Annual Report for the Almond Research Board 2003-2004

Correct Project Number: 03-JA-01

Project No. 95-JA1: Epidemiology and management of almond anthracnose and brown rot in California –
I. Pre- and postharvest studies on ecology and epidemiology of almond anthracnose;
II. New cultural and fungicide management practices for brown rot and anthracnose.

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Objectives

I. **Epidemiology**

A. Detection of fungal pathogens in plant tissue -
Evaluation of the ELISA detection kit and DNA-PCR as compared to fungal isolation on agar media for rapid diagnosis of blossom, foliar, and fruit diseases caused by species of *Colletotrichum, Monilinia,* or *Botrytis* in cooperation with farm advisors as a method.

B. Laboratory/greenhouse studies on infection processes -
   1) Environmental factors that may induce differentiation of germ tubes of *C. acutatum* into appressoria or infection structures that directly penetrate host tissue.
   2) Factors that may induce the hemi-biotrophic phase of the pathogen to shift into the necrotrophic or disease phase. Evaluation of the effects of plant stresses such as inoculum levels and darkness on anthracnose disease development under conducive or less conducive leaf wetness and temperature parameters.

C. Development of almond anthracnose in the field -
   1) Disease progress curves based on periodic disease evaluations and environmental monitoring using dataloggers.
   2) Evaluate a preliminary model for disease forecasting that is based on rainfall, wetness duration, and temperature.

D. Field evaluation of host susceptibility to blossom, leaf, fruit, and kernel diseases among almond cultivars -
   1) Varieties planted in the variety block at UC Davis will be sprinkler irrigated during bloom and evaluated for brown rot, shot hole, and other diseases.
   2) As part of collaboration with Dr. T. Gradziel, we will continue to evaluate fungi associated with kernel discolorations.

II. **Disease management strategies**

A. Continue to determine the potential for resistant populations of target organisms to develop by establishing EC_{50} and baseline sensitivity values and monitoring field populations.

B. Continue fungicide efficacy studies and evaluation of rotation programs for anthracnose management, as well as evaluations of new fungicides for brown rot and other diseases of almond.

1) Evaluate persistence and post-infection activity of selected fungicides in field inoculation studies for management of brown rot and anthracnose.

2) Evaluate alternate and every-other-row sprays in respect to fungicide residues and brown rot blossom blight incidence of inoculated blossoms.

SUMMARY

The incidence of Colletotrichum disease was initially low in both epidemic centers of Butte and Stanislaus-Merced Co. but with late spring rains in the Sacramento valley, the disease increased to epidemic proportions in some northern California orchards. Disease data obtained in field trials in Butte Co. indicated that three applications of the newly registered fungicide chlorothalonil (Bravo Ultra or Echo) and soon to be registered Pristine (BAS-516) reduced the disease to 0.8% - 4.3% as compared to 27.3% in the untreated control. In brown rot studies, the registered materials Laredo and Vangard, the nearly registered products Pristine, Scala, and Scala-Rovral, Elite, as well as the experimental materials USF 2010 and V-10116, were highly effective reducing brown rot incidence from 0% to 1.4% as compared to 19% in the control. Chlorothalonil was also highly effective in reducing shot hole suggesting optimal timing of applications at early and late petal fall for shot hole and anthracnose management. Alternate-row application strategies of fungicides for brown rot blossom blight control were evaluated again in 2003. Results verified 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 when fungicides are applied after pink bud. Results were correlated to reduced fungicide residues and the amount of open blossoms. Exposure of the pathogen to lower fungicide concentrations favors the selection of outlier populations that are less sensitive. Thus, 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 when used after pink bud. In our epidemiological research on *Colletotrichum* on almond, an analysis of covariance was used to compare regressions of leaf disease incidence on wetness period for four almond cultivars (e.g., Nonpareil, Wood Colony, Carmel, and NePlus Ultra) at three temperatures. Results of these studies indicated that Nonpareil had the lowest, whereas NePlus Ultra had the highest levels of disease for the cultivars evaluated at each wetness duration and at all temperatures studied. Interestingly, disease levels for each wetness period evaluated for Carmel and Wood Colony were dependent on temperature and this may explain some of the variable field ratings of cultivar susceptibility. We also demonstrated that discontinuous wetness periods were not conducive to disease development. If 24-h dry periods interrupted a total wetness period of 72-h after 24 or 48 h, disease levels were significantly lower than when the 72-h wetness period was continuous. These studies indicated that survival of the

germinating and penetrating fungus decreases during a period of dryness and that new inoculum needs to be re-introduced for increases in disease to occur. Disease development is also dependent on some host stress factor(s). In 2003, we found that the pathogen causes shifts in pH that increase the activity of its enzymes that cause disease. In healthy fruit, pH values of fruit tissues on average were 4.17 and in diseased tissue they were 7.96. This increase in pH was accompanied by a 16-fold increase in the ammonia concentration. Possibly, long wetness periods and pH shifts represent accumulative stresses allowing the pathogen to penetrate and transform from the biotrophic to a necrotroph phase. Furthermore, the long wetness period may prevent the host from maintaining its internal pH resulting in disease development.

Epidemiological models for predicting Colletotrichum disease of almond based on defined wetness and temperature conditions. In our epidemiological research on *Colletotrichum* on almond, an analysis of covariance was used to compare regressions of leaf disease incidence on wetness period for four almond cultivars (e.g., Nonpareil, Wood Colony, Carmel, and NePlus Ultra) at three temperatures. Results of these studies indicated that Nonpareil had the lowest, whereas NePlus Ultra had the highest levels of disease for the cultivars evaluated at each wetness duration and at all temperatures studied. Interestingly, disease levels for each wetness period evaluated for Carmel and Wood Colony were dependent on temperature. Thus, Carmel was susceptible like NePlus Ultra at low temperature (i.e., 15C) and more resistant like Wood Colony at high temperatures (20C, 25C); whereas Wood Colony was more resistant like Nonpareil at 15C and 20C but more susceptible at 25C. These temperature effects may explain some of the variable field ratings of cultivar susceptibility. We also demonstrated that discontinuous wetness periods were not conducive to disease development. If 24-h dry periods interrupted a total wetness period of 72-h after 24 or 48 h, disease levels were significantly lower than when the 72-h wetness period was continuous (Table 1). These studies indicated that not every spore survives a period of dryness during these early stages of infection and that new inoculum needs to be re-introduced for increases in disease to occur.

Table 1. Effect of interrupted wetness periods on development of Colletotrichum disease on leaves of almond cv. Carmel after inoculation with spores of *C. acutatum*.

Treatment No.	Treatment Sequence	Disease Incidence (%)	LSD Grouping
1	Continuous wetness (72 hr)	100	A
2	Wet (24 h) - Dry (24 hr) - Wet (24 h) - Dry (24 hr)	28.6	C
3	Wet (24 h) - Dry (24 hr) - Wet (48 h)	35.1	C
4	Wet (24 hr)	34.0	C
5	Wet (48 h) - Dry (24 hr) - Wet (24 h)	46.0	B
6	Wet (48 h)	43.0	B

* - Leaves were inoculated with a spore concentration of (10^6) using a hand-sprayer. Leaves were bagged to obtain the wetness period duration. Re-wetting was done with sterile water. Drying treatments were done by removing bags and air-drying under ambient conditions.

Determine factors that may induce the hemi-biotrophic phase of the pathogen to shift into the necrotrophic or disease phase. Some of our previous histological studies were recently published (Diéguez-Uribeondo et al., 2003). We had demonstrated that infection and initial penetration by *C. acutatum* occur within 12-24 h after inoculation at optimal temperatures between 20 and 25C. Wetness periods of ≥48 h, however, are needed for high disease levels to develop. Thus, additional factors are apparently necessary to trigger the change from the biotrophic to the necrotrophic (disease causing) phase of the pathogen. Modulation of the pH within host cells by the pathogen and a subsequent increase in pectolytic or glucanase enzyme activity have been demonstrated in other diseases caused by *Colletotrichum* and *Alternaria* spp. (Eshel et al. 2000, Prusky et al. 2001). Thus, shifts in pH, caused by the pathogen, can increase the activity of the pathogen's pathogenicity factors. In 2003, we found similar shifts in pH in inoculated almond fruit. In healthy fruit, pH values of fruit tissues on average were 4.17 and in diseased tissue they were 7.96. This increase in pH was accompanied by a 16-fold increase in the ammonia concentration. Possibly, long wetness periods and pH shifts represent the accumulative stress allowing the pathogen to penetrate and transform from the biotrophic to a necrotrophic phase. Furthermore, a long wetness period may prevent the host from maintaining its internal pH resulting in disease development. We will continue to evaluate this and other potential environmental stress factors that may lead to increases in disease severity.

Fungicide evaluations for management of Colletotrichum disease. In 2003, fungicides registered for Colletotrichum control included Captan, Abound, Flint, Laredo, and Ziram. Fungicides evaluated in field trials either in mixtures or rotations included chlorothalonil (Echo), pyraclostrobin (BAS-516 or Pristine), myclobutanil (Laredo), as well as the experimental materials USF 2010, USF 2004, and DOW 20EW. Disease data obtained in field trials in Butte Co. indicated that three applications of the newly registered fungicide chlorothalonil (Bravo Ultra or Echo), Pristine (BAS-516), as well as the experimental materials USF 2010 and USF 2004, reduced the disease to 0.8% - 4.3% as compared to 27.3% in the untreated control (Table 2).

Table 2. Efficacy of fungicide rotation programs for management of anthracnose on almond cv. Price in 2003.

No.	Treatment	Product rate/A	Incidence (%)	LSD Grouping
1	Control	---	27.3	a
2	BAS 516 38WG (Pristine)	0.92 lb	4.3	cd
3	DOW EXP 20EW	15.3 oz	11.3	bc
4	USF 2010 500SC	196.3 ml	2.3	de
5	USF 2010 500SC	163 ml	4.3	cd
6	USF 2004 500SC	101 ml	2.0	de
7	Laredo 2EC	12.8 oz	14.5	bc
8	Echo 720	4 pt	0.8	e

* - Treatments were applied using an air-blast sprayer (100gal/A). All trees received a Rovral bloom treatment at pink bud on 2-11-03. Subsequent treatment dates were: 2-19-03 (full bloom), 3-4-03 (petal fall), and 3-25-03 (shuck split).
*** - Evaluations on 5/21/03 were based on 100 fruit for each of 5 single-tree replications. Values followed by the same letter are not significantly different based on an analysis of variance and LSD mean separation ($P > 0.05$).