Epidemiology and Management of Almond Anthracnose and Brown Rot in California

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I. Pre- and postharvest studies on ecology and epidemiology of almond anthracnose;

II. New cultural and fungicide management practices for brown rot and anthracnose.

Objectives

I. Epidemiology
   A. Detection of fungal pathogens in plant tissue
      1. Detection of anthracnose and other diseases in almond orchards as a preharvest management tool using molecular assays.
         a. Evaluation of the ELISA detection kit in cooperation with farm advisors as a method for rapid diagnosis of anthracnose.
         b. Continue determining the accuracy of the ELISA detection kit as compared to DNA-PCR detection or isolation of the pathogen on agar media.
      2. Detection of postharvest anthracnose infections in whole and sliced kernels.
   B. Evaluation of the effects of microclimatic parameters such as leaf wetness and temperature on disease development in growth chamber and field studies.
   C. Histological studies on the initial infection process of *C. acutatum* on almond under different environments.
   D. Laboratory, growth chamber, and field evaluation of host susceptibility and temperature-wetness relationships for disease development on selected almond cultivars.

II. Disease management strategies
   A. Determination of potential development of resistant populations of target organisms by establishing EC_{50} and baseline sensitivity values and monitoring field populations.
   B. Continuation of fungicide efficacy studies and rotation programs for anthracnose management (dormant and in- season programs will be evaluated), as well as evaluations of new fungicides for brown rot, shot hole, and other diseases of almond.
   C. Orchard sanitation and irrigation practices for anthracnose management

Summary

Due to low rainfall in the spring of 2002, anthracnose incidence was low in both epidemic centers of Butte and Stanislaus-Merced Co. Disease data obtained in field trials and under controlled conditions in growth chambers were correlated to environmental parameters (temperature, rainfall, and other parameters). Epidemiological models for predicting disease on almond leaf and blossom tissue for several almond cultivars (Nonpareil, Wood Colony, Carmel, and NePlus Ultra) were developed for a range of temperatures and wetness periods. For leaves, wetness duration was a more critical parameter for disease development than it was for blossoms. Temperature, however, was of similar importance for both tissues with temperatures above 10°C being an absolute minimal requirement. Preliminary studies indicated that the effect of wetness period on fruit disease is similar as for leaves. Furthermore, we found that a concentration of 10^3 conidia/ml represents a critical threshold for disease development.

Digital image analysis of light micrographs and histological studies indicated that the internal light spot corresponds to the penetration peg of appressoria and that fungal penetration and initial colonization of almond tissues occurs within12 h after inoculation and incubation at 20°C. Colonization begins as subcuticular and intracellular growth in a biotrophic phase. The biotrophic phase is temperature-dependent and is shorter as temperatures increase. In fungicide efficacy studies, single-fungicide, mixtures, or rotation programs with Abound (azoxystrobin), BAS516 (mixture of pyraclostrobin and nicobifen), Bravo/Echo (chlorothalonil), Captan (captan), Elite (tebuconazole), Flint (trifloxystrobin), Laredo (myclobutanil), Orbit (propiconazole), Scala (pyrimethanil), and Ziram (ziram) were all very efficacious for disease control. Continued development of new materials is essential in developing fungicide management programs that are highly effective and that prevent the development of resistance within populations of *Colletotrichum* and other foliar fungal pathogens of almond.

For brown rot blossom blight control Vangard, Scala, Laredo, Eminent, BAS516, and Bravo were highly effective treatments. Alternate-row application strategies of fungicides for blossom blight control were also evaluated. Results indicated that fungicides had a reduced efficacy when the blossoms were collected from the far-side as opposed to the near-side of trees adjacent to the sprayer. Results were correlated to reduced fungicide residues and the amount of open blossoms. Furthermore, the exposure of the pathogen to lower fungicide concentrations favors the selection of outlier populations that are less sensitive to the fungicide as confirmed in laboratory studies. Alternate-row application programs may reduce disease management costs, but may be a high-risk practice that potentially leads to fungicide resistance in the field.

Epidemiological models for predicting Colletotrichum disease of almond based on defined wetness and temperature conditions. In previous growth chamber studies, we characterized the effects of microclimatic temperature and wetness duration on the amount of disease for almond blossoms and leaves. The results were presented graphically in last year's report. Statistical analyses were done this year to develop a

model for predicting the disease on almond. We analyzed disease severity data (number of lesions) for leaves and incidence data for blossoms of several cultivars including NePlus Ultra, Wood Colony, Carmel, and Nonpareil. These cultivars represent approximately 80% of the total almond crop in California. We selected the equations that best described the development of the disease for each tissue evaluated based on several statistical parameters (significance of the model, mean square error, coefficient of determination, and plots of residuals of actual and predicted values).

On blossoms, the models selected were very similar for each of the cultivars evaluated and reflect the overall similarity of blossom susceptibility between the cultivars. The models were:

$$\text{Wood Colony: } Y_b = 0.022TW + 0.190T^2 - 8.163;\ R^2 = 0.82$$
$$\text{Carmel: } Y_b = 0.024TW + 0.182T^2 - 7.925;\ R^2 = 0.80$$
$$\text{Nonpareil: } Y_b = 0.047TW + 0.112T^2 - 11.65;\ R^2 = 0.82$$

On leaves, the models selected were considerably different for each of the cultivars evaluated. This reflects the differences in susceptibility observed among the cultivars tested (refer to last year's report). The models for the most and least susceptible cultivars evaluated, respectively, were:

$$\text{NePlus Ultra: } Y_L = 0.01TW + 0.01T^2 - 0.38T - 0.067W + 2.4;\ R^2 = 0.90$$
$$\text{Nonpareil: } Y_L = 0.001TW - 0.001W^2 - 0.06W - 0.009T + 0.3;\ R^2 = 0.92$$

For the equations described above, Y_b is the percentage of diseased blossoms, Y_L is the number of lesions per leaf, W is wetness duration in hours, and T is the temperature (°C) during the wetness period. These equations show that the severity of disease both on leaves and blossoms increases with increasing temperatures during each wetness period. The temperature of 10°C (46 F) represents an absolute threshold with no disease developing below this temperature. The equations also reflect that the duration of wetness required for disease development depends on temperature. For example, at 20°C (68°F) the wetness duration threshold for development of disease was 12 h, whereas at 15°C (59 F) it was 24 h. Leaves and blossoms are affected differently by wetness durations. Longer wetness durations were required for disease development on leaves than on blossoms. Short wetness periods of 3 or more hours allowed disease development on blossoms for all temperatures evaluated above 10°C. These results confirm that different disease prediction models are required for different tissues (i.e., blossoms vs. leaves and fruit) and that a series of models will be required to describe disease in the field on leaves and potentially fruit for a number of cultivars ranging from low (e.g., Nonpareil) to high (NePlus Ultra) in disease susceptibility.

An assumption of the models developed was the presence of sufficient inoculum (e.g., 10^6 conidia/ml) to cause disease. The importance of inoculum availability in the manifestation of Colletotrichum disease has been previously studied by other researchers (Timmer and Zitko, 1993). In a prediction model for post bloom fruit drop of citrus, the effect of inoculum concentration was estimated based on the number of

infected blossoms before the first rain and the amount of rainfall (Timmer & Zitko, 1996). Based on inoculation studies on potted almond plants, we also found that inoculum concentration is critical for Colletotrichum disease development on this host. Our results indicate that a concentration of 10^3 conidia/ml represents another critical threshold for disease development. A model for forecasting Colletotrichum disease in the field would probably need to incorporate a factor to take into account the amount of inoculum. This potentially could be done with an index on disease history for a given orchard.

Histological studies on the initial infection process of *C. acutatum* on almond under different environments. Studies to evaluate the initial infection and host colonization strategies of *C. acutatum*, and the conditions favoring these processes were done using leaves and blossom petals. We investigated structures known as the internal light spots (ILS) of appressoria of the pathogen and the role of this structure in host penetration. These studies were conducted by using digital image analysis of light micrographs, scanning electron microscopy, and histological techniques. Comparative image analyses using depth relief mapping and line profiles of appressoria with and without ILS showed that the ILS corresponds to the penetration peg of appressoria (Fig. 1). Histological results confirmed fungal penetration and colonization of almond tissues within 36 h after inoculation and incubation at 20°C (Fig.2).

We have also studied the conditions that effect spore germination and formation of infection structures. Our results indicated that the time needed for appressorium and infection peg formation coincided with the wetness duration required for disease development at any selected temperature. This potentially provides an explanation for the importance of wetness duration on the development of the disease. On leaves, the number of lesions per leaf was positively correlated with the number of appressoria developed. On petals, however, this effect was even more pronounced. The delicate petals possibly allow more rapid development of the fungus than leaf surface tissues.

Our studies using classical histology and digital image analysis have allowed us to determine that *C. acutatum* colonizes its host at first subcuticularly and then intracellularly in a biotrophic phase. Subcuticular colonization has been related to the formation of quiescent infections in other hosts (Zaitlin et. al, 2000). The intracellular colonization also constitutes a relative period of latency, i.e. quiescent infection. Thus, the strategy of host colonization by *C. acutatum* includes two possible periods of latency previously not described. Furthermore, studies on the effect of temperature on symptom development after infection show that the biotrophic period is temperature-dependent. For example, at 20°C, the biotrophic stage lasts 4-5 days in leaves and 2 days in blossoms, whereas at 10°C it may last for 14-21 days in leaves and at least 7 days in blossoms. Thus, selected combinations of wetness durations and temperatures may be conducive for development of quiescent infections, but not for disease symptoms. We are currently investigating the factors that determine the switch from the biotrophic stage to a necrotrophic phase that lead to symptom development in almond.

Fungicide evaluations for management of Colletotrichum disease. In 2002, fungicides registered for Colletotrichum control included Captan, Abound, Flint, Laredo, and Ziram. Fungicides evaluated in field trials either in mixtures or rotations included: Abound (azoxystrobin), BAS516 (mixture of pyraclostrobin and nicobifen), Bravo/Echo (chlorothalonil), Captan (captan), Elite (tebuconazole), Flint (trifloxystrobin), Laredo (myclobutanil), Orbit (propiconazole), Scala (pyrimethanil), and Ziram (ziram). Chlorothalonil was evaluated based on the potential registration of this fungicide on almond in 2003. Due to little rainfall in the spring of 2002 disease incidence was low again. In one trial with rotations and mixtures using different fungicide classes, there was 2.8% disease incidence on fruit of the non-treated control. Five applications (in approximately 2-week intervals from pink bud in mid-February to mid-May) in each of the five rotation programs significantly reduced disease incidence to zero or near zero levels (Table 1). In another fungicide mixture/rotation trial using Orbit/Abound, Elite/Flint, Bravo, Captan, BAS516, and Laredo disease was also decreased to zero or near zero levels, as compared to 1.6% in the non-treated control.

Table 1. Efficacy of fungicide rotation programs for management of Colletotrichum disease on almond cv. NePlus Ultra.

			Application timing					Incidence of anthracnose	
No.	Treatment	Rate/A	PB	FB	PF	5-wk	7-wk	(%)	LSD
1	Control	---	---	---	---	---	---	2.8	a
	Rovral 4F	16 fl oz	@	---	---	---	---		
2	Laredo 2EC	12.8 fl oz	@					0.0	b
	Laredo 2EC/Flint 50WDG	10 fl oz/2 oz		@					
	Ziram 75WDG	8 lb			@				
	Flint 50WDG	3 fl oz				@	@		
3	Elite 45WP/Induce	6 oz/0.06%	@					0.0	b
	Elite 45WP/Flint 50WDG	2.3 oz/2oz		@					
	Captan 50WP	8 lb			@				
	Flint 50WDG	3 oz				@	@		
4	Orbit 3.6EC	4 fl oz	@					0.0	b
	Orbit 3.6EC/Abound 4F	2 fl oz/12.3 fl oz		@					
	Bravo 720SC	64 fl oz			@				
	Abound 2F	12.3 fl oz				@	@		
5	Orbit 3.6EC	4 fl oz/2 fl oz	@					0.3	b
	Vangard 75WG/Abound 2F	5 oz/12.3 fl oz		@					
	Bravo 720SC	64 fl oz			@				
	Abound 2F	12.3 fl oz				@	@		
6	Elite 45WP/Induce	6 oz/0.06%	@					0	b
	Scala 40F/Flint 50WDG	13.4 fl oz/2 oz		@					
	Bravo 720SC	64 fl oz			@				
	Flint 50WDG	3 oz				@	@		

* - Treatments were applied using an air-blast sprayer (100gal/A) at pink bud, full bloom, petal fall, and 5 and 7 weeks after petal fall.
** - Evaluations on 4/25 were based on 100 fruit for each of 4 single-tree replications.

Values followed by the same letter are not significantly different based on an analysis of variance and LSD mean separation ($P > 0.05$).

Fungicide evaluations for management of brown rot blossom blight and shot hole. Fungicides evaluated either as single-fungicides, in mixtures, or in rotations in field trials using two application timings (pink bud and full bloom) for control of blossom blight included: Abound (azoxystrobin), BAS516 (pyraclostrobin + nicobifen), Bravo (chlorothalonil), Elite (tebuconazole), Eminent (tetraconazole), Flint (trifloxystrobin), Laredo (myclobutanil), Orbit (propiconazole), Rovral (iprodione), Scala (pyrimethanil), Vangard (cyprodinil), and Ziram (ziram). In these trials, any of these applications was highly efficacious, significantly reducing the incidence of brown rot blossom and twig blight from that of the non-treated control (Table 2). For control of shothole, the same fungicides programs were used, however, no data could be obtained in 2002 due to very low disease levels.

Table 2. Efficacy of a bloom fungicide program for management of brown rot blossom blight of Drake almonds at the UC Davis experimental orchard.

No.	Treatment*	Rate/A	Application timing		Brown rot strikes	
			PB	FB	Incid. (%)**	LSD
1	Control	---	---	---	19.5	a
2	Bravo 720 SC	4 pts	@	@	5.2	bcd
3	BAS516 38WG	0.658 lb	@	@	5	bcde
4	BAS516 38WG	0.92 lb	@	@	2.4	cde
5	Eminent 125SL	16 fl oz	@	@	2.6	cde
6	Laredo 2EC	12 fl oz	@	@	6	bc
7	Vangard 75WG	5 oz	@	@	4	bcde
8	Elite 45WP + Rovral 4F	3 oz/8 fl oz	@	@	2.2	cde
9	Scala 400SC+ Rovral 4F	13.7 fl oz/8 fl oz	@	@	1	e
10	Elite 45WP+ Flint 50WDG	2.32 oz/2.08 oz	@	@	2.2	cde
11	Elite 45WP+ Flint 50WDG	2.89 oz/2.6 oz	@	@	2.6	de
12	BAS516 38WG	0.92 lb	@		3.6	cde
	Rovral 4F	16 fl oz		@		
13	Laredo 2EC	12.8 fl oz	@		2.6	cde
	Ziram 75WDG	8 lb		@		
14	Vangard 75WG	5 oz	@		9.4	b
	Bravo 720 SC	4 pts		@		
15	Vangard 75WG	5 oz	@		3	cde
	Orbit 3.6EC	4 fl oz		@		
16	Rovral 4F	16 fl oz	@		4.2	bcde
	Ziram 75WDG	8 lb		@		
17	Laredo 2EC	12.8 fl oz	@		6.6	bcd
	Vangard 75WG	5 oz		@		

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

** - Incidence of disease based on 100 shoot samples from each of five single-tree replications from each treatment. Values followed by the same letter are not significantly different based on an analysis of variance and LSD mean separation ($P > 0.05$).

Field studies to evaluate the pre- (protective) and post-infection ("kick-back action") efficacy of selected fungicides (Abound, Bravo, Elevate, Laredo, Rovral, Topsin, and Vangard) were conducted on cvs. Butte and Carmel. To evaluate the post-infection action, blossoms were collected and inoculated in the laboratory, and were then treated after one or two days. To evaluate the protective action of the fungicides, trees were treated in the field using an air-blast sprayer. Blossoms were then collected and inoculated in the laboratory. Similar results were obtained for the two almond cultivars and the data for cv. Butte are shown in Table 3. The results indicated that all fungicides evaluated had a "kick-back action" when treated one or two days after inoculation. Elevate was the least effective when applied 1 day after inoculation, reducing the incidence of anther infections from 32.9% in the control to 8.4%, whereas the remaining fungicides reduced the incidence to 0-0.5%. All fungicides also showed a good protective activity for pre-infection intervals of 1 to 3 days (Table 3). Thus, all fungicide classes currently registered for blossom blight control on almond have some pre- and post-infection activity. This indicates that under less conducive disease conditions (little rainfall) one full bloom application will be sufficient to protect the flowers from blossom blight.

Table 3. Efficacy of fungicides applied at full bloom before or after inoculation for management of brown rot blossom blight of cv. Butte almond*.

		Incidence of infected anthers (%) Inoculation of blossoms before or after treatment				
Fungicide	Rate	2 d before	1 d before	1 d after	2 d after	3 d after
Rovral 4F/oil	16 fl oz	40.2 bc	0 c	7.6 bc	16 b	2.1 c
Topsin 70WP	2 lb	19.3 c	0.3 c	14.7 bc	25.5 b	5.6 bc
Vangard 75WG	5 oz	39.5 bc	0.5 c	2.6 c	13.8 b	9.0 bc
Elevate 50WG	1.5 lb	54.8 b	8.4 b	27.2 b	22.1 b	15.0 b
Echo 720	64 fl oz	17.7 c	0 c	29.9 ab	22.2 b	15.6 b
Abound 2F	12.3 fl oz	41.7 bc	0 c	14.5 bc	19.9 b	16.0 b
Laredo 2F	12.8 fl oz	15.6 c	0 c	11.5 bc	20.2 b	16.1 b
Control	---	79.4 a	32.9 a	57.8 a	67.9 a	52.7 a

* - To determine the "kick-back action" of the fungicides, blossoms were detached from the trees, placed into wet vermiculite in plastic boxes, inoculated with conidia of *M. laxa* (15K/ml) and treated with fungicides in the laboratory using an aerosol spray bottle 1 or 2 days after inoculation. To determine the protective action of the fungicides, blossoms were treated in the field using an air-blast sprayer, and collected 1, 2, or 3 days after treatment. Blossoms were then inoculated with conidia of *M. laxa* and incidence of anther infections was evaluated after 4 days. Numbers within a column that are followed by the same letter are not significantly different from each other.

Evaluation of alternate-row spray programs for management of brown rot blossom blight. In additional trials in commercial and experimental orchards, we compared fungicide efficacy for control of blossom blight by spraying trees from only one side to evaluate an alternate-row spray program. When cv. Carmel blossoms were

treated using an air-blast sprayer with Vangard at 1 to 3% bloom, blossoms from the side of the tree directly facing the application (the near side) that were inoculated in the laboratory had an incidence of anther infections similar to that of blossoms that were collected from the opposite side of the tree (the far side, Table 4). When trees were treated at later bloom stages, however, a significant difference in disease level was found between near- and far-side blossoms. Thus, for cv. Peerless treated at 50% bloom, disease was 93.8% in the control, 2.9% for near-side, and 15.3% for far-side blossoms (Table 4). For cv. Butte treated at 70-80% bloom, disease was 98.5 in the control, 23.9% for near-side blossoms and 68.1% for far-side blossoms.

Similar results were obtained with Laredo-treated blossoms. When trees were first treated at an early bloom stage and then at full bloom from the opposite row, differences in disease between near- and far-side blossoms were smaller, and sometimes not significant. Fungicide residues that were determined after a single application for blossoms in the two tree locations were significantly lower in the far-side blossoms as compared to the near-side blossoms. The exposure of the pathogen to lower fungicide concentrations is likely to favor the development of fungicide-resistant populations. This was confirmed in laboratory in vitro studies using a new method, the spiral gradient endpoint method.

In these studies fungicide thresholds can be determined where no more fungal colonies develop. Preliminary results of these experiments indicated that confluent growth of *M. fructicola* ended at 0.06 mg cyprodinil (Vangard)/L (ppm). Outlier colonies developed up to 4 mg/L, well above the 1.5-mg/L residues of the opposite-side blossoms. For *M. laxa*, confluent growth ended at a higher concentration of 0.12 mg cyprodonil/L and outliers continued to develop at much higher concentrations up to 20 ppm. These results indicate that alternate-row-spraying with the single-site mode-of-action fungicide cyprodinil may allow populations of species of *Monilinia* to survive, resulting in decreased sensitivity of the pathogens to the fungicide. Other fungicides such as myclobutanil (Laredo) were also evaluated with similar results and thus, the specific fungicide should not be emphasized but rather the practice. Thus, alternate-row application strategies to reduce disease management costs may be a high-risk practice that potentially leads to fungicide resistance.

Table 4. Efficacy of fungicides in alternate row spray programs - Blossoms treated with cyprodinil and sampled on near and far side of tree.*

Location on tree	Incidence of anther infections (%)		
	Carmel 1-3% bloom	Peerless 50% bloom	Butte 70-80% bloom
Untreated	82.8 a	93.8 a	98.5 a
Near side	8.2 b	2.9 c	23.9 c
Far side	11.7 b	15.3 b	68.1 b

* - Trees at different bloom stages were treated commercially (cvs. Carmel and Peerless) or experimentally (cv. Butte) on one side using an air-blast sprayer at a rate of 5 oz/100 gal. Blossoms were collected from the near (sprayer-facing) and far (sprayer-opposite) sides of the trees, placed into plastic boxes with vermiculite, inoculated with conidia of *Monilinia laxa* (1.5 x 10^4/ml), and incubated at 20C for 3-4 days.

** - Numbers within a column that are followed by the same letter are not significantly different from each other.

References

Timmer, L.W., and S.E. Zitko. 1992. Relationships of environmental factors and inoculum levels to the incidence of postbloom fruit drop of citrus. Plant Dis. 77:501-504.

Timmer, L.W., and S.E. Zitko. 1996. Evaluation of a model for prediction of postbloom fruit drop of citrus. Plant Dis. 80:380-383.

Zaitlin, B., E. I. Zehr, and R.A. Dean. 2000. Latent infection of peach caused by *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*. Can J. Plant Path. 22:224-228.