Annual Report 1999

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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.

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

Cooperating: H. Förster, D. Thompson, Beth Teviotdale, J. Connell (Butte Co.), Lonnie Hendricks (Merced Co.), Mark Freeman (Fresno Co.), P. Verdegaal (San Joaquin Co.), and Roger Duncan (Stanislaus Co.).

SUMMARY

In 1999, incidence of anthracnose was low in most locations, however, the disease remained detectable in most orchards where damage had occurred in previous years. The two genotypes of the causal pathogen *Colletotrichum acutatum* (genotype P and genotype G) continued to be isolated from counties in the mid- and northern portions of the almond production regions of the state. Low temperatures and low rainfall for most of the spring were not conducive for disease development and emphasize the importance of a forecasting system for the disease. Thresholds of minimum temperature and wetness periods are being established and histological observations on the infection process of the anthracnose pathogen were initiated. In field inoculation studies, long wetness periods (96 hr) after inoculation were required for disease development. Wetness periods of 30 hr, however, were sufficient to produce non-visible quiescent infections of the fungus. This observation correlated with preliminary results of histological studies that indicated that after a 24 hr-wetness period the fungus had produced appressoria and apparently penetration pegs. *C. acutatum* was found to overwinter in almond branches with dieback symptoms. Dieback of the majority of branches that were tagged in 1998 advanced during the 1999 season. In order to define environmental conditions that lead to postharvest anthracnose kernel damage, almond kernels that were inoculated in the laboratory with mycelium of the fungus were incubated for up to six months at selected humidities. Kernel colonization occurred at kernel moisture levels higher than 10.6% after 6 months. No growth of the fungus was observed below kernel moisture contents of 10.2%. This is consistent with our previous report (1998) where studies were conducted for 3 months. Rapid methods for the detection of *C. acutatum* in infected almond tissue were evaluated. A method that is based on the detection of specific fungal proteins and that requires little technical expertise was quite accurate in detection of the disease and will be further developed to be used by farm advisors and PCAs. In fungicide research, Abound was registered for both, anthracnose and Alternaria leaf spot. Efficacy trials were continued for anthracnose management. Break, Elite, Indar (at 4 oz), Abound, and Flint were the most efficacious fungicides. Rotations of Break, Elite or Abound with Captan were highly effective. The effect of pruning on the incidence of anthracnose was evaluated. Trees that were pruned in 1998 to remove all dead twigs and branches larger than 1 cm in diameter had significantly less anthracnose in 1999 as compared to non-pruned trees. For brown rot control, Elite and Stratego were most efficacious in reducing the incidence of spur blight. For shothole control the most efficacious compounds were Abound, Flint, and the experimental compounds TM-417 and RH168737. In addition to this research, a new almond fruit disease and associated branch dieback caused by the fungus *Phomopsis amygdali* was reported for the first time in California.

INTRODUCTION

In recent years, almond anthracnose has become a widespread and serious problem in the major almond growing regions in California. Symptoms of the disease occur on blossoms, developing and maturing fruit, and on spurs and branches with often extensive leaf yellowing and dieback throughout the spring and summer, if conducive conditions persist. All almond varieties are susceptible to anthracnose, but varietal differences have been observed. Disease incidence has been correlated with rainfall and warmer temperatures. Infected mummified fruit has been identified as inoculum sources for new spring infections. Preliminary studies to determine the cause of tree dieback symptoms associated with almond anthracnose indicated that phytotoxic compounds secreted by the fungus might be responsible for leaf yellowing and branch dieback. Thus, the disease can cause direct pre- and postharvest losses of the almond crop by infecting fruit and kernels, as well as potentially indirect losses by causing shoot dieback and tree decline.

Previously, the fungal pathogen was characterized and identified as *Colletotrichum acutatum* with two distinct sub-populations occurring in California that differ in their cultural pigmentation (pink vs. gray), pathogenicity on wound-inoculated almond fruit, sensitivity to benomyl at low conidial concentrations, and in their DNA fingerprints. In 1997, the disease was also found to cause postharvest problems on the 1996 crop. Laboratory studies indicated that postharvest damage to almond kernels was probably the result of preharvest epidemics that were not managed. This information was summarized in a paper published in Phytopathology (see attached, Vol. 89: 1056-1065).

Several fungicides with efficacy against anthracnose have been identified. Applications with Abound, Flint, Elite, Break, or Bravo resulted in the lowest fruit disease incidence in single-fungicide programs. Rotation programs using Abound, Break or Elite with Bravo or Captan were very effective in reducing incidence of fruit anthracnose. These field evaluations are ongoing.

Brown rot blossom blight caused by *Monilinia* species is another major disease of almond in California that potentially can cause extensive crop losses. We continued our research on the management of this disease by evaluating the newest fungicides available. Some of the new fungicides have a "reduced risk" classification by the US-EPA. "Reduced risk" fungicides either increase the adoption of integrated pest management or they have reduced toxicity to humans, reduced potential for contamination of the environment, or reduced toxicity to non-target organisms. Of the seven fungicides classified to date as "reduced risk", we have evaluated Abound, Elevate, Flint, and Vangard.

OBJECTIVES

I. Epidemiology
   - A. Detection of pre- and postharvest infections of *Colletotrichum acutatum* in plant tissue.
   - B. Evaluation of the effects of microclimatic parameters such as leaf wetness and temperature on disease development in laboratory and field studies.
   - C. Histological studies on the initial infection process of *C. acutatum* on almond under different environments.
   - D. Laboratory and field evaluation of host susceptibility and temperature-wetness relationships for disease development on selected almond cultivars.
   - E. Evaluation of kernel moisture content as a factor in establishing postharvest kernel damage.

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

MATERIALS AND METHODS

Fungal isolations. Fungi were isolated from selected symptomatic tissues of almond including fruit, leaves, peduncles, spurs, and branches. For fungal isolations, infected tissue was surface-sterilized with 400 mg/liter hypochlorous acid (HOCl) for 1 min and plated onto potato dextrose agar (PDA) amended with rifampicin (20 mg/liter) and ampicillin (130 mg/liter) to inhibit bacterial growth. Cultures were maintained on PDA (up to 1 year) or cryogenically.

Inoculation of almond fruit in the field. Fruit bearing branches of Carmel and Mission almond trees were either wound-inoculated or non-wound-inoculated by spraying with conidial suspensions of *C. acutatum* (genotype P; 10^5 or 10^6 conidia/ml). Branches were then bagged for 30 or 96 hr and disease was evaluated after 4 weeks.

Molecular detection of* C. acutatum *in infected almond tissue. DNA was isolated from almond tissues (blossoms, fruit, leaves, mummies) using a procedure recently developed for stone fruit tissues (Förster & Adaskaveg, 1999). PCR reactions were performed using *C. acutatum* species-specific primers (Adaskaveg & Hartin, 1997). Reaction products were separated by electrophoresis in 1.5% agarose using 0.5 x Tris-borate-EDTA buffer at 10-15 V/cm. Samples were considered infected by *C. acutatum* when the specific DNA band was present. For detection of anthracnose-infected almond tissues by an enzyme-linked immuno-sorbent assay (ELISA), a commercially available kit (Adgen Agrifoods Diagnostics, UK) was adapted. In this assay, proteins of the plant extract are first bound to the wells of a microtiter plate. Fungus-specific antibodies are then added that bind to the fungal proteins in the wells. Subsequently, a secondary antibody with an enzyme attached specifically binds to the first antibody. Positive samples are identified by a blue color reaction that is mediated by the enzyme that is attached to the secondary antibody.

***Evaluation of kernel moisture content as a factor in establishing postharvest kernel damage by* C. acutatum.** In order to continue defining environmental conditions that lead to postharvest kernel damage, almond kernels that were inoculated in the laboratory with agar disks of fungal cultures were incubated for up to 6 months at selected relative humidities using different glycerol solutions. For this, almond kernels (cultivar Carmel) were split in two halves along the sutures and were sterilized by autoclaving for 20 min. Kernels were then placed on sterile wire racks in plastic boxes containing 200 ml of a glycerol solution. These solutions were adjusted to different refractive indices that correspond to selected relative humidities. The boxes were sealed with silicon sealant or plastic tape and the kernels were allowed to equilibrate for 7-10 days at 20-24°C. The kernels were then inoculated with mycelial agar plugs (5 mm diameter, 2 mm thick) of an isolate of *C. acutatum* (genotype P). Three boxes were prepared for each solution and each box contained three replications for each inoculation method with 10 kernel halves each and three replications for measuring kernel moisture contents with 20 kernel halves each. The boxes were incubated at 20-24°C for up to 6 months. Kernels were checked weekly for fungal growth. When fungal growth was observed, aliquots of the non-inoculated kernels were ground using a food processor and passed through a 12-mesh sieve. The moisture content of the ground almond kernels was determined using a moisture balance (Mettler LP16). Kernels of treatments that did not show any fungal growth after 6 months of incubation were processed for determining their moisture content. To test the viability of the fungus in these latter treatments, the dried-up agar plugs were removed from the kernels and plated on PDA. If no growth occurred after 5 days the fungus was considered nonviable. This experiment was done twice.

Initial histological studies on the infection process of* C. acutatum *on almond. Detached almond leaves (*cv.* Nonpareil) were drop-inoculated with spore suspensions of *C. acutatum* (genotypes P or G) and were incubated at 10, 15, 20 or 25C. Samples (approximately 3 x 3 mm) were taken after selected time periods, cleared in saturated chloralhydrate, and were then examined under the light microscope.

Field evaluation of fungicides and evaluation of cultural practices for the development of management strategies. In field trials for control of anthracnose the following compounds were evaluated in selected spray programs using single-fungicide applications, rotations, or tank mixes: Abound (azoxystrobin), Indar (fenbuconazole), Rally (myclobutanil), Break (propiconazole), Elite (tebuconazole), Captan (captan), Flint (trifloxystrobin), Thiram (dimethyl-dithiocarbamate), Stratego (a mixture of propiconazole and trfloxystrobin), and TM-417 (a numbered experimental compound). To evaluate the effect of pruning, trees in a commercial orchard were pruned in 1998 to remove all dead twigs and branches larger than 1 cm in diameter and trees were treated with six applications of Captan.

For control of brown rot blossom blight and shothole two fungicide applications (at pink bud and full bloom) were done using an air-blast sprayer, calibrated to deliver 100 gal/A. Fungicides evaluated included: Abound (azoxystrobin), Indar (fenbuconazole), Break (propiconazole), Elite (tebuconazole), Captan (captan), Stratego [a pre-mixture of Flint and Break (trifloxystrobin and propiconazole)], TM-417 (iminoctadine), Vangard (cyprodonil), Flint (trifloxystrobin), an experimental compound (RH 168737), Rovral (iprodione), and PD 100-Dipel.

RESULTS AND DISCUSSION

Epidemiology. In 1999, incidence of anthracnose was low in most locations, however, the disease remained detectable in most orchards where damage had occurred in previous years. Low temperatures and low rainfall for most of the spring were not conducive for disease development and emphasize the importance of a forecasting system for the disease. Temperature, leaf wetness, and relative humidity for two orchards in Merced and Butte Co., respectively, are shown in the graphs of Figs. 1-2. After spring rainfalls ceased by early to mid April, no further increase in disease incidence was observed in the two orchards that were monitored over the season (Table 1). Infected almond blossoms were found in some orchards during petal fall (March). Blossom infections may be more important than previously considered in initiating epidemics. Thus, disease progress will be more closely monitored during the early portion of the 2000 growing season and will be correlated with local weather data. With overall low disease incidence, the two genotypes of the causal pathogen *C. acutatum* (genotype P and genotype G) still continued to be isolated from counties in the mid- and northern portions of the almond production regions of the state.

Field inoculation studies with isolates of the pink genotype using non-wounded fruit were conducted to determine conducive wetness period durations and inoculum concentrations. No significant difference was observed between inoculum concentration levels (10^5-10^6 spores/ml), 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% disease incidence, respectively. In contrast, no difference between length of wetness period was observed when fruit were wound-inoculated. Symptomless, non-wound inoculated fruit were harvested, surface sterilized, and incubated under high humidity. Within 14 days, these fruit developed lesions from which *C. acutatum* was isolated. Non-inoculated fruit were still symptomless. These studies suggest that the anthracnose fungus is capable of causing non-visible, quiescent infections in almond fruit. Thresholds of minimum temperature and wetness periods are being established during current research.

Studies were conducted to determine if previous season's branch dieback would persist and advance during the current growing season. For this, branches with dieback were marked in 1998 at harvest time (September) in two orchards (a Price orchard in Butte Co., and a Carmel orchard in Merced Co.). Branches were tagged between the dead and healthy looking tissue and the length of dieback was measured. In May of 1999 dieback of these branches was measured again. Our results indicated that dieback of most of these branches (52.4% in the Price orchard and 70% in the Carmel orchard) advanced. On average, new dieback that had developed between September of 1998 to May of 1999 increased by 15.4% and 41.5% of the total dieback in the Price and Carmel orchards, respectively. This difference between the two orchards could be explained by the different almond varieties used or by the different locations with their different climatic conditions.

Initial histological studies on the infection process of* C. acutatum *on almond. Studies to evaluate infection processes of the two subpopulations of the fungal pathogen were initiated by inoculating detached almond leaves. Isolates of the pink genotype, that are able to grow at higher temperatures as 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 both genotypes germinated and formed appressoria and infection pegs after 11 hr. Isolates of the gray genotype, that are able to grow at lower temperatures compared to the pink isolates, completed conidial germination and appressorium formation within 1 day at 10 C. Micrographs of the infection structures are illustrated in three figures on the enclosed reduced-size copy of our poster that was presented at the Annual Almond Industry Conference in December of 1999. Thus, our inoculation and histological studies indicated that wetness periods of 24-30 hr are sufficient for the fungus to penetrate the host plant and produce quiescent infections, however, longer wetness periods (30 hr) are necessary for disease development (see previous page). To better understand these processes, more detailed epidemiological and histological studies are in progress.

Molecular detection of* C. acutatum *in infected almond tissue as a preharvest management tool. Two molecular methods that are based on the detection of either fungal DNA or fungal protein were evaluated for the detection and identification of anthracnose caused by *C. acutatum* in diseased almond tissue (blossoms, leaves, or fruit) in California. In these studies, we compared the accuracy of these two molecular methods to the standard isolation method (assuming that the standard method was always 100% accurate). A total of 114 samples were processed in this study. Overall, the DNA method using specific primers in PCR reactions was accurate 74% of the time, whereas the protein method using an ELISA assay was accurate 83% of the time. Similar accuracies were obtained using blossoms or young, mature, or mummified fruit tissues. Considering that more than one sample should be assayed from a specific location, the probability that the disease will be detected is very high. A lower accuracy was obtained using leaf tissue. Possibly, compounds in the leaves interfered with the molecular assays. Although the isolation method is highly accurate, long incubation times of 5-6 days and expertise in the identification of fungi are required. The DNA based system can be completed in 1-2 days, however, much expertise is required. To be used in field diagnostics, samples will have to be analyzed by a commercial lab. The protein system can be completed overnight or in about 18 hours and can be done onsite by a farm advisor or PCA with minimal laboratory equipment and expertise. Thus, this method will be further evaluated in field studies with farm advisors in 2000. If successful, kits for detection of other fungi will be developed and utilized for improved disease diagnosis and management programs.

***Evaluation of kernel moisture content as a factor in establishing postharvest kernel damage by* C. acutatum.** Postharvest damage of almond kernels in storage was first reported in 1997. The pathogen was isolated from processed kernels in storage, predominantly from kernels with internal brown or purplish discolorations. Recovery rates from shriveled or gummy kernels were much lower. In order to define environmental conditions that lead to postharvest kernel damage, almond kernels that were inoculated in the laboratory with mycelium of the fungus were incubated for up to six months at selected humidities. In these studies, kernel colonization by the anthracnose pathogen occurred at kernel moisture levels higher than 10.6% after 6 months. No growth of the fungus was observed below kernel moisture contents of 10.2% after 6 months. This is consistent with our previous report (1998) where studies were conducted for 3 months. Thus, longer incubation times did not increase colonization of the kernels. Although the fungus was nearly 100% viable after 3 months at any of the kernel moisture contents evaluated, viability of the fungus decreased significantly to less than 3% after 6 months of incubation. As concluded last year, these results indicate that postharvest damage to almond kernels was most likely a result of preharvest epidemics that were not managed.

Evaluations of fungicides and cultural practices for management of anthracnose. Alternative fungicides in addition to the currently registered compounds need to be identified in order to design rotation programs using fungicides with different modes of action. These rotation programs are more likely to reduce the potential of development of resistant pathogen populations. Fungicides evaluated in field trials either alone or in rotations included: Abound (azoxystrobin), Break (propiconazole), Captan

(captan), Elite (tebuconazole), Flint (trifloxystrobin), Indar (fenbuconazole), Rally (myclobutanil), Thiram (dimethyl-dithiocarbamate), a pre-mixture of Flint and Break (trifloxystrobin and propiconazole), and TM-417 (iminoctadine). No fungicide program eradicated the disease. Six applications (in approximately 2-week intervals from mid-February to late April) of either Abound, Flint, Elite, Break, Indar, Stratego, Thiram or TM-417 (high rate only) significantly decreased disease incidence to about the same levels as compared to the untreated control (Figs. 3 and 4). Rotation programs with Rally, Captan and Abound; Rally, Break and Abound; Break, Captan and Abound; Elite and Abound; Elite and Flint; or Abound, Break and Elite with Captan were all very effective in reducing anthracnose incidence (Fig. 5). There were no differences in yield, however, due to low disease incidence (Fig. 5). Yield differences were reported in our 1998 Annual Report when high disease incidence occurred in our trial site. Results of rotations using Abound, Break, or Elite with Captan are shown in Fig. 6. Results of epidemiological studies to evaluate temperature and wetness relationships for disease development will help to improve timing of fungicide applications in an anthracnose management program.

In 1999, an emergency registration, Section 18, for propiconazole (Break 3.6EC) was obtained at the beginning of the season for management of almond anthracnose from bloom to 90 days PHI. Additionally, by mid-April, a Section 24C-Special Local Need registration of azoxystrobin (Abound 2F) was obtained for anthracnose and Alternaria leaf spot. Although the captan label was initially changed for use only up to five-weeks-after petal fall, the label was revised again to allow use of the fungicide up to 30 days PHI.

The effect of pruning on the incidence of anthracnose was evaluated in a commercial orchard. Trees were pruned in 1998 to remove all dead twigs and branches larger than 1 cm in diameter. Six applications of Captan were applied during the growing season of 1999. Disease incidence on pruned trees was significantly reduced as compared to non-pruned trees. Disease incidence of non-pruned trees was 11.6%, whereas incidence of pruned trees was 7%. This result correlates with our isolation and epidemiological studies that demonstrated that the fungus may colonize the woody parts of the tree and cause dieback. The fungus apparently can overwinter in infected branches because, as we described in the *Epidemiology* section of this report, dieback may further advance in the following growing season. Infected branches may also serve as inoculum sources. Sporulation has been observed on infected peduncles.

Fungicide evaluations for management of brown rot blossom blight and shot hole. In these trials two applications (at pink bud and full bloom) with Elite or Stratego (a pre-mixture of Flint and Break) were most efficacious in reducing the incidence of spur blight (Fig. 7). For control of shothole, the same fungicides (except Indar) were evaluated using four applications (at pink bud, full bloom, petal fall and shuck split). The most efficacious compounds for reducing shot hole incidence of almond fruit were Abound, Flint, Elite, Break, TM-417, and RH 168737 (Fig. 8). No data for leaf symptoms could be obtained due to hail damage.

Occurrence of a new disease of almond in California. A new almond fruit disease and associated branch dieback caused by the fungus *Phomopsis amygdali* was found in California in the 1998 season. The pathogen is known from other parts of the world on almond and is found on peach in the eastern United States. In 1999, we confirmed the pathogenicity of the organism. Fruit symptoms included extensive grayish brown discolored and shriveled hulls, often associated with a clear gum secretion and shriveled kernels. Effected fruit frequently abscised. The disease was found mostly in the Sacramento valley but a low incidence was also detected in the central San Joaquin valley. The disease was not detected in 1999 on almond fruit but was found persisting in twig cankers in effected orchards. High rainfall and warm temperatures characterize the environmental conditions conducive for the disease on other crops. This information was published in a note in Plant Disease (Vol. 83:1073, 1999).

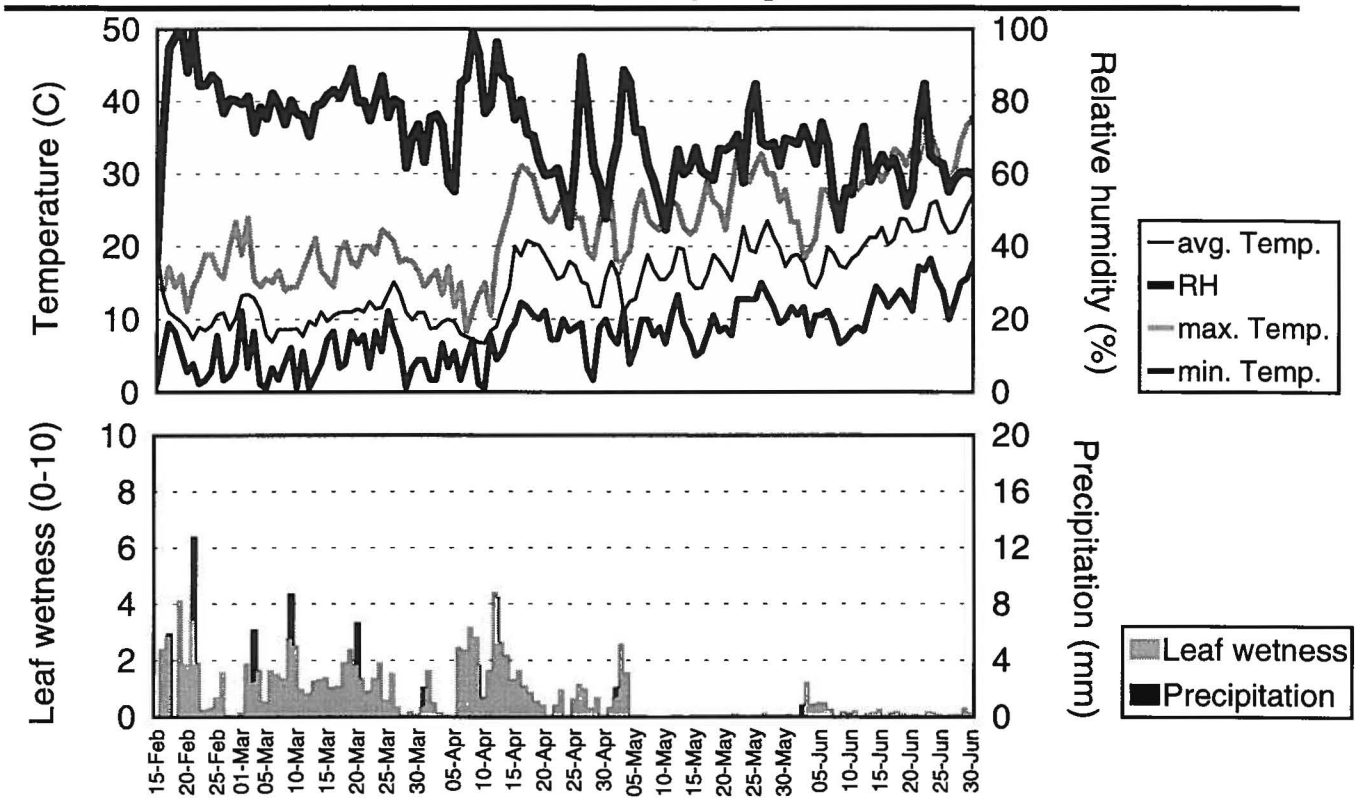
Extension Article Published. An extension article on diseases of almond blossoms, leaves, and fruit was published in 1999 by the UC ANR publications (Publication 21588). This is an important field guide to the identification of several new diseases not previously included in other publications.

Table 1: Almond anthracnose incidence in three orchards during the growing season of 1999

Orchard/location	Date	Disease incidence
Orchard 1/Butte Co.	04/21/1999	
	05/05/1999	31.30%
	05/15/1999	
	06/18/1999	31.20%
Orchard 2/Butte Co.	04/21/1999	
	05/05/1999	16.50%
	05/18/1999	17.60%
	06/18/1999	16.50%
Orchard 1/Merced Co.	05/25/1999	11%

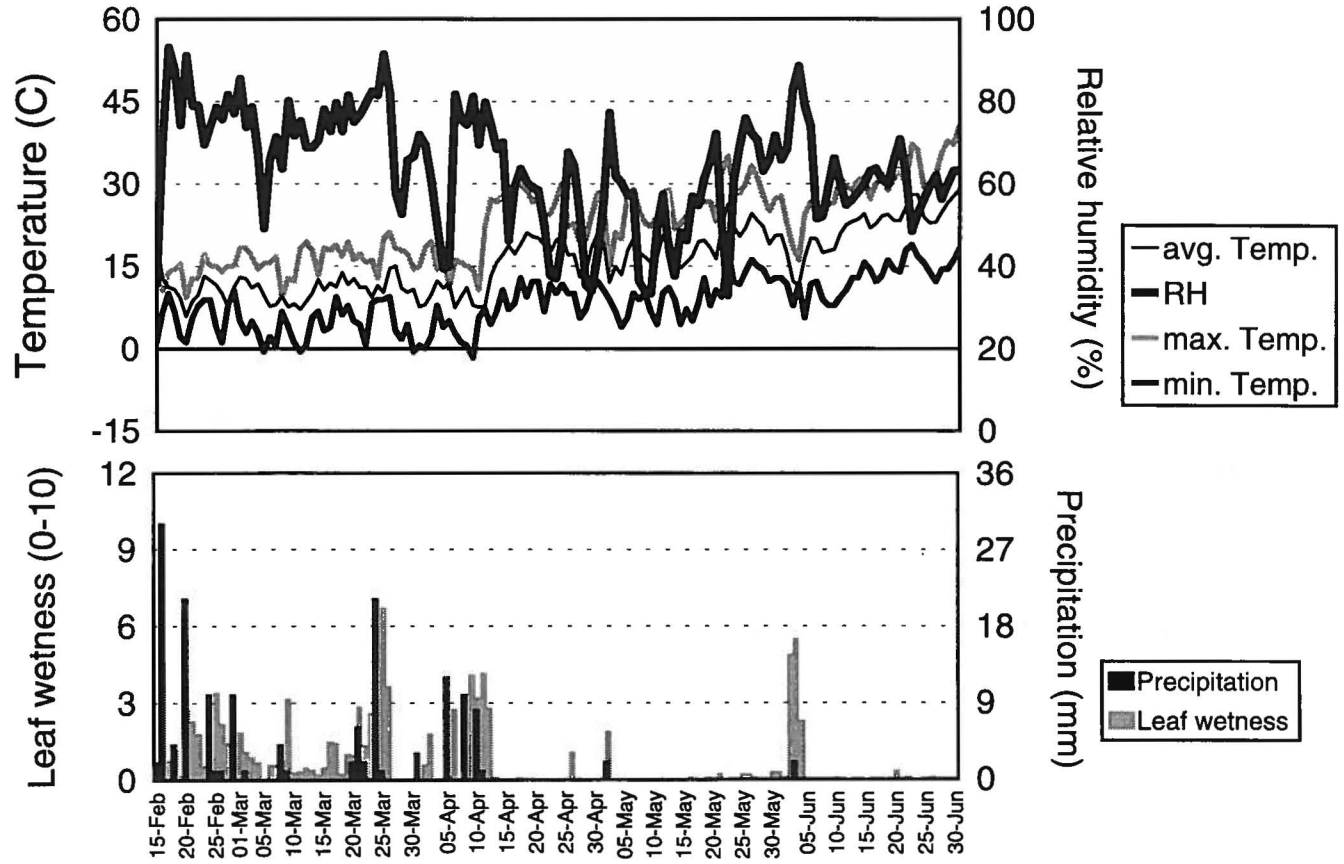
Evaluations were based on 150 spur evaluations for each of four single-tree replications

Fig. 1 : Temperature, relative humidity, and leaf wetness in an orchard in Merced Co. in the spring of 1999



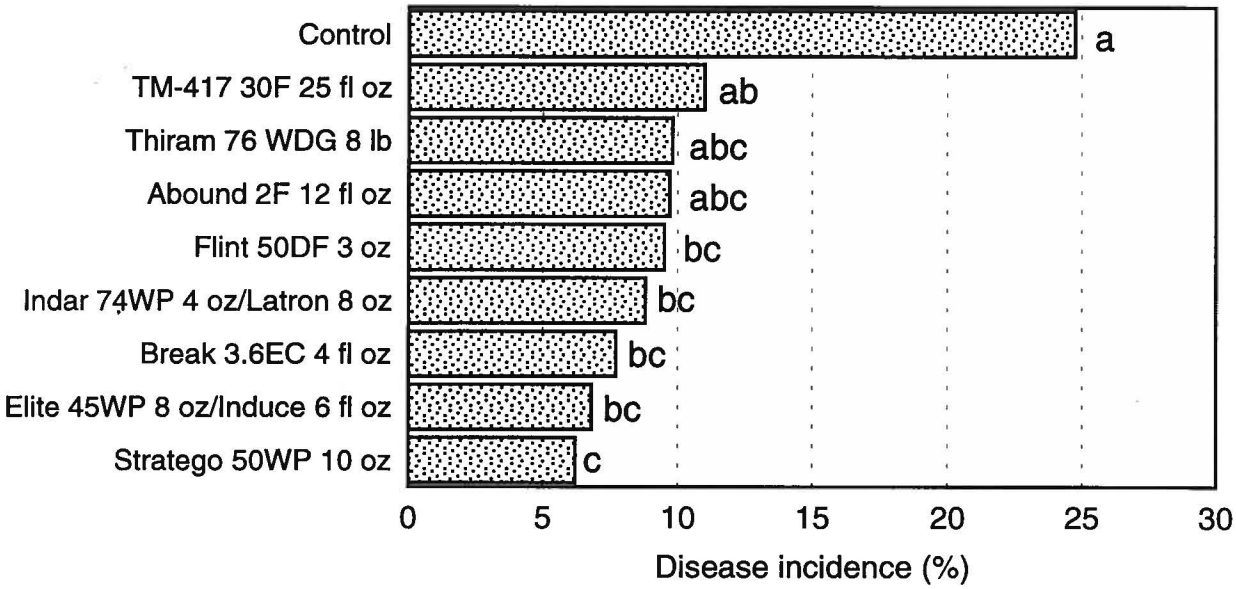
Relative humidity (RH) and leaf wetness values are the daily averages.

Fig. 2: Temperature, relative humidity, and leaf wetness in an orchard in Butte Co. in the spring of 1999



Relative humidity (RH) and leaf wetness values are the daily averages.

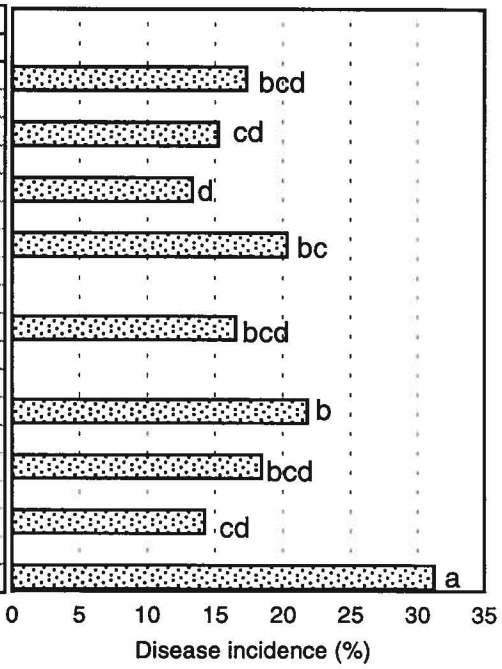
Fig. 3: Efficacy of fungicide treatments for management of almond anthracnose on Price almond in Butte Co. 1999



Treatments were applied on 2/24 (pink bud), 3/3 (full bloom), 3/17 (petal fall), 3/31 (shuck split), 4/7 (5-wk after petal fall), and 4/21(7-wk after petal fall) using an air-blast sprayer (100 gal/A). Evaluations were done on May 5 and are based on 150 spur evaluations for each for four single-tree replications.

Fig. 4: Efficacy of fungicide treatments and programs for management of anthracnose on NePlus almond in Butte Co. 1999

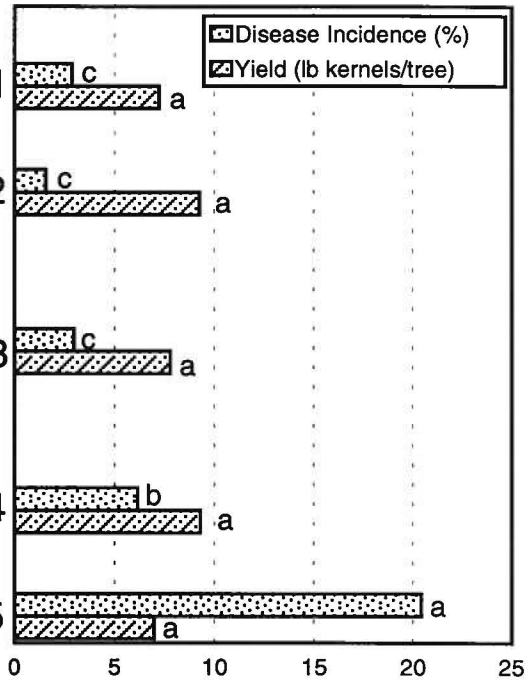
Treatment/ Formulation	Product/Acre	Application dates 2/12	2/24	3/10	3/24	4/3	4/14
Break 3.6EC	4 oz	@	@	@	@	@	@
Break 3.6EC	8 oz	@	@	@	@	@	@
Indar 75WP + Latror	6 oz/8 fl oz	@	@	@	@	@	@
Elite 45WP+ Induce	8 oz/6 fl oz	@	---	@	---	@	---
Abound 2F	15 oz	---	@	---	@	---	@
Elite 45WP+Induce	8 oz/6 fl oz	@	---	@	---	@	---
Flint 50WG	3 oz	---	@	---	@	---	@
Abound 2F	15 oz	@	@	@	@	@	@
TM-417 30F	25 fl oz	@	@	@	@	@	@
TM-417 30F	50 fl oz	@	@	@	@	@	@
Check	---	---	---	---	---	---	---



Treatment dates correspond to pink bud, full blom, petal fall, shuck split, 5-wk after petal fall, and 7-wk after petal fall. Treatments were applied using an air-blast sprayer (100 gal/A). evaluations were done on May 5 and were based on 150 spur evaluations for each of four single-tree replications.

Fig. 5: Efficacy of fungicide programs for management of anthracnose on Merced almond in Stanislaus Co. 1999

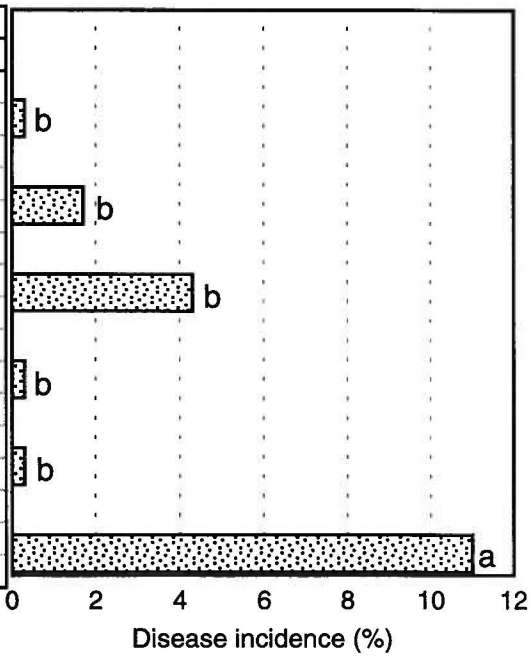
Program No.	Treatment/Formulation	Product/Acre	Application times 1st	2nd	3rd	4th	5th
1	Rally 40W/Captan 50WP	4 oz/6 lbs	@	@	@	@	—
	Abound 2F	12.5 fl oz	—	—	—	—	@
							—
2	Rally 40W/Captan 50WP	8 oz/9 lbs	@	@	@	@	—
	Abound 2F	12.5 fl oz	—	—	—	—	@
3	Rally 40W	8 oz	@	—	—	—	—
	Captan 50WP	9 lbs	—	@	—	—	—
	Break 3.6EC/Ziram	4 oz/8 lbs	—	—	@	—	—
	Break 3.6EC	4 oz	—	—	—	@	—
	Abound 2F	12.5 fl oz	—	—	—	—	@
4	Break 3.6EC	4 oz	@	—	—	—	—
	Break 3.6EC/Ziram	4 oz/8 lbs	—	@	—	—	—
	Captan 50WP	9 lbs	—	—	@	—	—
	Break 3.6EC	4 oz	—	—	—	@	—
	Abound 2F	12.5 fl oz	—	—	—	—	@
5	Check	—	—	—	—	—	—



Treatments were applied using an air-blast sprayer (100 gal/A). Evaluations were based on 120 fruit evaluations for each of four replicated blocks containing four trees per replication.

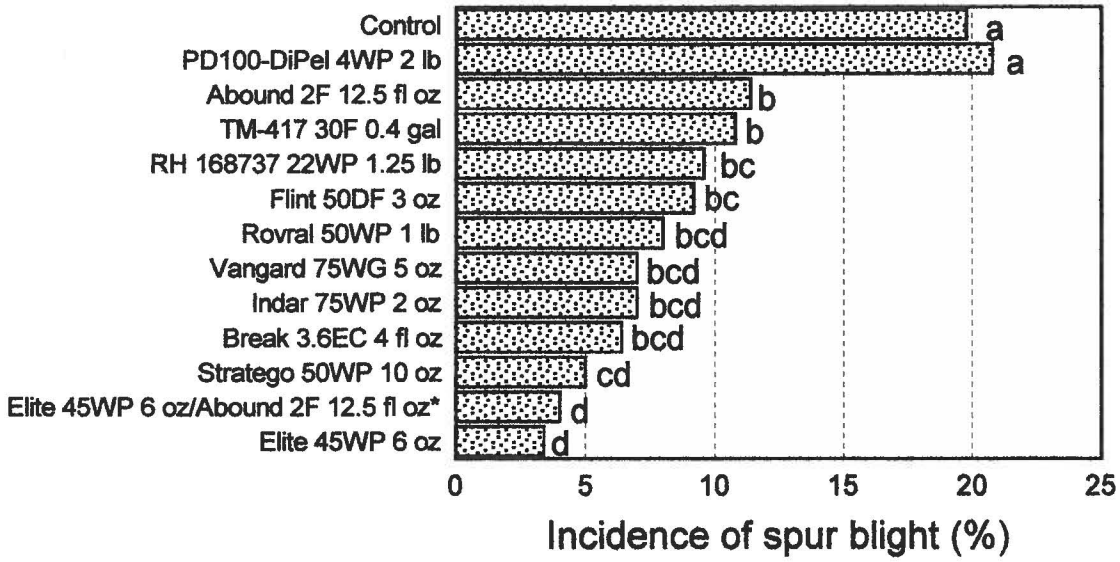
Fig. 6: Efficacy of fungicide programs for management of anthracnose on NePlus almond in Merced Co. 1999

Treatment/ Formulation	Product/Acre	Application dates 2/17	2/26	3/12	3/29	4/27
Abound 2F	15 oz	@	@	—	@	@
Captan 50WP	9 lbs	—	—	@	—	—
Abound 2F	15 oz	@	@	@	—	@
Captan 50WP	9 lbs	—	—	—	@	—
Break 3.6EC	8 oz	@	@	—	@	@
Captan 50WP	9 lbs	—	—	@	—	—
Break 3.6EC	8 oz	@	@	@	—	@
Captan 50WP	9 lbs	—	—	—	@	—
Elite 45WP	8 oz	@	@	—	@	@
Captan 50WP	9 lbs	—	—	@	—	—
Check	—	—	—	—	—	—



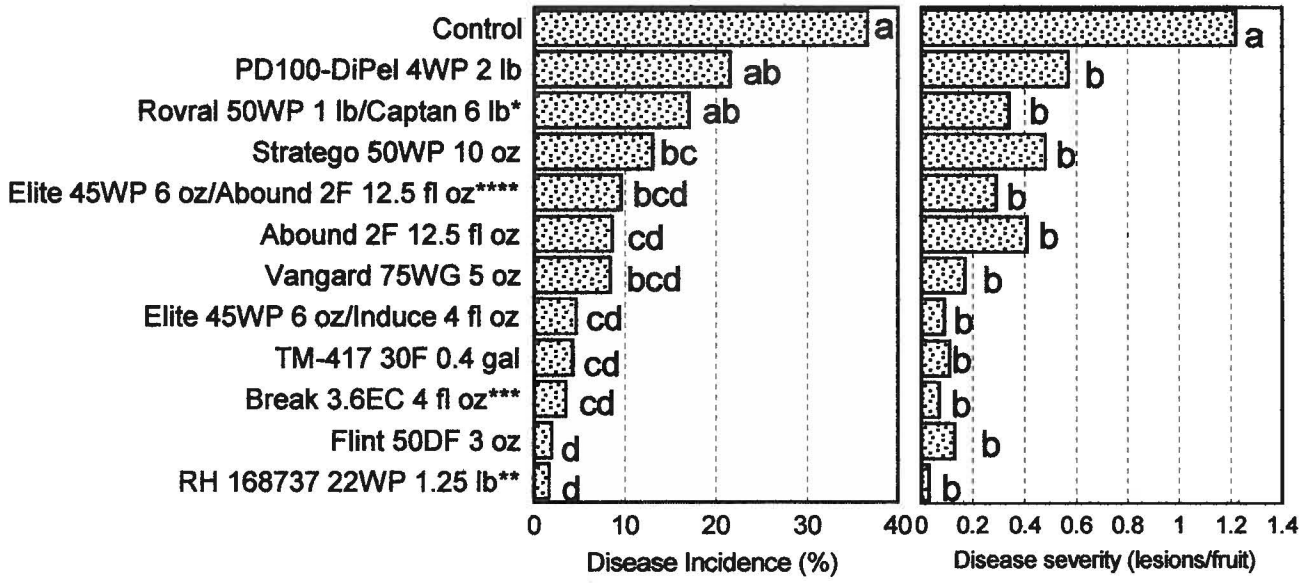
Treatment dates correspond to pink bud, full blom, petal fall, shuck split, and 5-wk after petal fall. Treatments were applied using an air-blast sprayer (100 gal/A). Evaluations were done on May 25 and were based on 100 spur evaluations for each of four single-tree replications.

Fig. 7: Efficacy of fungicide treatments for management of brown rot blossom blight and resulting spur infections of almond in Solano Co. 1999



Treatments were applied on 2/22 (pink bud) and 3/2 (full bloom) using an air-blast sprayer (100 gal/A). In treatment * Elite was applied at pink bud, and a mixture of Elite and Abound was applied at full bloom. Evaluations were done in April. Incidence is the number of infected spurs of the total number of spurs evaluated (100 spurs for each of five single-tree replications.

Fig. 8: Efficacy of fungicide treatments for management of shot hole of almond fruit in Solano Co. 1999



Treatments were applied on 2/22 (pink bud), 3/2 (full bloom), 3/10 (petal fall), and 3/30 (shuck split) using a black-pack air-blast sprayer (100 gal/A). Evaluations were done May 28. Incidence is the number of infected fruit of the total number of fruit evaluated (25-30 fruit for each of four single-tree replications.

* Rovral was applied 2/22, 3/2, and 3/30. Captan was applied 3/2, 3/10, and 3/30.

**Applications were done 2/22 and 3/2 only.

*** Applications were done 3/2, 3/10, and 3/30 only.

**** Elite was applied 2/22 and 3/2. Abound was applied 3/2, 3/10, and 3/30.

:st Report of Fruit Rot and Associated Branch Dieback of Almond in California Caused by a *Phomopsis* Species Tentatively Identified as *P. amygdali*. J. E. Adaskaveg and H. Förster, Department of Plant Pathology, University of California, Riverside 92521; and J. H. Connell, University of California Cooperative Extension, Butte Co. Plant Dis. 83:1073, 1999; published on-line as D-1999-0916-01N 1999. Accepted for publication 15 September 1999.

A fruit rot of almond (*Prunus dulcis* (Mill.) D. Webb.) was observed in an orchard in Durham, CA (Butte County), in June of 1998 after an unusually wet spring with a total precipitation of 17.2 cm for April and May. Disease incidence on fully developed fruit of almond cv. Sonora was nearly 90% in the lower tree canopy by July. Almond cv. Nonpareil grown in alternate rows in the same orchard was much less affected. Fruit symptoms included extensive grayish brown discolored and shriveled hulls, often associated with a clear gum secretion and shriveled kernels. Affected fruit frequently abscised. Leaf symptoms and branch dieback were not associated with the disease in 1998. In May of 1999, however, extensive twig dieback was observed on almond cv. Sonora in the same orchard. Isolations from more than 100 symptomatic fruit were conducted from 9 sampling sites in the 9-ha orchard. Based on morphological characteristics, the same fungus was isolated from 93% of the fruit. The fungus also was isolated consistently from samples exhibiting twig dieback. During a major disease survey conducted in 1998, the fungus was only incidentally isolated from almond fruit from other California orchards. Ascomata were not observed in vivo or in vitro. The fungus produced alpha and beta spores in pycnidia when cultured on potato dextrose agar. Spore measurements were obtained from 10 spores for each of 3 isolates obtained from fruit or twig dieback of almond cv. Sonora. Conidial dimensions of fruit and twig isolates were very similar. Based on spore ::s, with alpha spores measuring 5.3 to 7.5 (to 8) × 1.7 to 2.5 µm and ..ta spores measuring12.8 to 29.8 × 0.6 to 0.7 µm, the fungus was tentatively identified as *Phomopsis amygdali* (Del.) Tuset & Portilla (2). Previous reports on this fungus (2), however, indicated that beta spores are not produced in culture, and disease symptoms have not been observed on fruit. The fungus was morphologically different from other species of *Phomopsis* reported from almond and other *Prunus* species, including *P. mali* Roberts, *P. padina* (Sacc. & Roum.) Died., *P. parabolica* Petrak, *P. perniciosa* Grove, *P. pruni* (Ellis & Dearn.) Wehm., *P. prunorum* (Cooke) Grove, *P. ribetejana* Camara, and *P. stipata* (Lib.) Sutton (3). Field inoculation studies were performed in May of 1999 on almond cvs. Carmel and Mission. Almond fruit were wounded (2 × 2 × 2 mm) or left unwounded and were sprayed with water (control) or a suspension of alpha spores (10^5 spores per ml). Branches were bagged for 4 days to maintain high humidity. Fruit symptoms on cv. Carmel were observed after 4 weeks on wounded and nonwounded inoculated fruit, and *P. amygdali* was successfully reisolated from diseased tissue. No symptoms were observed in the control treatment for almond cv. Carmel or in any treatment for cv. Mission. This is the first report of *P. amygdali* causing a late spring and summer fruit rot and associated branch dieback of almond in North America (1).

References: (1) D. F. Farr et al. 1989. Fungi on Plants and Plant Products in the United States. The American Phytopathological Society, St. Paul, MN. (2) J. J. Tuset and M. T. Portilla. Taxonomic status of *Fusicoccum amygdali* and *Phomopsis amygdalina*. Can. J. Bot. 67:1275, 1989. (3) F. A. Uecker. 1988. A World List of *Phomopsis* Names with Notes on Nomenclature, Morphology, and Biology. Mycologia Memoir No. 13. J. Cramer, Berlin.

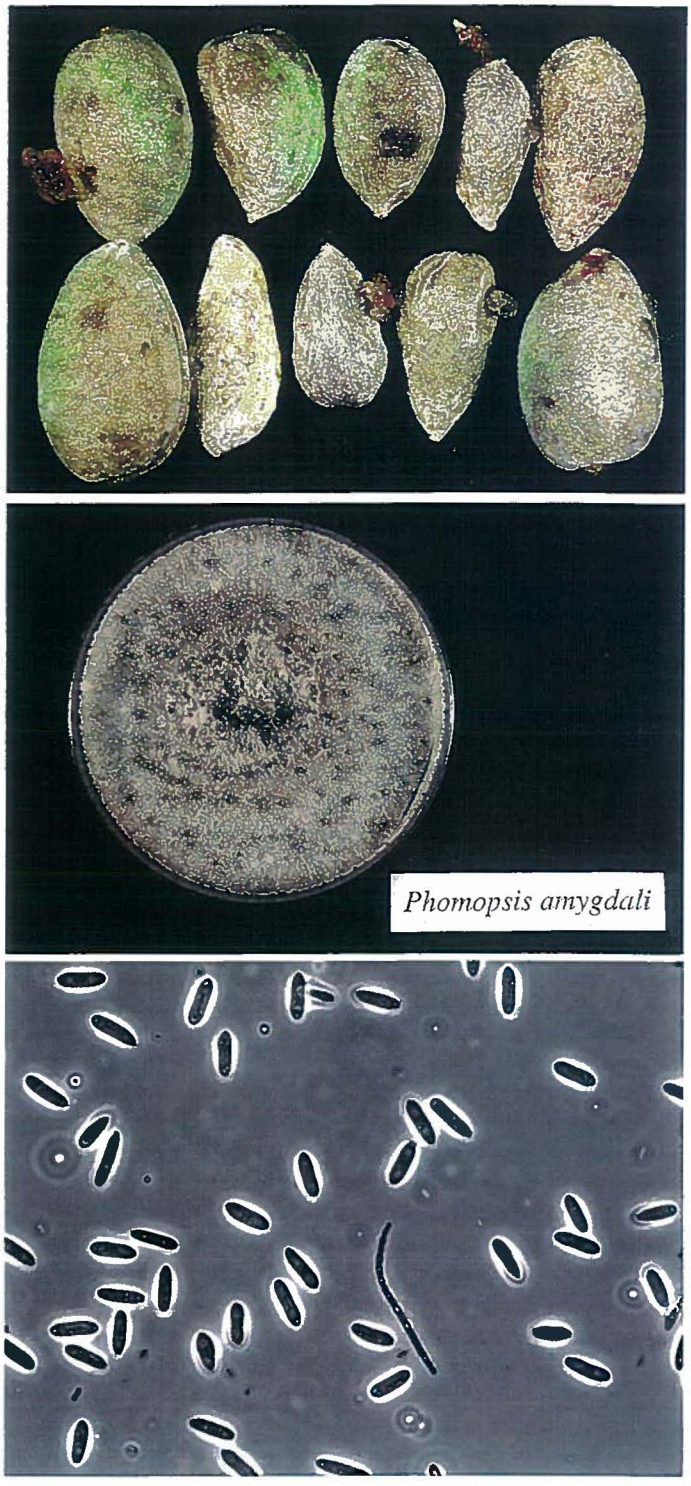


Figure legend. Phomopsis fruit rot of almond. *Top photo*: Extensive grayish brown discolored and shriveled hulls. Lesions are often associated with a clear gum secretion (actual size). *Middle photo*: Culture of *Phomopsis amygdali* on potato dextrose agar. Note small fructifications (pycnidia) scattered on the surface of the plate (0.7x). *Bottom photo*: Alpha conidia and filiform beta conidium of the fungus as observed using phase contrast microscopy (1500x).

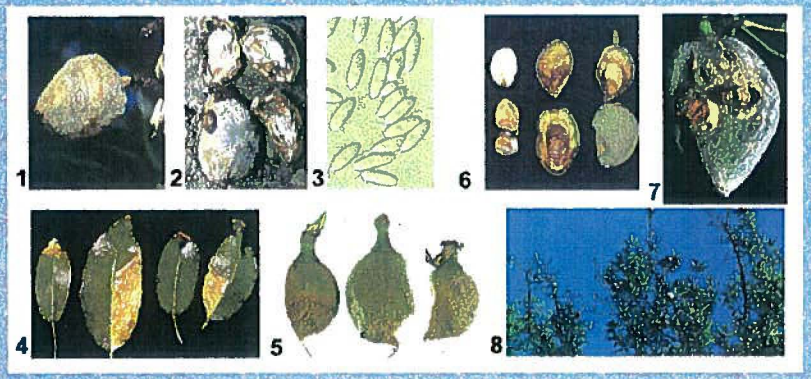
Epidemiology and management of almond anthracnose in California

J. E. Adaskaveg, H. Förster, and D. Thompson, Department of Plant Pathology, University of California, Riverside
Cooperating: J. H. Connell, UCCE, Butte Co., R. Duncan, UCCE, Stanislaus Co., and M. Freeman, UCCE, Fresno Co.

SUMMARY

In 1999, incidence of anthracnose was low in most locations, however, the disease remained detectable in most orchards where damage had occurred in previous years. The two genotypes of the causal pathogen *Colletotrichum acutatum* (genotype pink and genotype gray) continued to be isolated from counties in the mid- and northern portions of the almond production regions of the state. Low temperatures and low rainfall for most of the spring were not conducive for disease development and emphasize the importance of a forecasting system for the disease. Thresholds of minimum temperature and wetness periods are being established and histological observations on the infection process of the anthracnose pathogen were initiated. Rapid methods for the detection of *C. acutatum* in infected almond tissue were evaluated. A method that is based on the detection of specific fungal proteins and that requires little technical expertise was quite accurate in detection of the disease and will be further developed to be used by farm advisors and PCAs. In fungicide research, Abound was registered for both, anthracnose and Alternaria leaf spot. Break, Elite, Indar (at 4 oz), Abound, and Flint were the most efficacious fungicides for anthracnose management. Rotations of these fungicides with Captan were highly effective. A new almond fruit disease and associated branch dieback caused by the fungus *Phomopsis amygdali* was reported for the first time in California.

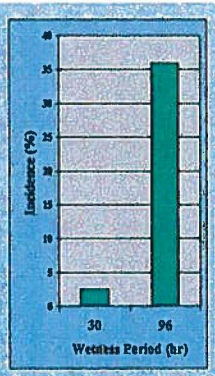
Anthracnose symptoms:
1) Overwintering fruit mummy,
2) Cracked open mummy with white mycelium of the fungus inside,
3) Conidia of *C. acutatum*,
4) Leaf symptoms,
5) Symptoms on immature fruit,
6) Internal symptoms of fruit and kernels,
7) Late stage fruit symptoms,
8) Dieback of branches.



- Epidemiology -
Microclimatic parameters for disease development

In field inoculation studies, long wetness periods after inoculation were required for disease development. Wetness periods of 30 or 96 hr resulted in 2.5 or 36% disease incidence, respectively (Fig. at right). Wetness periods of 30 hr, however, were sufficient to produce non-visible quiescent infections of the fungus. These infections became visible, when symptomless, inoculated fruit were detached and incubated in the laboratory under high humidity (see Fig. far right). *C. acutatum* was successfully isolated from these infections. In the field, low seasonal accumulation of wetness duration was correlated with low levels of natural incidence of anthracnose.

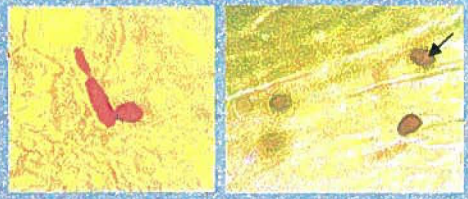
The results of these studies indicate that wetness period requirements for disease establishment may be cumulative following formation of infection structures by the fungus.



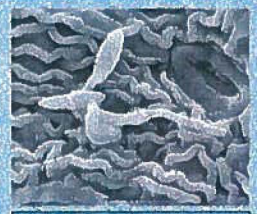
Left: Incidence of fruit with anthracnose lesions 30 days after fruit were inoculated in the field with conidia of *C. acutatum* and treated with either 30 or 96 hrs of wetness.
Top: Activation of quiescent infections of *C. acutatum* after fruit were inoculated in the field, harvested symptomless after 30 days, and incubated for 7-14 days in the laboratory under high humidity.

Histological studies on the initial infection process of *C. acutatum* on almond

Following drop-inoculation of detached almond leaves in the laboratory, after 11 hr at 20C spores of both *C. acutatum* genotypes germinated and formed appressoria and infection pegs (Figs.). At 10C spores of the pink genotypes required longer incubation periods of up to 3 days to complete formation of these infection structures as compared to the gray genotype.



Light micrographs of germinated *C. acutatum* conidia on almond leaves after 2 days of wetness. Left: Germinated conidium with appressorium and secondary conidiation. Right: Germinated conidia with pigmented appressoria. The penetration peg is the light spot (arrow).



Scanning electron micrograph of a germinated septate conidium of *C.acutatum* on an almond leaf with germ tube, appressorium, and secondary conidiation

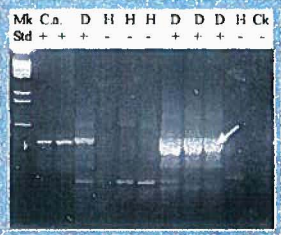
Molecular assays for the detection of anthracnose

SAMPLE	ISOLATION	ELISA Reaction	PCR
Positive Check	Standard	+	+
Negative Check	Standard	-	-
Healthy Almond Fruit	-	-	-
Diseased Almond Fruit	+	+	+
Diseased Almond Fruit	+	+	+
Healthy Almond Blossom	-	-	-
Diseased Almond Blossom	+	+	+
Healthy Almond Blossom	-	-	-

SUMMARY	ISOLATION	ELISA	PCR
Detection Method	Fungal Growth	Color Rxn	Electrophoresis
Accuracy	100%	83%	74%
Time required	5-6 days	20 hr	2 days
Expertise	++	+	+++

- Detection of *C. acutatum* in infected almond tissue -

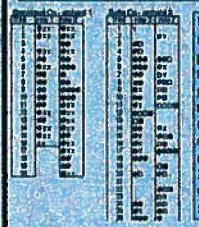
Molecular assays and evaluated to detect anthracnose infections in almond tissues including mummies, blossoms, fruit, leaves, and woody tissues. An immunoassay (ELISA) and a DNA-PCR method were compared to standard fungal isolation (Table at left). Comparisons assumed that the standard method was always 100% accurate. Field studies with farm advisors are planned in the 2000 growing season. If successful, kits for detection of other fungi will be developed and utilized for improved diagnosis and management programs.



Agarose gel of PCR amplification products from fungal mycelium of the pathogen (C. a.), and from diseased (D) and healthy (H) fruit tissue. The check had no DNA. The first lane is the DNA molecular weight marker (Mk Std). The arrow points to the *C. acutatum*-specific DNA band in the diseased tissues.

- Epidemiology II -
Pathogen populations and additional sources of inoculum

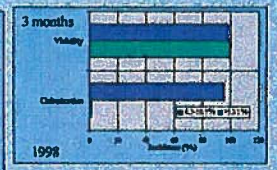
To date, we have identified mummified fruit and peduncles (top left) as the major sources of overwintering inoculum of the pathogen. Blossoms (top right), leaves, and woody tissues are less likely sites for fungal survival.

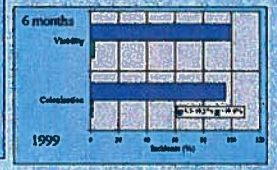


Using molecular technology, we have identified two sub-populations of the pathogen - Genotype P and Genotype G that have been correlated with pink and gray cultural phenotypes. In the field, some orchards had mostly Genotype P (black circles), whereas in other orchards both genotypes can be found (black circles and open squares). Diagrammatic maps on the left illustrate two rows in each of two orchards where fruit isolates were characterized.

- Postharvest studies -

Almond kernel colonization and fungal viability was evaluated at different kernel moisture contents. After 3 months (Fig. top right), the fungus was 100% viable regardless of kernel moisture content but only actively colonized kernels when moisture content was above 13.1%. After 6 months (Fig. bottom right) viability of the fungus decreased to less than 3% for kernel moisture contents less than 10.2%. These results indicate that postharvest damage to almond kernels is likely due to preharvest infections.



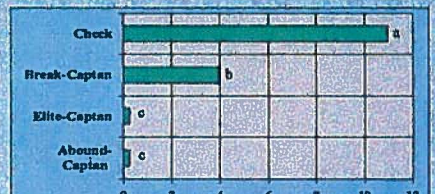


Management of anthracnose with fungicides - We continued to evaluate new fungicides for management of anthracnose. The most promising were the strobilurins (Abound, Flint) and the sterol inhibitors (Break, Elite, Indar) (see Table lower left). Because of the single-site mode of action of all of these fungicides, we also continued evaluation of fungicide rotation programs to prevent resistant populations from developing to any one of these classes of fungicides. Programs included rotations of the representative classes with captan (see Fig. lower right). An emergency registration for control of anthracnose was obtained for Break and a full registration was obtained for Abound for anthracnose and Alternaria leaf spot in 1999. Also, captan was renewed under the RED of EPA.

Efficacy of fungicide treatments for management of almond anthracnose on Price almond in Butte County

Treatment/Formulation	Product/Acre	Disease Incid. (%)	LSD
Break/Flint 62.5WP	10 oz	6.2	c
Elite 45DF+ Induce	8 oz/6 fl oz	6.8	bc
Break 3.6EC	4 oz	7.6	bc
Indar 75WP + Latron	4 oz/8 oz	8.8	bc
Flint 50WG	3 oz	9.5	bc
Abound 2F	12 oz	9.7	bc
Thiram 76WDG	8 lbs	10.7	bc
TM-417 30F	25 fl oz	11	ab
Check	---	16.5	a

* - Treatments were applied at pink bud, full bloom, petal fall, shuck split, 5-wk, and 7 after petal fall.
** - Evaluations (June 18) were based on 150 spur evaluations for each of four single-tree replications.



Efficacy of fungicide programs for management of almond anthracnose. Treatments were applied at pink-bud, petal fall, shuck split, and 5 wks after petal fall using an air-blast sprayer (100 gal/A). Abound 2F, Break 3.6EC, Elite 45DF, and Captan 50WP were applied at 15 oz, 8 oz, 8 oz, and 9 lb per acre, respectively. Evaluations were done on May 25, 1999.