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Prepared for the California Almond Board

Project No. 95-JA1: Management of Almond Anthracnose in California
I. Detection and Identification of the Causal Pathogen, II. Epidemiology, and III. New Management Practices.

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OBJECTIVES

I. Collection of isolates
   A. Survey of isolates from major almond growing regions within California
   B. Isolation and culturing of causal anthracnose pathogen
II. Identification of *Colletotrichum* spp.
   A. Cultural studies on morphology
   B. Molecular characterization
   C. Detection of *Colletotrichum* species in plant tissue using molecular probes
III. Preliminary management strategies
   A. In vitro sensitivity of fungal isolates to selected fungicides
   B. Develop control strategies for winter of 1995 and spring of 1996

INTRODUCTION

In the spring of 1995, a serious outbreak of anthracnose occurred in the Sacramento and San Joaquin Valleys and in August we initiated an emergency research project on the management of this potentially serious disease. In California, anthracnose of almond was first reported in 1916 (Czarnecki, 1916) and in 1925 (Taylor and Philip, 1925) as a leaf and fruit pathogen. Although the disease was unofficially observed in the mid-1980's (J. Connell, personal communication), the disease was consistently observed and reported to the Almond Board of California by Adaskaveg and Ogawa in 1992-1994. In recent years, disease incidence was observed in Butte, Merced, and Stanislaus Co. In the spring of 1995, anthracnose had become widespread. Carmel and NePlus were the most commonly infected cultivars but the disease has also occurred on Nonpareil and probably other cultivars. The disease symptoms include orangish, circular, and sunken lesions on young fruit. Fruit often do not drop and the infections continue to develop into the spurs and shoots. Shoots often dieback probably due to girdling of stem tissue by the fungal infection. Leaves attached to fruit spurs often wilt and remain attached (similar to brown rot). Symptoms are generally observed 2-3 wk after petal fall as shriveled fruit that become light rusty orange, and appear like almond blanks. Later and into the summer, killed shoots similar to brown rot infections become apparent. In 1992 and 1993, Koch's postulates were performed by us in both

laboratory and field inoculation studies demonstrating causality and pathogenicity of the isolated *Colletotrichum* species.

RESULTS AND DISCUSSION

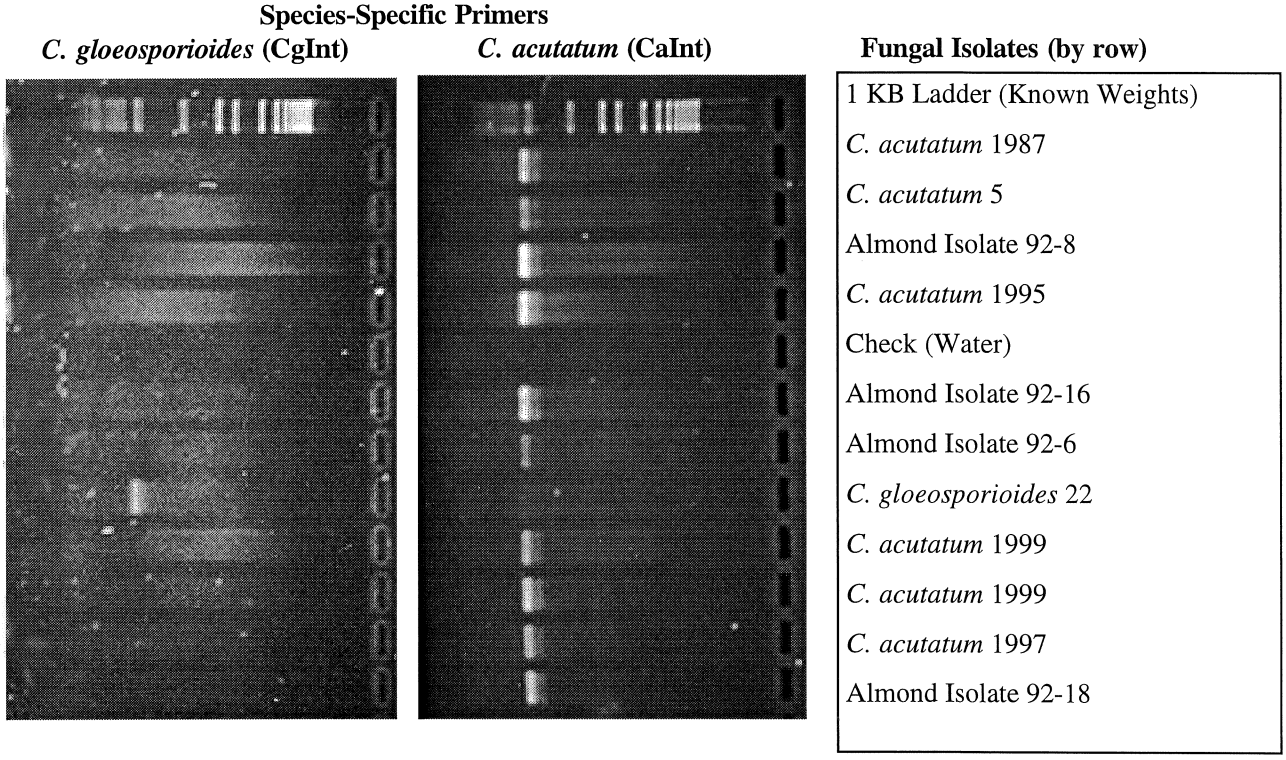
Collection and morphological comparisons of isolates. In an initial survey, forty isolates were collected from 4 orchards from north, central, and southern almond growing regions of California. Initial morphological data of conidia obtained from PDA cultures of almond isolates indicated that conidia were hyaline, aseptate, and fusiform measuring 8-17 x 2.5-4 *u*m. In mass, conidia were orange or salmon color. These were similar in description to that published for conidia of *C. acutatum* and to the stock cultures from strawberry of this species. Conidia of stock cultures of *C. gloeosporioides* from citrus were more elliptical and slightly larger 9-20 x 3-5 *u*m. Because of the overlapping size of conidia, species identification is difficult based on conidial morphology.

The genus *Colletotrichum* contains an extremely diverse collection of fungi including both saprophytes and plant pathogens. Currently, there are at least 39 accepted species within the genus. The anthracnose fungus isolated from almond in Israel has been identified as *C. gloeosporioides* (*Glomerella cingulata* (Stonem.) Spaulding & Schrenk) by Katan and Shabi (1983). Several species including *C. gloeosporioides*, *C. acutatum*, and *C. fragariae*, however, are closely related and are more recently known to be part of disease complexes of cultivated crops such as strawberry. Variable morphological characteristics and difficulty in recognizing characteristics distinguishing these organisms has led to uncertainty in their identification. Recently, identification of *Colletotrichum* species has been improved using molecular technology (Mills et al. 1994).

Amplification of Colletotrichum species-specific DNA from fungal mycelium. Species-specific target primers were synthesized for *C. gloeosporioides*, *C. acutatum*, and *C. fragariae* based on published DNA-sequence information for *Colletotrichum* species. Following PCR amplification, PCR products were separated by electrophoresis in agarose gels containing ethidium bromide and viewed on a UV transilluminator. Figure 1 shows results of electrophoretic separation of DNA amplification by species specific primers. **Thus, at his time, our preliminary results indicate that the species collected from almond is *C. acutatum* and not *C. gloeosporioides* as previously indicated.**

A collection of samples from dead leaves and fruit were evaluated in the fall of 1995. Cultures were obtained using standard isolation methods and grown on PDA. DNA was extracted using standard purification methods and PCR amplification and product separation was conducted as described previously. All 15 fruit isolates were again identified as *C. acutatum*. Three isolates obtained from leaves did not produce PCR products with any of the known primers. Thus, other isolates could be other species of *Colletotrichum*. Additional studies are required to determine the identity of these isolates. It is possible that these leaf isolates obtained from senescing leaves are saprophytic. Mummified fruit isolates were all identified as the *C. acutatum*. Because anthracnose affects both blossoms and fruit, isolations of from these tissues would most likely provide the pathogen. Thus, this data is consistent with our other collections obtained from infected fruit during the spring and summer of 1992-1995.

Figure 1. Electrophoretic agarose gel of DNA amplification products of species-specific target primers (CgInt or CaInt and ITS4). Bands indicate positive identification of almond isolates as compared to known species of *Colletotrichum*.



Preliminary disease management strategies. Chemical control strategies are dependent on the species of *Colletotrichum* (Bernstein and Miller, 1995). For example, captan and chlorothalonil are reported to be effective for control of anthracnose on peaches. Benomyl, however, is only effective on *C. gloeosporioides* and not *C. acutatum*. Potentially, with correct identification by surveyed regions, effective control strategies could be developed relatively rapidly. Specific control strategies will be developed based on *Colletotrichum* species and the sensitivity of *Colletotrichum* species to the fungicides evaluated. Studies planned include:

A) In vitro sensitivity of fungal isolates to selected fungicides. Selected fungicides including captan, chlorothalonil, benzimidazoles, and iprodione will be evaluated in vitro.

B) Beneficial cultural practices for fall and winter of 1995 will be considered. The effects of removing dieback branches to reduce inoculum will be considered.

C) Efficacy of fungicides for control of anthracnose for winter of 1995 and spring of 1996. Standard fungicide trials to determine efficacious compounds and correct timing of application.

Growers who suspect they have anthracnose in their orchards should contact their county farm advisor for correct positive identification of the disease. **Based on published information on control of anthracnose on other crops, general management guidelines include removal of**

mummified fruit and dieback branches to reduce inoculum and, because the disease probably initiates similar to jacket rot, applications of captan from full bloom to petal fall should be beneficial.

References

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