Developing Improved Strategies for Management of Replant Problems

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Objectives:

1. Determine the biological causes of replant disease (RD).
2. Develop improved management strategies for RD and other replant problems.

Interpretive Summary:

Replant disease (RD) is manifested by moderate to severe suppression of tree growth and productivity in successive plantings of almond and other stone fruit orchards. This report summarizes the background and current status of our research on: 1) causes of RD and 2) new approaches for management of RD and other replant problems.

Previously, we presented evidence that RD is mediated by microorganisms in the soil (for example heating RD soil to 140 °F for at least 30 min or fumigating it with chloropicrin prevents the disease). Further work identified specific fungi (*Cylindrocarpon* sp., *Fusarium* spp.), stramenopiles (*Pythium* spp.), and bacteria

(*Rhizobium* spp.) found more commonly in RD-affected roots than in healthy roots. It was determined that RD occurs in the absence of plant parasitic nematodes, and that fumigants most effective for controlling nematodes (i.e., 98% methyl bromide or Telone II) are less effective than chloropicrin (CP) or mixtures of it with other fumigants for preventing RD. Spot fumigation treatments applied using GPS-controlled shank injection and subsoil drip fumigation for control of RD were developed to help reduce fumigant costs and emissions and are now being perfected. However, soil fumigation is increasingly regulated and costly, and additional replant management strategies with minimal reliance on fumigants are needed.

Currently, we are pursuing more definitive evidence on the causes of RD (Objective 1). The approach being used is: 1) to see whether associations between certain microbes and the disease hold up as we survey healthy and RD-affected trees across multiple orchards and years, 2) to increasingly use culture-independent (i.e., PCR/DNA-based) methods to broaden the scope of our microbial examinations (many organisms cannot easily be detected by culture isolations), and 3) to conduct repeated tests of pathogenicity for organisms isolated in association with the disease. Since our last report, we completed a culture-independent (PCR/DNA-based) analysis of fungal populations associated with RD at a replant trial near Firebaugh, CA (details below). Also, from the same site, we are completing culture-independent examination of stramenopiles (fungal-like organisms including *Pythium*, *Phytophthora*, and many other diverse organisms that are seldom detected in culture), and bacteria. Previously, only culture-based isolations were completed with samples from the Firebaugh trial. Four greenhouse trials were completed to test the pathogenicity of fungi and *Pythium* isolates that were isolated most frequently from RD-affected. In these trials, only some of the *Cylindrocarpon destructans* isolates and an isolate of *Pythium helicoides* caused disease on Nemaguard rootstock peach seedlings.

Our current work on control measures for replant problems (Objective 2) is focused on reduced fumigant dependence. Growth and yield data were collected in 2009 from trials established in 2007 to assess interaction of crop rotation treatments with pre-plant soil fumigation. Although a rotation with Sudan grass after removal of the old orchard significantly improved tree growth in the first growing after replanting, the benefit did not result in larger tree canopies or greater yield in 2009 (details below). Therefore, this trial provided no evidence that growers will benefit economically from a short-term Sudan rotation before replanting. In contrast, spot treatments significantly improved 2009 tree growth and yields. Greenhouse and orchard trials are now being established to evaluate efficacy of spot treatments with seed meal and nitrogen-based amendments for control of RD. These types of amendments, when applied in sufficient amounts and under appropriate conditions, have provided good control of other soilborne diseases. We are working with Steve Fennimore and Bob Weimer to develop an auger system to apply steam and these amendments to tree planting sites (also see Fennimore et al.). Additional work in support of improved RD control measures was completed under a project led by B. Holtz, which, in a field trial, explores interactions of in-place orchard grinding with spot drip fumigation (see Holtz et al. for details). We conducted culture-based root isolations to determine how orchard grinding (as compared to tree pushing and burning) and spot drip fumigation (as compared to no fumigation) affect incidences

of suspected RD pathogens and other soilborne pathogens. Sampling from these plots did not detect elevated pathogen levels associated with the "grind" treatment, but a complex of fungi, including *Cylindrocarpon*, was found most frequently in non-fumigated plots.

Results and Discussion:

Objective 1. Determining causes of RD**.**

The 2009 culture-independent examinations of fungi associated with roots from the 2007 Firebaugh replant trial (root samples collected in 2007 were frozen for subsequent PCR/DNA testing) revealed pronounced shifts in fungal incidence associated with RD (**Figure 1**). *Cylindrocarpon destructans*, two species of ectomycorhizae, a *Fusarium* sp., and several other fungi were most frequently found in RD-affected roots, whereas several other fungi were found most frequently in healthy roots (**Figure 1**). These results are important because they guide us in targeting genotypes for further analysis (pathogenicity tests, and specific, quantitative PCR for more accurate quantification of the suspected pathogens). The culture-independent results (**Figure 1**) generally agree with our previous culture-dependent results (see Browne et al., ABC Final Report, 2007/08) but also provide important complementation. For example, *Cylindrocarpon destructans* was associated with RD in both culture-independent and culture-dependent examinations at the Firebaugh site, but each method also detected fungi not detected by the other method (see Browne et al., ABC Final Reports, 2007/08 and 2008/09).

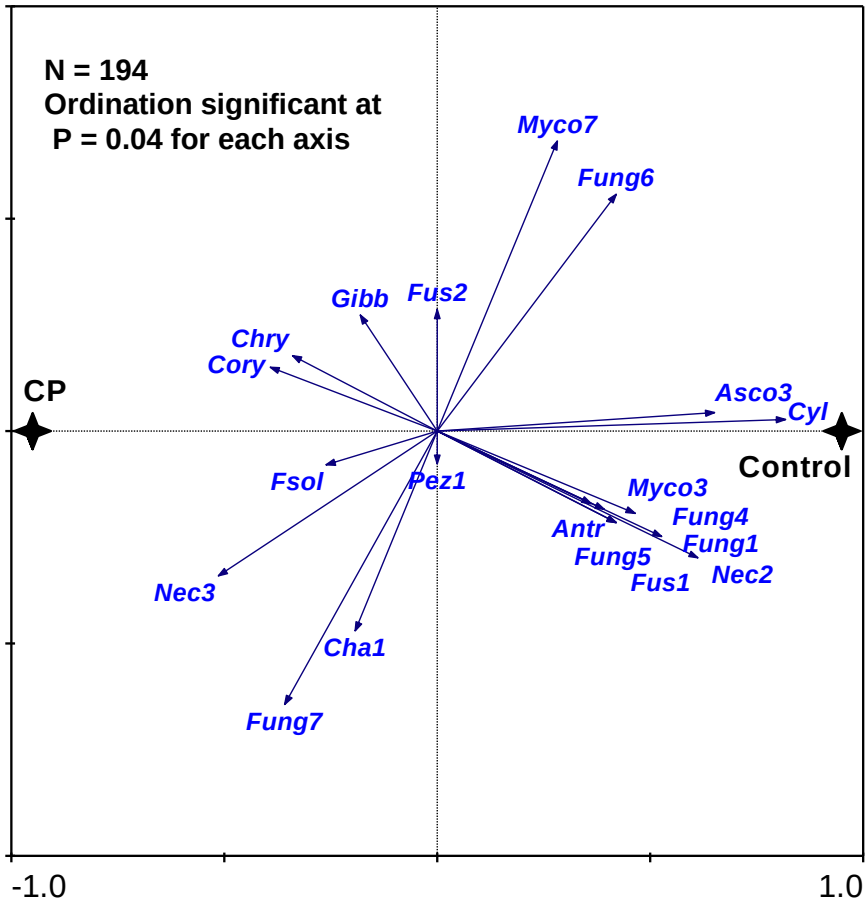

Figure 1. Ordination of culture-independent fungal incidence, Firebaugh replant trial, planted 2007. To interpret, keep in mind that proximity of the symbols representing the pre-plant treatments (the stars) and the abbreviations representing the associated fungi (text at arrow points) indicates the degree of statistical association. Treatment abbreviations: CP, chloropicrin (trees in this treatment were healthy); Control, no fumigation (trees in this treatment were RD-affected). Fungal abbreviations: Antr, *Antrodia camphorata*; Asco 3, Uncultured ascomycete clone; Cha 1, *Chaetomium globosum*; Chry, *Chrysosporium pseudomerdarium*; Cory, *Corynascus sepedonium*; Cyl 1, *Cylindrocarpon destructans*; Fsol, *Fusarium solani*; Fung 1, Uncultured endophytic fungus; Fung 4, uncultured soil fungus clone; Fung 5, Uncultured soil fungus clone; Fung 6, Fungal endophyte; Fung 7, Uncultured fungus isolate; Fus 1, *Fusarium* sp.; Fus 2, *Fusarium* sp; Gibb, *Gibberella avenacea*; Myco 3, Uncultured ectomycorrhiza (Laccaria) isolate; Myco 7, Uncultured ectomycorrhiza (Laccaria) isolate; Nec 2, *Phaeonectriella lignicola*; Nec 3, *Bionectria* sp.; Pez 1, uncultured Pezizomycotina.

Selected results from 2009 greenhouse pathogenicity tests are shown below (**Tables 1, 2**). In these tests, Nemaguard peach seedlings were grown in soil infested with the test isolates as well as in non-infested (control) soil. Before use in the trials, the soil (Hanford Sandy Loam, collected from around RD-affected trees) was amended with course sand to facilitate water drainage (2 field soil: 1sand, by vol.). In separate treatments, the soil was either autoclaved or not autoclaved before use (this was done to allow for potentially important interactions between inoculants and other soil microbes in non-autoclaved soil). Results are shown for both autoclaved and non-autoclaved soil where the autoclaving affected responses to inoculation. Pathogenicity was demonstrated for an isolate of *Cylindrocarpon destructans* (**Table 1**) and an isolate of *Pythium helicoides* (**Table 2**). *Pythium* sp. 1 significantly reduced shoot growth (**Table 2**). Note that other isolates did not cause disease. These results suggest that at least some isolates of *C. destructans* and *P. helicoides* can contribute to RD.

Table 1. Pathogenicity of fungi and oomycetes associated with RD (experiment 1)

Inoculant	Top fresh wt. (g)	Root fresh wt. (g)	Root cortex necrosis (%)	
			Soil autocl. before inoc.	Soil not autocl. before inoc.
Control (sterile substrate)	7.4	5.1	27	70
Cylindrocarpon destructans-1	*2.1*	*1.1*	*88*	90
Cylindrocarpon destructans-2	6.1	3.6	65	69
Cylindrocarpon sp.	7.0	4.8	*72*	54
Fusarium oxysporum-1	6.5	4.2	37	79
F. oxysporum-2	8.3	5.7	30	74
F. oxysporum-3	7.6	4.8	26	81
F. solani-1	6.3	4.4	*75*	66
F. solani-2	6.6	4.4	61	73
F. solani-3	6.7	4.5	31	75
F. solani-4	7.8	5.3	53	78
F. solani-5	7.9	5.5	26	81
F. solani-6	5.3	3.3	42	80
F. solani-7	5.7	3.6	53	79
F. solani-8	5.8	3.8	30	77
F. solani-9	6.8	4.7	31	71
F. solani-10	8.7	6.1	35	78
Nectria haematococcus	5.5	3.3	66	79
Pythium sp.-1	8.4	5.5	49	73
Pythium sp.-2	6.1	4.3	28	75
Pythium sp.-3	9.9	7.1	40	67
Trichoderma sp.-1	7.5	4.9	45	71
Trichoderma sp.-2	6.5	4.1	38	73
Unculutred Ascomycete-1	7.6	5.0	31	62
Uncultured Ascomycete-2	8.9	5.8	24	64
Uncultured Ascomycete-3	9.4	6.0	21	73
Uncultured fungus-1	7.7	4.6	36	67

[a]Values (means) bracketed with asterisks differ significantly from the control.

Table 2. Pathogenicity of RD-associated *Pythium* isolates in Hanford Sandy Loam soil collected from a peach orchard affected by RD

Soil treatment before inoculation	Inoculant	Top fresh wt. (g)	Root fresh wt. (g)	Root cortex necrosis (%)
Soil autoclaved	Control (sterile substrate)	31.8	23.1	10
	Pythium sp.-1	36.9	29.7	9
	Pythium sp.-2	44.4	35.5	14
	Pythium sp.-3	43.1	34.3	29
	Pythium sp.-4	32.5	31.9	8
	Pythium helicoides	*0.7*	*0.8*	*100*
Soil not autoclaved	Control (sterile substrate)	36.1	24.0	77
	Pythium sp.-1	*16.7*	13.5	64
	Pythium sp.-2	28.4	19.4	64
	Pythium sp.-3	35.2	26.1	68
	Pythium sp.-4	37.3	23.2	58
	Pythium helicoides	*10.3*	*6.7*	83

[a] Values (means) bracketed with asterisks differ significantly from the control.

Objective 2. Improved control strategies for replant problems**.**

Results are shown from one of two orchard replant trials testing interactions of crop rotation with pre-plant fumigation treatments for management of RD. The results shown are for peach, but the trees were planted on the prevalent almond rootstock, Nemaguard. Tree growth in the first year after planting was measurably improved (P<0.02) by a short pre-plant rotation with Sudan grass, but by 2009, the rotation did not significantly affect the proportion of photosynthetically active radiation (PAR) absorbed by the canopy or the first year's fruit yield (**Table 3**). The temporary growth benefit in the peach trees following Sudan grass rotation was relatively small. In the same trial, the 8.3-ft.-wide strip treatment with Telone C35 (a mixture of 61% 1,3-dichloropropene and 35% chloropicrin) and the GPS-controlled spot shank treatments with chloropicrin (CP) both produced good responses in the replanted trees, as measured by the increase in tree circumference in 2008, PAR absorbed in 2009, and fruit yield in 2009. Trees responded less favorably, although acceptably, to strip treatments with methyl bromide, GPS-controlled and drip-applied spot treatments with Telone C35 and Inline (a drip formulation of Telone C35). Based on these results, short-term crop rotation with Sudan grass alone is not expected to prevent RD. The fact that the spot treatments with Inline were not as effective as the strip treatment with Telone C35 or the spot treatment with CP, suggests that the limited area of treatment may be a problem unless a highly effective fumigant for control of RD, such as CP, is used. Economic analysis of the treatments presented here as well as those of many other orchard replant trials will be made available on a website dedicated to the Pacific Area-Wide Pest Management Program for Integrated MB alternatives.

Table 3. Interactive effects of pre-plant fumigation treatments and crop rotation with Sudan grass on performance of peach on Nemaguard rootstock

Fumigation treatment (Oct 2007)	Fum. per treated acre (lbs)	Fum. per orchard acre (lbs)	Sudan grass (Jul-Sep 2007)	Increase in trunk cir. 2008 (cm)	PAR absorption July 2009 (%)	Mkt. fruit yield July 2009 (kg)
Control (non fumigated)	0	--	no	3.9	1	2.0
			yes	7.1	6	5.3
MB, shank strip	400	168	no	10.4	17	12.1
			yes	9.5	15	10.0
Tel. C35, shank strip	540	227	no	12.5	20	21.1
			yes	13.9	21	20.3
Tel. C35, sh. spot 5x 6'	540	81	no	9.7	10	12.5
			yes	11.0	13	14.3
Inline, drip spot, 4' dia	540	43	no	9.1	10	9.3
			yes	9.6	10	10.0
Chlorop. sh. spot 5x6'	400	60	no	10.5	14	14.7
			yes	11.6	16	16.1
None, yeast extract	0	--	no	5.5	5	3.6
			yes	6.6	5	5.5
MSD, 95% CI:				*3.5*	*6*	*9.2*
P value, fumigation trt:				*<0.0001*	*<0.0001*	*<0.0001*
P value, rotation trt:				*0.02*	*0.16*	*0.45*

In the trial testing interaction of orchard removal practice (tree pushing and burning vs. grinding in place) and pre-plant fumigation treatment (non-fumigated control vs. pre-plant spot fumigation at tree sites using single sub-drip tubes fed by 1 gph drip emitter discharging 18" below soil surface), culture-based isolations provided no indication that grinding favored pathogen attack of new roots in the replanted trees (**Table 4, Figure 2**). *Cylindrocarpon* was detected at greater incidence in roots from non-fumigated plots than from fumigated plots, especially when the roots were bleached to remove surface inhabitants (**Table 4, Figure 2**). *Trichoderma* was isolated at greatest incidence from roots of fumigated plots.

In the remainder of the current project year, we will focus on testing spot treatments with nitrogen-based and seed-meal-based amendments. We intend to compare effects of the non-fumigant spot treatments with effective soil fumigation treatments. We also intend to initiate trials evaluating rootstocks for tolerance to RD, which will provide further support for non-fumigant based approaches to managing orchard replant problems.

Table 4. Summary of results of culture-based isolations from roots of replanted trees, Holtz et al. trial testing effects of orchard residue management x spot drip fumigation

Treatment			Genera detected, number of isolates total (N), and isolate distribution (%) among treatments									
Pre-plant Fumigation	Orchard removal	Root sample	*Cylindrocarpon (N=14)*	*Fusarium (N=144)*	*Gliocladium (N=4)*	*Macrophomina (N=6)*	*Mortierella (N=120)*	*Mucor (N=9)*	*Penicillium (N=7)*	*Pythium (N=25)*	*Rhizoctonia (N=7)*	*Trichoderma (N=90)*
Control	burn	bleach	43	2	0	17	3	0	14	4	29	0
		rinse	14	20	0	0	23	67	29	48	14	10
	grind	bleach	14	22	0	0	0	0	0	4	0	0
		rinse	7	24	50	0	35	22	29	20	0	7
Spot drip	burn	bleach	0	4	0	0	0	0	0	4	0	6
		rinse	0	6	0	33	9	11	14	8	29	48
	grind	bleach	21	7	0	17	0	0	0	0	29	3
		rinse	0	15	50	33	29	0	14	12	0	27

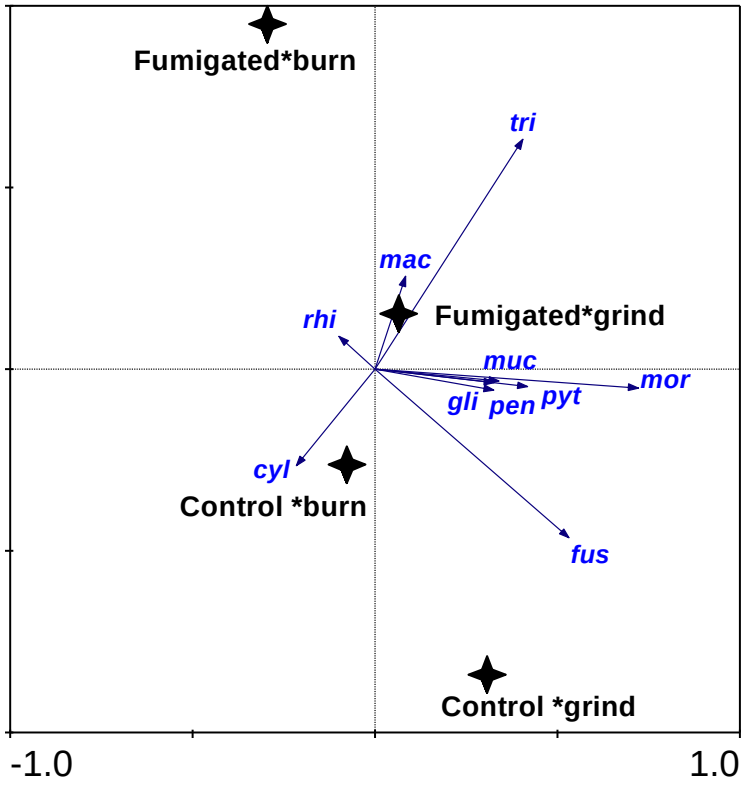

Figure 2. Ordination of culture-detected fungal incidence associated with fumigation and orchard residue treatments in trial of Holtz et al. Abbreviations are as follows: cyl, *Cylindrocarpon*; fus, *Fusarium*; gli, *Gliocladium*; mac, *Macrophomina*; mor, *Mortierella*; muc, *Mucor*; pen, *Penicillium*; pyt, *Pythium*; rhi, *Rhizoctonia*; and tri, *Tricoderma*.