## Annual Report 2000

*Prepared for the Almond Board of California* 



## **Objectives**

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- 1. EPIDEMIOLOGY
	- A. Detection of *Colletotrichum* species in plant tissue
		- 1. Detection of anthracnose 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 field studies.
	- C. Laboratory, greenhouse, and field evaluation of host susceptibility and temperature-wetness relationships for disease development on selected almond cultivars.
	- D. Histological studies on the initial infection process of C. *acutatum* on almond under different environments.
- 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 high rainfall in the spring of 2000, anthracnose continued to cause losses in both epidemic centers of Butte and Stanislaus-Merced Co. Disease evaluation data are being used to describe disease progress curves and to model anthracnose of almond blossoms and fruit. Disease incidence data obtained in field trials or in controlled conditions in growth chambers are being correlated to environmental parameters

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(temperature, leaf wetness, rainfall, relative humidity) and will be the basis for the development of a disease prediction model. A rapid detection method for anthracnose infected tissues based on an ELISA assay that was developed by us last year was further evaluated in a collaborative study with farm advisors. These tests were very successful with a high degree of accuracy in correctly diagnosing the disease. This assay is important for rapidly diagnosing the disease and may be essential for developing a forecasting model that is dependent on critical inoculum concentrations for disease development to occur. In fungicide research, Abound was widely used in the first full year that the compound was registered on almonds. In our efficacy studies, the fungicide provided excellent disease control especially in rotations with captan or ziram. Comparatively, ziram was equivalent to captan as a broad spectrum rotational or companion fungicide with Abound. In greenhouse studies evaluating both pre-and postinfectional efficacy of Abound, the fungicide was most effective as a preventative treatment but also provided very good control within 24 hours of an infection period. Fungicide application 2 to 3 days after an infection period resulted in substantial loss of efficacy. Comparative fungicide trials with experimental compounds indicated that Elite, Flint, *A-9325,* and Indar provided disease control similar to Abound and Captan. Continued development of these 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. These compounds were also effective for brown rot management. Vangard was highly effective for brown rot and it was also effective against shot hole. The most efficacious compounds for reducing shot hole incidence were Abound, Rovral, and Rovral/Captan mixtures.

Epidemiology - Field evaluations of disease progress and relationships to environmental parameters. In three field sites in Butte, Stanislaus, and Merced Co., disease progress curves during the spring season were developed for blossoms and fruit of  $cv$ . Merced and NePlus Ultra. At each site, dataloggers were used to record environmental parameters (leaf wetness, temperature, and relative humidity) and disease incidence was evaluated weekly on selected non-fungicide treated trees. Anthracnose blossom blight, which is easily confused with brown rot blossom blight, was positively identified in selected samples by fungal isolation or by the ELISA assay (see below). At all three sites rainfall was abundant in late winter until March 20 (Julian day 79; see Fig. 1 for the trial site at CortezlMerced Co.). Although only two to four rainy periods occurred for the remaining spring season at each site, high leaf wetness values were recorded throughout this period (data not shown). Incidence of anthracnose blossom blight increased to 30% in the Cortez orchard (Fig. 1) and to 15 (cv. NePlus Ultra) or 72% (cv. Merced) at the other two locations by the end of March. Incidence of fruit anthracnose increased to 29% in the Cortez orchard (Fig. 1) and to 3.2 (cv. NePlus Ultra) or  $13\%$  (cv. Merced) at the other two locations. The decrease in incidence in mid-May (Julian day 132) could be attributed to a storm that caused many of the diseased fruit to drop. These disease data will be used to describe disease progress curves and to model anthracnose of almond blossoms and fruit.

Epidemiology - Field and growth chamber evaluations of disease progress after inoculation and wetness/temperature relationships. The effects of wetness period duration and temperature on disease incidence on leaves and blossoms of Nonpareil, Carmel, Wood Colony, and NePlus Ultra almond were evaluated. In growth chamber studies, leaves of potted plants were inoculated with an isolate of the pink genotype of C. *acutatum* at a concentration of  $10^6$  conidia/ml and were kept wet for 1, 24, 48, or 72 hr at 8, 15, or 20 C (46, 59, or 68 F). Disease increased linearly with longer wetness period duration for all cultivars and temperatures evaluated (Fig. 2). Total disease incidence on leaves was lower at lower temperatures as compared to higher temperatures for each cultivar after a 14-day incubation period. Amongst the cultivars evaluated, NePlus Ultra was most susceptible with numbers of lesions per leaf generally three to six times higher than for Nonpareil for each wetness period evaluated at 8 to 20 C. Carmel and Wood Colony were intermediate in disease susceptibility as compared to NePlus Ultra and Nonpareil. Thus, differential host susceptibility was demonstrated under defined environments, and the results correlated with field observations and field studies done with fruit in previous years. The effect of wetness duration at three temperatures (8, 15, and 20C) on incidence of leaf anthracnose on Carmel almond is illustrated in Fig. 3. We also initiated inoculation studies with almond blossoms (cultivars Carmel, Nonpareil, and Wood Colony). In field studies, disease incidence increased linearly as wetness periods were increased from 1 to 72 hours for all three almond cultivars evaluated. The statistical comparison of the three cultivars indicated that although

disease increased similarly, Nonpareil had the lowest and Wood Colony had the highest incidence of blossom infections. These studies will be repeated in 2001 with lower inoculum concentrations. Shorter incubation times were required for disease symptoms to develop on blossoms as compared to leaves or fruit, indicating that blossoms are much more susceptible to anthracnose than are leaf or fruit tissues. Thus, incubation periods of approximately four weeks were necessary in previous years' field studies before symptoms developed on non-wounded, inoculated fruit as compared to 7 days in the current year's blossom studies. For fruit inoculations, no significant difference was observed between inoculum concentration levels  $(10<sup>5</sup> - 10<sup>6</sup>$  conidia/ml) in last year's field studies, however, the duration of the wetness period was critical. Wetness periods of 30 or 96 hr resulted in an average of 2.5% or 36% incidence of fruit decay, respectively. Data generated from controlled-environment studies in addition to disease progress data obtained in the field will be the basis for the development of a disease prediction model. Differential susceptibility of different almond tissues (blossoms, leaves, fruit) will require that separate models have to be developed for each type of tissue. Because high levels of quiescent infections (as detected in detached fruit) did not always result in a high incidence of fruit lesions in the field, a bi-modal model may also need to be developed, with separate models for infection and for expression of disease symptoms.

*Histological studies.* Studies to evaluate infection processes of the two subpopulations of the fungal pathogen were continued by inoculating detached almond leaves. In 1999 we indicated that isolates of the pink genotype, that are able to grow at higher temperatures compared to the gray isolates and that predominate in most areas with anthracnose epidemics, required longer incubation periods of up to 3 days at 10 C to complete conidial germination and formation of appressoria and infection pegs. At 20 C, conidia of the pink genotype germinated and formed appressoria after 11 hr; whereas isolates of the gray genotype completed conidial germination and appressorium formation after 24 hr at 10 and 20 C. In 2000, appressorium formation was studied in more detail using an isolate of the pink genotype. Microscopic observations indicated that appressoria mature quickly after being formed. Thus, by 12 hr after inoculation 95% of the appressoria were mature as indicated by melanization and by 24 hr 92% of the appressoria exhibited and internal light spot. Image modeling analysis of serial light micrographs provided evidence that this internal light spot corresponds to an infection peg. An evaluation of number of appressoria on diseased and non-diseased inoculated leaves indicated that diseased tissue had three times the number of appressoria than non-diseased tissue. This information is critical for developing a forecasting model of anthracnose on almond because inoculum thresholds are apparently needed to establish disease but not infections. More detailed histological studies are in progress and will be correlated with our field research.

Detection of anthracnose in almond orchards as a preharvest management tool using molecular assays. In 1999, we reported that a protein or ELISA assay had an accuracy of 83% for correctly identifying *Colletotrichum-infected* tissues, assuming that the standard isolation method of growing the fungus from plant tissue was 100% accurate. The ELISA assay method results in a color reaction for positive samples and is an overnight process. In contrast, the standard isolation method is accurate but requires long incubation times (5-6 days) and expertise in fungal identification. In 2000, we continued to evaluate the ELISA system in cooperation with John Edstrom, Roger Duncan, and Joe Connell. Using split-samples in comparative studies with these farm advisors, and comparing isolation data and ELISA results we found that after some experience the ELISA method was an effective tool for diagnosing the disease. Considering only "false negatives" (negative ELISA tests with positive fungal isolation of the same sample) the ELISA assay had an accuracy of 100% and 95% in our tests and 90% and 67% for tests done by the farm advisors for blossom and fruit samples, respectively. We expect greater accuracy in farm advisor evaluations as familiarity with the procedure increases. False positives (positive ELISA tests with negative fungal isolations of the same tissue) occurred at a higher incidence but were not of great concern because the ELISA test utilized a bigger tissue sample and is highly sensitive based on an enzymatic reaction. Thus, isolation procedures that utilize only a fraction of the tissue utilized in the ELISA method may overlook the presence of the fungus.

Fungicide evaluations for management of anthracnose. Consistent periods of rainfall occurred from Feb. through mid-March in Durham (Butte Co.) and Modesto (StanislauslMerced Co.), CA, in the spring of 2000. Rainfall patterns were similar for both counties with low precipitation from mid-March through mid-April.

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Temperatures were moderate with High/Low temperatures between 70 and 35 F through March 15 in both locations. Temperatures increased in early April.

In 2000, fungicides registered for anthracnose control included Captan, Abound, and Rally. Fungicides evaluated in field trials either alone or in rotations included: Abound (azoxystrobin), Flint (trifloxystrobin), Elite (tebuconazole), Indar (fenbuconazole), Rally (myclobutanil), Captan (captan), Ziram (ziram), and a pre-mixture of trifloxystrobin and propiconazole (A-9325). None of the fungicide programs eradicated the disease. Five applications (in approximately 2-week intervals from mid-February to late April) of either Abound, Flint, Elite, Indar, or BreakJFlint, significantly decreased disease incidence to about the same levels as compared to the untreated control (Table 1). Incidence of blossom anthracnose in the untreated control was 43%. Because anthracnose blossom blight can easily be confused with brown rot blossom blight, the presence of anthracnose was confirmed by fungal isolations and by an ELISA assay that specifically detects *Colletotrichum* species. Incidence on the treated trees ranged from 7.3 to 15.3%. Incidence of fruit anthracnose in the untreated control was 12.3%. Again, all treatments evaluated significantly reduced fruit anthracnose, and there was no difference between the treatments. Incidence on the treated trees ranged from 1.5 to 3.5%.

Rotation programs that began at pink bud and continued throughout the spring (5 applications total) with Rally or Abound mixed with Captan or Ziram were all effective in reducing anthracnose incidence as compared to the control (Table 2). The mixture of myclobutanil with captan was less effective, reducing incidence of blight from  $43\%$  in the control to  $8.3\%$ . Incidence in the other rotations was less than  $1\%$ . Incidence of fruit anthracnose with 3.1% in the control was low in this orchard. All programs reduced the incidence to near zero levels. The second rotation program in Butte Co. also included fungicide mixtures (Table 3). Incidence of blossom blight and fruit anthracnose in the untreated control was 15.8 and 28.3%, respectively. All six rotation programs were efficacious in reducing blossom blight and fruit rot and there was no significant difference between the programs. In Merced Co. several selected fungicides were rotated with captan treatments. Anthracnose blossom blight that had 30.2% incidence in the control was reduced similarly by all rotations to 5.3% or lower (Table 4).

Studies to evaluate the pre- (protective) and post-infection ("kick-back action") efficacy of Abound were conducted in the greenhouse with almond leaves or in the lab using detached fruit. A fungicide application three days after inoculation reduced the efficacy by approximately 60% on leaves and 50% on fruit as compared to the protective treatment that provided nearly complete control (Fig. 4). Still, a fungicide application one day after inoculation provided greater than 80% control, whereas two days after inoculation, the fungicide provided approximately 60% control on both leaves and fruit. In similar studies using Break (propiconazole), comparable results were obtained.

**Fungicide evaluations for management of brown rot blossom blight and shot hole.** Fungicides evaluated in field trials using two applications (at pink bud and full bloom) for control of blossom blight included: Indar (fenbuconazole), Elite (tebuconazole), Rovral (iprodione), RovrallCaptan (captan), Abound (azoxystrobin), Flint (trifloxystrobin), Vangard (cyprodonil), and TM-417 (iminoctadine). In these trials, applications of any of these fungicides were highly efficacious, reducing the incidence of brown rot blossom and twig blight to less than 1.2% while the control treatment had an incidence of 14.2% (Table 5). Vangard was the best treatment with 0% incidence of brown rot. Reports from growers, however, indicated that some fungicide programs that included Vangard resulted in minor phytotoxicity on leaves. Oils are known to be incompatible with Vangard on other crops. We have not seen any injury in our trials, however, we will continue to investigate this possibility in 2001. The experimental compound RH168737 with 1.8% had the highest incidence among the treatments. Shothole on leaves was very low in 2000. Thus, evaluations were only done on fruit. For control of shothole, the same fungicides were effective using four applications (at pink bud, full bloom, petal fall, and shuck split) (Table 6). The most efficacious compounds for reducing shot hole incidence were Abound, Rovral, and Rovral/Captan mixtures.



Table 1. Efficacy of fungicide treatments for management of anthracnose on almond cv. Price in the spring of 2000.

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\* - Treatments were applied using an air-blast sprayer (100gal/A) at early pink bud, pink bud, full bloom, petal fall, shuck split, and 5 weeks after petal fall.

\*\* - Evaluations were based on 100 blossoms or 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  $(P > 0.05)$ .

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Table 2. Efficacy of fungicide mixture or rotation programs for management of anthracnose on almond cv. NePlus Ultra in 2000.

'- Treatments were applied using an air-blast sprayer (100gal/A) at early pink bud, pink bud, full bloom, petal fall, shuck split, S weeks after petal fall, and mid-spring (grower applied treatments). Note that Program 1 and 2 are mixtures except for the last application in Program 1.

\*\* - Evaluations were based on 100 blossoms on 3/22 or 400 fruit on 5/7 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 ( $P > 0.05$ ).



Table 3. Efficacy of fungicide rotation programs for management of anthracnose on almond cv. NePlus Ultra in 2000

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• - Treatments were applied using an air-blast sprayer (100ga1/A) at pink bud, full bloom, petal fall, shuck split, and S weeks after petal fall. \*\* - Evaluations were based on 100 blossoms on 3/22 or 400 fruit on 5/4 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 ( $P > 0.05$ ).



Table 4. Efficacy of fungicide rotation programs for management of anthracnose on almond cv. NePlus Ultra in 2000

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"- Treatments were applied using an air-blast sprayer (100ga1/A) at pink bud, full bloom, petal fall, shuck split, and 5 weeks after petal fall. \*\* - Evaluations were based on 100 blossoms on 3/22 or 100 fruit on 4/28 for each of 4 single-tree replications. Blossom blight was mostly caused by anthracnose, however, brown rot was also present. Values followed by the same letter are not significantly different based on an analysis of variance and LSD mean separation ( $P > 0.05$ ).



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

\* - Treatments were applied using an air-blast sprayer at a rate of 100 gallA. In the Rovral/Captan treatment, Captan was only applied at full bloom.

\*\* - Incidence of disease based on 100 shoot sample 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 ( $P > 0.05$ ).

Table 6. Efficacy of a bloom fungicide program for management of shothole of Drake almond fruit at the UC Davis experimental orchard.

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\*, Treatments were applied using an air-blast sprayer at a rate of 100 gallA. In the Rovral/Captan treatment,

Rovral was applied at pink bud and full bloom. All other treatments were also applied at pink bud and full bloom. \*\* - Incidence of disease based on a 25-fruit sample from each of five single-tree replications from each treatment. Severity is based on the average number of spots per fruit. Values followed by the same letter are not significantly different based on an analysis of variance and LSD mean separation ( $P > 0.05$ ).

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Leaves were inoculated with conidia (106 conidia/ml) of an isolate of C. *acutatum.* Wetness periods were provided by bagging plants for 1, 24, 48, or 72 hours and then incubting for 2 weeks without additional wetness at 20C.

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Fig. 3. Effect of wetness period duration and temperature on incidence of leaf anthracnose on Carmel almond Growth chamber studies under defined environmental conditions



Thresholds for minimum temperature and wetness period duration are being determined. Disease increased linearly with longer wetness period duration for all cultivars and temperatures evaluated. The optimum temperature for disease development was 20C.

## Fig. 4. Efficacy of azoxystrobin (Abound 2F) for control of fruit and foliar anthracnose of almond cv. Carmel

Regression of disease reduction on time of fungicide application



Leaf inoculations were conducted in the greenhouse. Inoculations of detached almond fruit were done in the laboratory.