and confirmed this year was that an increase in the DSV value was followed by an increase in disease 21 to 27 days later (latent period) when temperatures were above 60-62F. In disease management studies all fungicides evaluated significantly reduced the incidence and severity of disease. Overall, Abound, Pristine (BAS516), and Flint (strobilurin fungicides) were the most effective fungicides. Application strategies based on a 15 to 20-point increase in 7-day DSV values resulted in a significantly lower incidence of disease than in the control. Disease incidence in these treatments, however, was significantly higher than in a calendar-based program. Thus, the forecasting strategy will need to be modified using either a lower threshold for the 7-day DSV or cumulative DSV values. Isolates of *Alternaria* spp. resistant to strobilurin fungicides in laboratory studies were found in three orchards in Kern Co. These isolates had EC_{50} values more than 100 times higher than those of the sensitive baseline isolates. Lack of efficacy and crop losses, however, were not associated with the presence of less sensitive isolates in our test orchards at this time. Thus, we cannot confirm field resistance at this time but a shift in fungicide sensitivity among isolates of *Alternaria* spp. has been documented.

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*. The disease occurs throughout the almond production areas in the central valleys of California but is most serious in the lower San Joaquin valley. The disease is most severe is areas where dew forms, the air is stagnant, and temperatures are high. Under favorable conditions for disease development, trees can be completely defoliated by early to mid-summer.

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, models for forecasting Alternaria diseases on other crops could also be evaluated on almond. For the last three years we have established disease progress curves in several orchards in Kern Co. and investigated correlations between the actual disease increase and increase in Disease Severity Values (DSV) using the DSV model for black mold of tomatoes caused by *A. alternata*. The disease severity in this model is determined based on the number of hours of wetness within defined temperature ranges. In both 2001 and 2002, an increase in the DSV value was followed by an increase in disease 21 to 27 days later. In 2003, studies were done in two orchards (a cv. Sonora orchard on Pond Ave.; and a cv. Carmel orchard off of Kimberlina Ave. in Kern Co.). Field dataloggers were used with sensors recording leaf wetness, temperature, rainfall, and relative humidity. Orchards were evaluated periodically for disease development from May to the end of August. Ten to fifteen terminal branches from the four quadrants of each tree were collected 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. In both orchards low levels of disease were first observed in mid-June that increased to 56.8% (Pond Ave. site) and 68% (Kimberlina Ave. site) at the end of the evaluation period. When parameters were graphed out together with the cumulative and 7-day DSV values, a correlation was evident between disease increase and increase in the 7-day DSV values (Figs. 1-2). Thus, an increase in the 7-day DSV values was followed by an increase in disease 21 to 27 days later when temperatures were above 60-62F. In addition, the graphed lines of the cumulative DSV values closely paralleled the actual cumulative increase in disease indicating that DSV values accurately describe the season-long disease progression. In the Kimberlina Ave. orchard, 3 infection periods were identified as indicated by vertical arrows A-C in Fig. 1. Infection periods B and C were characterized by a rapid increase in the accumulation of DSV and subsequently, by a rapid increase in observed disease (B'-C') approximately 25 days later. Infection period A, however, was not followed by an increase in disease probably because temperatures during this increase were below 62F. Thus, the interpretation of the DSV model will have to be adjusted by only considering values that accumulate above 62F. This will be evaluated using previous and future data sets. In the Pond Ave. orchard, several infection periods were evident by the 3 to 4 peaks in the 7-day DSV plot. The beginning of the increase in the 7-day DSV values >10 is shown by an A and the subsequent increase in disease is shown by an A'. Again, the latent period was approximately 25 days as in the Kimberlina plot. The cumulative DSV did not increase as fast as in the Kimberlina orchard (Fig. 2) but paralleled the season-long disease progression. Still, by the end of the evaluation period the disease incidence values were similar between the two orchards test sites. Thus, the DSV model looks very promising as a tool for predicting infection periods for the Alternaria pathogen on almond and, with additional adjustments, probably can be used to time fungicide applications (see below).

Trials were again set up in the spring of 2004 at two locations in Kern Co. and results are pending.

Fungicide evaluations for management of Alternaria leaf spot of almond. Two field trials were established in 2003 in Kern Co. with a history of the disease. Fungicides evaluated as single-fungicide applications or in mixtures or rotations included: Abound (azoxystrobin), Cabrio (BAS500; pyraclostrobin), Pristine (BAS516; mixture of pyraclostrobin and nicobifen), Echo (chlorothalonil), Elite (tebuconazole), Flint (trifloxystrobin), and the experimental compound V-10114 (Tables 1 and 2). Chlorothalonil was evaluated because it was registered on almond in 2003. Two applications of each treatment were done in each orchard using an air-blast sprayer, except for the rotation of Bravo with Pristine where a total of three applications was done. In the first orchard most treatments were applied in a calendar-based program. One Abound spray program was based on the DSV model. In the second orchard all treatments were applied based on the DSV model. All DSV model sprays followed increases in the 7-day DSV values of 15-20 points (at temperatures >62F) and were done on May 30 and June 17. For disease evaluation on July 31 or August 27, leaves of 30 terminal branches of each tree were assessed for disease in the field. For the laboratory evaluation, 10-15 terminal branches were collected from each tree and were scored for incidence and severity (number of lesions/leaf) of disease.

Table 1. Efficacy of fungicide treatments for management of Alternaria leaf spot on almond cv. Sonora in Kern Co.

			Application date			Disease incidence	
No.	Treatment	Prod Rate/A	3-28	4-23	5-14	%	LSD
1	Control	---	---	---	---	48.5	a
2	V-10114 1.6SC		---	@	@	14.2	bc
3	Abound 2F	12.8 fl oz	---	@	@	13.9	bc
4	BAS516 38WG	0.92 lb	---	@	@	2.6	d
5	Flint 50WDG	3 oz	---	@	@	12.9	bc
6	Elite 45WP/Flint 50WDG	2.9 oz/2.6 oz	---	@	@	10.3	c
7	Echo 720	4 pts	@	---	---	2.0	d
	BAS516 38WG	0.92 lb	---	@	@		
8	Abound 2F	12.8 fl oz	DSV Model			25.2	b

* - Treatments were applied using an air-blast sprayer (100gal/A) on 4 single-tree replications. DSV model sprays were applied on 5-30 and 6-17-03.

** - Trees were evaluated for disease on 7-31-03. For this, leaves from 30 terminal branches of each of the four tree quadrants were rated for incidence of disease. Values followed by the same letter are not significantly different based on an analysis of variance and LSD mean separation ($P > 0.05$).

In the first orchard where most treatments were applied in a calendar-based program, Pristine was the most effective fungicide (Table 1). The Abound spray program that was based on the DSV model had significantly less disease than the control treatment but it was less effective than the other treatments. In the second orchard, however, where all treatments were applied based on the DSV model, all treatments statistically performed similar, significantly reducing incidence and severity of the disease from the control (Table 2). The performance of two commercial applications with the registered fungicide Flint based on the DSV model was also evaluated. Disease was assessed in late summer and treatment effects were evident with a reduction from 67.7% in the control to 17.6% in the Flint treatment.

Thus, as in 2002, all strobilurin fungicides including Pristine and Abound as well as Flint were effective fungicides against Alternaria leaf spot in our 2003 trials. Effective alternatives for management of this disease are becoming increasingly important. Resistant pathogen populations appear to be common in Kern Co. where we evaluated three orchard populations for their sensitivity against azoxystrobin (Table 3). EC_{50} values for conidial germination among sensitive isolates ranged from 0.009 to 0.129 ppm azoxystrobin, whereas conidia of resistant isolates still germinated at concentrations of 50 ppm. Lack of efficacy and crop losses in our orchard plots, however, were not associated with the presence of less sensitive isolates. Abound and Flint, both strobilurin fungicides, both effectively reduced the incidence of disease. Thus, we cannot confirm field resistance at this time but a shift in fungicide sensitivity has been documented. Resistance

Table 2. Efficacy of fungicide treatments for management of Alternaria leaf spot on almond cv. Carmel in Kern Co.

			Field evaluation		Laboratory evaluation			
No.	Treatment	Product Rate/A	Incid. (%)	LSD	Incid. (%)	LSD	Severity (Avg. Lesions/Leaf)	LSD
1	Control	---	85.8	a	67.7	a	2.7	a
2	Abound 2F	12.8 fl oz	7.0	b	14.4	b	0.3	b
3	Flint 50WDG	3 oz	15.6	b	29.4	b	0.8	b
4	BAS500 22.2WG	9.5 oz	18.0	b	23.9	b	0.6	b
5	BAS516 38WG	0.92 lb	16.5	b	16.9	b	0.4	b

* - Treatments were applied using an air-blast sprayer (100gal/A) on 4 single-tree replications. Sprays were done according to the DSV model on 5-30 and 6-17-03.

** - Trees were evaluated for disease on 8-27-03. For this, leaves of 30 terminal branches of each of the four tree quadrants were rated for incidence of disease. For the laboratory evaluation, 10-15 terminal branches were collected from each tree and a random sample of 100 leaves were scored for incidence and severity (number of lesions/leaf) of disease. Values followed by the same letter are not significantly different based on an analysis of variance and LSD mean separation ($P > 0.05$).

management strategies that include fungicide rotations between different classes of fungicides and mixtures have to be implemented. For this, effective fungicides have to be available. Although one of the components of Pristine (pyraclostrobin) is a strobilurin like azoxystrobin where cross-resistance is likely to occur, the second component boscalid belongs to a different class of fungicides. Boscalid was evaluated in our 2001 field trial and was also shown to be effective against the disease. Pristine has recently been federally registered, with a California registration pending. Thus, an alternative to Abound and Flint will be available for use in 2004. Still, we will continue to evaluate new materials against Alternaria leaf spot in our trials next year. The numbered compound V-10114 represents a different class of fungicide but may not be developed in the United States.

In 2004 field trails for the evaluation of fungicides were again established again in two locations. Several new materials were included in these studies such as polymixin (Polyoxin-B), kasugamycin (Kasumin), and difenoconazole (Score). Results from these trials are pending.

Table 3. Occurrence of azoxystrobin-insensitive isolates of *Alternaria* spp. in three almond orchards in Kern Co. - Summer 2003

Orchard	No. of isolates evaluated	No. of sensitive isolates	EC_{50} (ppm)	No. of resistant isolates	EC_{50} (ppm)
1	20	16	0.009 - 0.129	4	>50
2	9	7	0.018 - 0.069	2	>50
3	20	0	---	20	>50

Azoxystrobin sensitivity was based on inhibition of conidial germination in a spiral gradient dilution assay.