Supporting Improved Strategies for Management of Replant Problems

Project No.: **07-PATH1-Browne/Kluepfel**

Project Leader: **G.T. Browne**
USDA-ARS
Department of Plant Pathology
University of California at Davis
One Shields Ave.
Davis, CA 95616
(530)754-9351
gtbrowne@ucdavis.edu

Project Cooperators: D. Doll, L. Schmidt, D. Kluepfel, S. Upadhyaya, B. Holtz, B. Hanson, B. Lampinen, J. Connell, J. Edstrom, S. Metcalf, P. Schrader, S. Cutter, and N. Goodell; Paramount Farming, Agriland Farming, and TriCal, Inc.

Objectives:

1. Determine the biological causes of and environmental contributions to replant disease (RD).
2. Support development of new management strategies for RD and other replant problems.

Interpretive Summary:

This project is dedicated to elucidating the causes of replant disease (RD) and developing economical ways to control it. Replant disease results in growth suppression of almond trees that are planted without precautions on land with a recent history of almond or other stone fruit production. The disease is initiated in the developing “feeder roots” of trees shortly after planting. The outer portions of affected roots become dark, and many of them die or fail to elongate. This results in a stunted, poorly developed root system, which fails to support optimal tree growth. In severe cases trees are killed or fail to grow in the first season after planting. Yield loss is proportional to the degree of growth suppression.

Replant disease and nematode parasitism should be viewed as two separate types of replant problems. Several lines of previous research have indicated that RD of almond is caused by biological agent(s) other than nematodes. We have shown that RD occurs widely, often in the absence of plant parasitic nematodes, although it may compound replant problems caused by nematodes. When we discuss replant problems, we are referring to those affecting entire young orchards, not just individual trees used to

replace missing tree sites in existing orchards. RD is a risk for all almond and other stone fruit growers managing successive generations of these crops on the same land.

Objective 1. To date this year our work under Objective 1 has focused on identifying organisms associated with RD in non-fumigated plots in previous replant trials near Chico, Parlier, and in a new large commercial orchard trial near Firebaugh. The recent trial is supported by the Pacific Area-Wide Pest Management Program for Integrated Methyl Bromide Alternatives (PAW-MBA) and is comparatively testing alternative strip, spot, and broadcast fumigation treatments with alternative fumigants.

Key steps in determining the cause of any biologically caused plant disease include finding, purifying, and identifying organisms that are consistently associated with it, determining whether the purified suspect organisms reproduce the disease after they are used to inoculate plants, recovering the inoculated organisms from the diseased inoculated plants, and confirming that the recovered organisms are in fact those used for inoculation. It is these diagnostic steps that we are continuing for microbial populations from many trees from several orchards affected by RD. The work is costly and time-consuming, but it is gradually providing important leads.

We are completing a greenhouse trial with isolates of bacteria that were detected more frequently from RD-affected almond trees than from healthy trees as well as with isolates of bacteria that were detected more frequently from healthy trees than from RD-affected trees. In the trial, seedlings of Nemaguard peach rootstock were inoculated with the test bacteria and then planted into autoclaved and non-autoclaved portions of Hanford Sandy Loam soil from a field with a history of peach rootstock affected by RD. The greenhouse trial is designed to determine whether individual isolates of the bacteria or combinations of them suppress or stimulate growth of the peach seedlings. The inoculated seedlings were planted into the soil after it received one of the following two treatments: 1) autoclaving, which is expected to minimize interactions between the inoculants and other microbes in the soil and facilitate evaluation of direct effects of the inoculant on seedlings, and 2) a non-autoclaved control, which is expected to facilitate evaluation of interactions with other microbes naturally common in the soil.

In addition to the greenhouse trial with previously collected bacteria, we collected additional fungi and bacteria from healthy and RD-affected trees in the 2006 replant trial near Firebaugh. As in previous trials, we are identifying these organisms by DNA fingerprinting (PCR amplification and sequencing) and microscope examinations (see comprehensive report to the Almond Board for 2006/07, Browne et al.). The composition of the microbe populations is being examined using ordination analysis, a statistical approach to determining which organisms are most likely to contribute to or suppress RD. An example of an ordination plot is given in Fig. 1. In this plot, fungal community members detected on roots on healthy and RD-affected trees are shown. In addition, the plot accounts for whether the root samples were surface sterilized with bleach solution or merely rinsed in sterile water before culturing to detect fungi. We use bleaching on some root samples because it favors access to fungi and bacteria residing inside the roots; bleaching can lessen competition from microbes residing on the root surfaces. In the diagram, the proximity of each organism's symbol (the symbols labeled

with fungal name abbreviations) to treatment symbols (symbols labeled to indicate pre-plant soil fumigation with chloropicrin, the non-fumigated control; and whether the roots were bleached or merely rinsed after sampling) is proportional to the abundance of the organism in roots in the treatment categories. From Fig. 1 it is apparent that *Trichoderma* (symbol labeled "*Tricho*-1") was common in and on roots of healthy trees in the fumigated plots, whereas it was seldom detected on the diseased roots. Conversely, *Cylindrocarpon* (symbol labeled "*Cyind*") and some species of *Fusarium* (symbols with labels including "*Fus*") were common in roots from the RD-affected trees and seldom detected among roots from the healthy trees. We have used DNA sequencing to more precisely identify representatives of each putative species of these organisms. The additional steps of pathogenicity testing will be required to learn whether the associations of these organisms are merely coincidental or are truly contributing to RD or its suppression. The steps of establishing healthy and diseased trees in replicate fumigated and non-fumigated plots, microbe sampling from resulting healthy and RD-affected trees, and pathogenicity testing of suspect organisms is being repeated at multiple orchards and times after planting.

Objective 2. We have concentrated this year's work under Objective 2 (improved management strategies) on GPS-controlled spot fumigation and orchard testing of short-term crop rotations. The Upadhyaya and Browne labs have worked with Holtz, Edstrom, and USDA at Parlier to establish GPS-controlled spot fumigation treatment test plots in three new orchard trials (at Nickels soils lab, Agriland Farming Madera Co., and USDA-ARS Parlier). Data documenting first-year tree responses to the spot treatments in comparison with conventional strip and broadcast treatments will be obtained with support from the PAW-MBA program and will be available after summer 2008. It is expected that the GPS-controlled spot treatments will offer an economical and environmentally desirable way to prevent RD.

We also established test plots for pre-plant spot fumigation treatments applied by subsurface drip. The drip application method uses the basic surface (or subsurface) irrigation system designated for the replanted orchard; the only major modification of the system required is temporary and includes attachment of a "spike" of "spaghetti tubing" to carry water from the irrigation tubing to a depth of about 20 inches beneath the soil surface (Fig. 2). After drip fumigation, the "spike" can be replaced with either surface drip emitters or microsprinklers. Tree performance data following this treatment will be available in 2008.

In collaboration with Brad Hanson, USDA-ARS, Parlier, pre-plant fumigation treatments were applied in October 2007 to the appropriate plots in our short-term cover crop rotation trial. The complete list of treatments is given below (Table 1), and the trial will be planted with almond trees in January 2008. The purpose of this trial is to see whether short term rotations such as those given below can suppress RD sufficiently to avoid the need for pre-plant soil fumigation. Previous results in microplots suggested that such benefits may occur. Hanson is an agronomist and will work with Browne to document effects of the crop rotations on soil structure and nutritional management.

Table 1. Pre-plant treatments in crop rotation × pre-plant fumigation replant trial[a]

	Preplant Cropping sequence					
		2007				
Treatment	1999-Oct. 2006	Winter	Spring	Summer	Fall	October
1	Peach on N.G.	Peach on N.G.	Peach on N.G.	Peach on N.G.	Fallow	No fumigation
2	Peach on N.G.	Peach on N.G.	Peach on N.G.	Peach on N.G.	Fallow	Fumigation[b]
3	Peach on N.G.	Fallow	Fallow	Fallow	Fallow	No fumigation
4	Peach on N.G.	Fallow	Fallow	Fallow	Fallow	Fumigation
5	Peach on N.G.	Mustard	Mustard	Fallow	Fallow	No fumigation
6	Peach on N.G.	Mustard	Mustard	Fallow	Fallow	Fumigation
7	Peach on N.G.	Wheat	Piper sudan	Piper sudan	Fallow	No fumigation
8	Peach on N.G.	Wheat	Piper sudan	Piper sudan	Fallow	Fumigation

[a]Nonpareil and Monterey almond trees on Nemaguard rootstock will be planted in all treatments in 2008; [b]Chloropicrin400 lb/a.

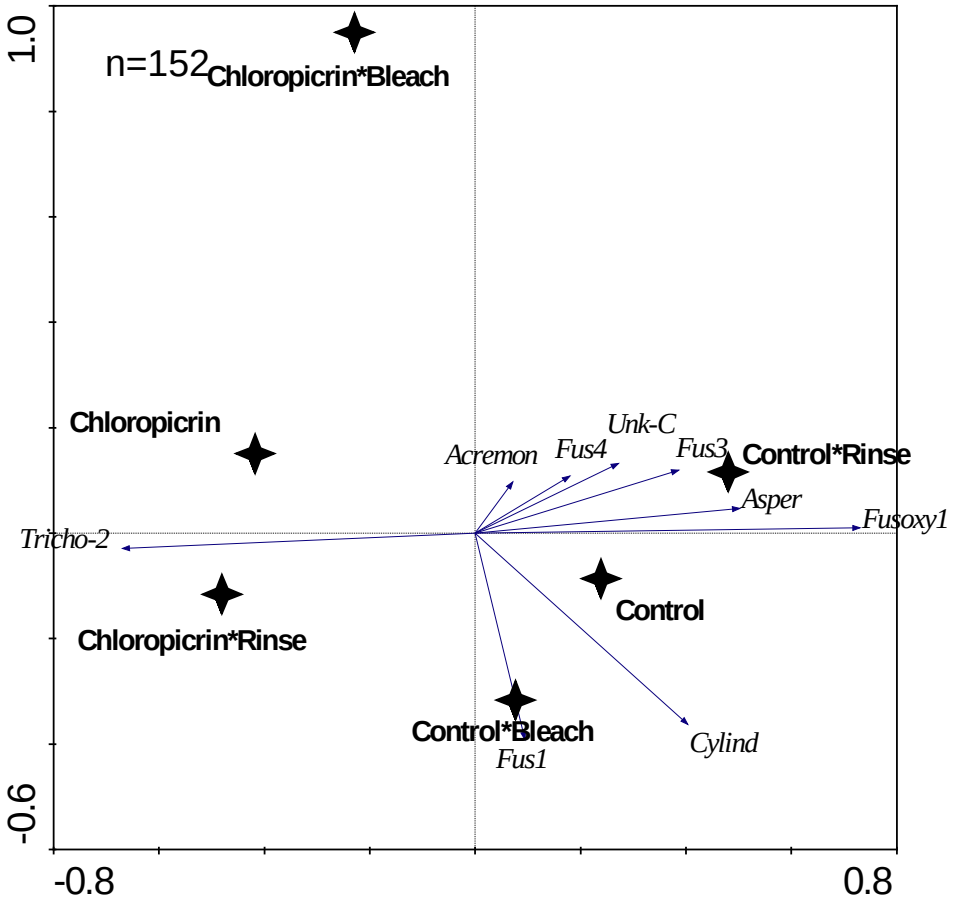

Fig. 1. Ordination diagram for fungal population sampled from healthy and RD-affected trees near Firebaugh in 2007.

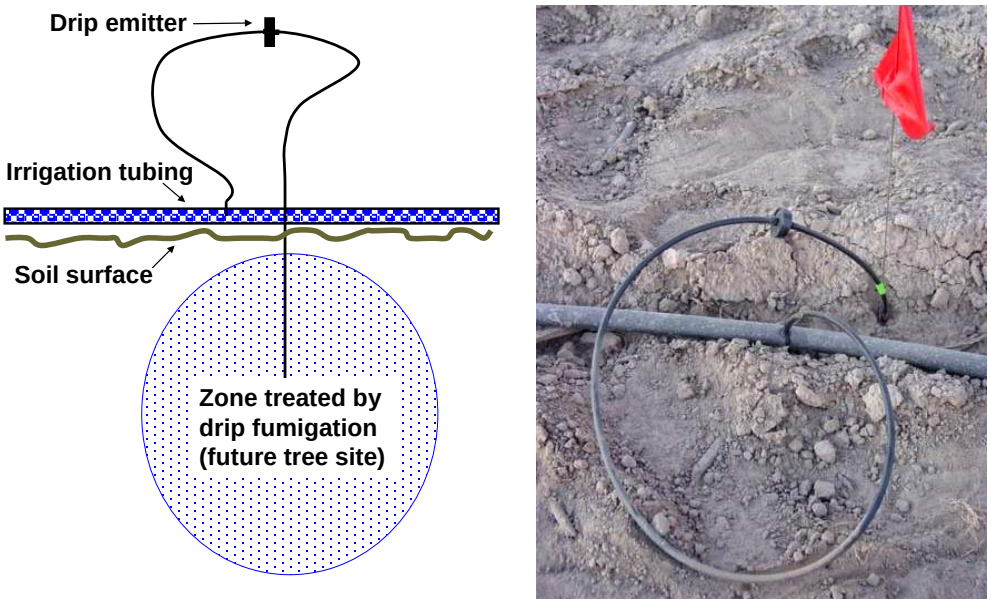

Fig. 2. Side-view diagram (left) and field photo (right) of spot drip fumigation set up used for treating tree sites (*experimental only*).