Developing Improved Strategies for Management of Replant Problems

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Objectives:
Key criteria for sustained economic production of almonds include the ability to efficiently replace old, unproductive orchards and quickly regain good crop production. These needs are often thwarted by replant disease (RD) (a moderate to severe suppression of root and shoot development caused by a host-specific soilborne microbial complex associated with cultivation of *Prunus* species) and other replant problems (i.e., *Armillaria*, *Phytophthora*, *Verticillium*, nematode parasitism, or poor soil physical or chemical properties) related to previous production practices. We hypothesize that: 1) that specific soil microbes, alone or in combination, cause RD and 2) improvement of RD management strategies with less reliance on soil fumigants is feasible and necessary. Our specific research objectives are to:

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:

Objective 1. Determining causes of RD

Identifying microbe suspects that may contribute to replant disease
To isolate and identify soil microbes that may cause RD, we are sampling roots from replicate healthy and RD-affected almond trees in their first year of growth in multiple orchard replant trials. Among other treatments, the trials include multiple plots of soil pre-plant fumigated with chloropicrin and multiple plots of soil left non-fumigated as

controls. Replanted trees in the former plots stay free from RD and grow vigorously, while those in the latter plots are suppressed by RD and grow slowly or not at all. We are using culture-based and DNA-based detection methods, followed by DNA sequencing, to identify microbes present in healthy and RD-affected roots.

Bacterial suspects

Using the approach described above, we commonly detected bacteria in the genus *Rhizobium* in roots of RD-affected trees in replant trials near Parlier, but the organism was seldom detected in roots from healthy trees in the same trials or from roots from replant trials near Chico. Conversely, bacteria in the genera *Pseudomonas* and *Variovorax* were detected more frequently from roots of healthy trees than from those of RD-affected trees in Parlier and Chico replant trials.

Fungal and oomycete suspects

Fungi detected preferentially from RD-affected trees in 2008 included *Cylindrocarpon* sp., several different species of *Fusarium*, *Psathyrella* sp., and *Aspergillus* spp. *Pythium*, an oomycete, also was detected more from roots of RD-affected trees than from healthy trees. *Trichoderma* sp. was isolated preferentially from roots of healthy trees. (Details of the microbial data are on the enclosed CD; Browne et al., 2008.)

Testing pathogenicity of the suspects

In 2008 we tested the ability of bacterial, fungal, and oomycete suspects to cause RD in greenhouse trials. This was done to distinguish between opportunistic organisms that may be simply taking advantage of already-sick roots and organisms capable of initiating the disease. The tests occurred in a greenhouse with potted Hanford Fine Sandy Loam (HFSL) from an orchard affected by RD, and Nemaguard peach rootstock seedlings were used as test plants. The soil was diluted with coarse sand (1 part sand:2 parts HFSL) to improve water drainage. Also, before inoculation with test inoculants of bacteria, fungi, or oomycetes, half of the soil was autoclaved to kill the resident microbes and half was left non-autoclaved. The autoclaved treatment permitted evaluation of inoculant pathogenicity without the complexity of the resident soil microbe community, whereas the non-autoclaved treatment evaluated inoculant pathogenicity in the presence of the resident soil microbes, thereby allowing for possibly important interactions with them. The peach rootstock test plants were inoculated with bacteria by root dipping and soil drenching and inoculated with fungi and *Pythium* by mixing the soil with inoculum substrate colonized by the test organisms (colonized substrate was added to make up 1, 5, and 10% of the final soil volume). After inoculation, the plants were watered and fertilized as needed and allowed to grow for 2 months. Non-inoculated controls were included. Growth and health of the rootstock was used to assess inoculant pathogenicity.

The bacterial inoculants used in the greenhouse trial did not reproduce levels of peach seedling growth suppression or stimulation consistent with the tree health status the bacteria had been associated with in orchard trials (Table 1). For example, *Rhizobium* isolates, which were associated with RD in orchards, did not induce growth suppression in the greenhouse trials (Table 1). However, in the greenhouse trial, plant growth was

suppressed in the non-autoclaved soil compared to that in the autoclaved soil, suggesting that causes of RD remained in the non-autoclaved soil (Table 1).

In contrast, some of the fungal inoculants were aggressively pathogenic in the greenhouse. Although the experiment will not be complete for several weeks, two isolates of *Cylindrocarpon* ("*Cyl1*" and "*Cyl2*") have caused severe growth suppression and killed a high proportion of Nemaguard test seedlings at the 1% and 5% soil infestation level. We isolated and identified *Cylindrocarpon* from several of the plants that were killed, thereby completing Koch's postulates, which comprise an established set of criteria for demonstrating that an organism can cause a disease. We will continue our systematic investigation of RD causes.

Objective 2. New management strategies for RD

Spot fumigation methods

For orchard replanting, one approach to minimizing fumigant emissions and getting maximum benefit out of every pound of fumigant applied is to use spot treatments focused on tree planting sites. Economical ways to deliver spot treatments are needed, and we have been testing the following: 1) using a global positioning system (GPS) with special software and hardware developed by S. Upadhyaya that turns a shank fumigation rig on and off appropriately as it travels with its shanks down in future tree rows so that 5 x 6 ft. "rectangles" of soil centered on tree sites are treated, and 2) using sub-drip spot fumigation, in which irrigation tubing ultimately used to irrigate the replanted orchard is used to deliver pre-plant fumigants in water to a soil depth of 18" to 24" at each tree planting site. The latter approach temporarily attaches a "spaghetti" tube and drip emitter in series onto the irrigation tubing at each tree site; the integrated drip emitter stays above the soil surface, while the distal end of the spaghetti tube to which the emitter is attached is inserted to a soil depth of 18" to 24" depth. The tubes deliver water-emulsified fumigant to zones approximately 30" in diameter from the point of discharge, and little or no wetting reaches the soil surface (see Figure in 2007 Proceedings of the Almond Board, Browne et al.). Before tree planting, the spaghetti tube can be withdrawn and connected to a microsprinker or left connected to the drip emitter.

Spot shank and spot drip fumigation treatments applied in 2007 as described above were nearly as effective as conventional strip and broadcast treatments, as measured by 1st-year tree growth until Aug 2008 (Tables 2-4). We will be collecting additional growth and yield data to examine the long-term effectiveness of the spot treatments compared to strip and broadcast treatments.

Effects of short-term crop rotation, fallowing, and amendments

Two orchard replant trials examining interactions of crop rotation and fallowing with preplant soil fumigation and a yeast extract amendment were planted in Feb 2008. The first trial involved planting peach after plum (both on Nemaguard rootstock) and included the spot and strip fumigation treatments described above as well as a yeast extract treatment (yeast extract was applied as a root spray and soil drench at planting); each of these treatments was applied in combination with 1) a sudan grass rotation

(August-September) and 2) no rotation (i.e., an August-September fallow was used). The second trial included the eight possible treatment combinations of four preplant fallow/rotation treatments: 1) no fallow; 2) “1-year” fallow; 3) “1-year” rotation of wheat and sudan grass; and 4) a 1-year rotation of mustard; and two pre-plant fumigation treatments: 1) a non-fumigated control; and 2) a strip treatment with chloropicrin at 400 lb / treated acre.

The preliminary tree growth data from the fallow/rotation/amendment/fumigation trials indicate moderate to strong benefits from the pre-plant fumigation treatments and lesser but significant benefits from pre-plant rotation and fallow treatments (Tables 3, 4). In the first trial (Table 3), the positive growth responses to chloropicrin, Telone C35, and Inline treatments were stronger than those following MB. There was no practical benefit from the yeast extract amendment. We will monitor effects of all of the pre-plant treatments on future growth, crop yield, and overall production economics.

Examining potential of “no-burn” approaches to orchard replanting

We are assisting B. Holtz and D. Doll in an orchard replant trial they have established to test a new “no-burn” approach to orchard replacement. Trees in half of the trial plots were ground in place with an “Iron Wolf” machine, and those in the other plots were pushed out and burned. On these two tree residue treatments we are superimposing two separate preplant fumigation treatments: 1) a non-fumigated control and 2) subsurface drip spot fumigation treatment with Inline (similar to that described above). The fumigation treatments were applied in late October 2008, and the orchard will be replanted in January 2009. Growth responses of the trees will be monitored as well as effects of the treatments on soil properties and the soil and rhizosphere microbial communities. Microplots will be established in spring 2009 to further examine effects of the orchard residue management strategies on plant health and soil properties.

Table 1. Effects of bacterial inoculants and soil autoclaving on Nemaguard peach, fall 2008 greenhouse trial*

Bacterial inoculants	Soil autoclaving	Shoot fresh wt. (g)	Root fresh wt. (g)	Estimated root cortex necrosis (%)
Control	-	6.9	6.1	68
	+	11.6	12.4	16
Flavobacterium sp.	-	7.8	7.5	69
	+	13.6	12.9	21
Pseudomonas sp.1	-	3.2	2.3	74
	+	9.9	9.7	14
Pseudomonas sp.c	-	5.9	4.2	58
	+	13.2	11.8	16
Rhizobium sp.1	-	4.5	4.2	63
	+	15.1	14.1	12
Rhizobium sp.c	-	6.8	5.4	67
	+	12.9	12.5	20
Rhizobium sp.A-1	-	6.3	6.1	63
	+	12.9	12.7	22
Rhizobium sp.A-c	-	6.0	3.4	72
	+	11.8	11.7	15
Variovorax sp.1	-	5.4	4.4	73
	+	9.7	9.6	25
Variovorax sp.c	-	8.1	7.5	71
	+	10.9	8.5	12

*Interaction of bacterial inoculant × soil autoclaving treatment significant for shoot and root fresh weights (P=0.04 and 0.006, respectively), but not for percent root cortex necrosis (P=0.4). For percent root cortex necrosis, only the effect of soil autoclaving treatment was significant (P<0.0001).

Table 2. Early growth responses of almond trees planted in Jan 2008 following strip, spot and broadcast fumigation treatments at the site of an old almond orchard, Madera County*

Trt.	Fumigant, rate per treated acre	Treated area	Fumigant per orchard acre (lb)	Increase in trunk diameter by 29 Aug 2008 (mm)
m1	Control	None	0	16.8
m2	Methyl bromide, 400 lb/A	Row strip (38%)	152	16.7
m3	Telone II, 340 lb/A	Row strip (38%)	129	17.6
m4	IM:Chloropicrin (50:50), 400 lb/A	Row strip (38%)	152	22.9
m5	Chloropicrin, 400 lb/A	Row strip (38%)	152	23.9
m6	Chloropicrin, 300 lb/A	Row strip (38%)	114	22.7
m7	Chloropicrin, 200 lb/A	Row strip (38%)	76	19.1
m8	Telone C35, 544 lb/A	Row strip (38%)	207	22.1
m9	Pic-Clor 60, 400 lb/A	Row strip (38%)	152	21.9
m10	Chloropicrin, 400 lb/A	Tree square (11%)	44	22.0
m11	Telone C35, 544 lb/A	Tree square (11%)	60	19.7
m12	Telone C35, 544 lb/A	Broadcast (100%)	544	22.2
Minimum significant difference (according to 95% confidence intervals):				*5.6*

*IM=iodomethane. Pic-Clor=proprietary mixture of chloropicrin (59%) and 1,3-dichloropropene (39%). Effect of pre-plant fumigant treatment significant at *P=0.001*

Table 3. Early growth responses of almond trees planted in Feb 2008 following combinations of pre-plant soil fumigation and sudan grass rotation treatments, USDA-ARS, Parlier

Fumigation treatment (Oct 2007)	Fumigant per treated acre (lbs)	Fumigant per orchard acre (lbs)	Sudan grass rotation (Jul-Sep 2007)	Disease severity rating (0 to 5 scale) (7 Jul 2008)	Increase in trunk diameter by 30 Aug 2008 (mm)
Control	0	--	no	1.5	7.9
			yes	0.9	14.1
MB, by conventional shanks to 8'-wide row strips	400	168	no	0.9	22.6
			yes	0.5	20.4
Telone C35, by conventional shanks to 8'-wide row strips	540	227	no	0.3	28.3
			yes	0.3	31.9
Telone C35, by GPS-controlled shanks to 5x 6' tree spots	540	81	no	0.7	21.4
			yes	0.5	24.2
Inline, by single drip emitters to 4'-dia. tree spots	540	43	no	0.8	20.8
			yes	0.6	21.3
Chloropicrin, by GPS-controlled shanks to 5x6' tree spots	400	60	no	0.6	24.2
			yes	0.2	26.5
None, yeast extract root spray and drench at planting	0	--	no	1.2	10.4
			yes	1.0	14.0
Minimum significant difference (according to 95% confidence intervals):					*8.9*

*Disease rating scale: 0= no disease, vigorous growth; 1= moderate stunting; 2= severe stunting; 3=severe stunting and wilting or leaf discoloration; 4= tree near death; 5=dead tree. For increase in trunk diameter, the main effect of fumigation treatment was significant ($P<0.0001$), the main effect of crop rotation treatment was significant at ($P=0.05$), and the interaction of fumigation × crop rotation treatment was not significant ($P=0.6$).

Table 4. Early growth responses of almond trees planted in Feb 2008 following combinations of pre-plant fallow, crop rotation, and fumigation treatments, USDA-ARS, Parlier

Pre-plant fumigation treatment	Pre-plant cropping	Increase in trunk diameter by 30 Aug 2008 (mm)
Control	Peach	12.2
	Fallow	15.7
	Mustard	16.7
	Wheat-Sudan	15.9
Chloropicrin 400 lb/A	Peach	24.9
	Fallow	28.1
	Mustard	29.5
	Wheat-Sudan	30.1
Minimum significant difference (according to 95% confidence intervals):		*5.9*

* The main effect of fumigation treatment was significant (*P<0.0001*), the main effect of crop rotation/fallow treatment was significant (*P=0.0006*), and the interaction of fumigation × crop rotation treatment was not significant (*P*=0.8).

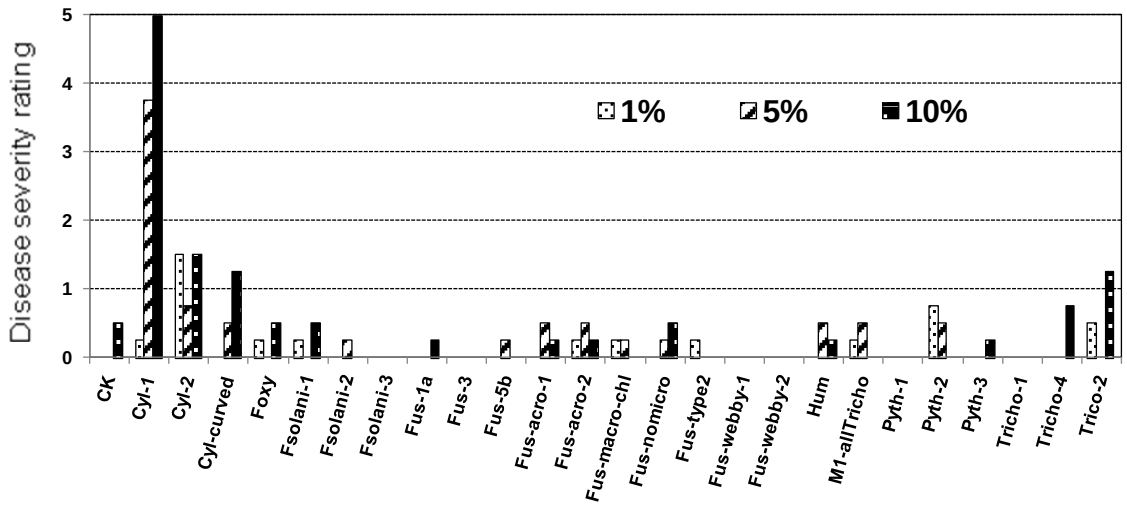

Figure 1. Effect of fungal and *Pythium* inoculants on health of Nemaguard peach seedlings (using a 0 to 5 scale in which 0= healthy and 5 = dead). Percentages in the legend indicate the portion of the soil mixture volume consisting of inoculum substrate colonized by the tested inoculants. Note the pathogenicity of "Cyl-1" and "Cyl-2" isolates (*Cylindrocarpon* sp.).