Developing Improved Management Strategies for Replant Problems

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Background

Replant disease (RD) and other replant problems such as plant parasitic nematodes can seriously reduce cumulative nut yield in successive almond plantings. When almond orchards are replaced, RD suppresses root development and thereby slows the rate of canopy development. In severe cases RD kills trees. Evidence suggests that a soilborne complex of microorganisms causes RD, but many of the important details remain unresolved. RD is a separate problem from nematode damage.

Pre-plant soil fumigation can prevent RD and other replant problems, but all soil fumigants face tremendous regulatory pressures. This project is 1) using traditional and DNA-based methods to unravel the causes of RD, and 2) testing and improving non-fumigant-based strategies for controlling replant problems.

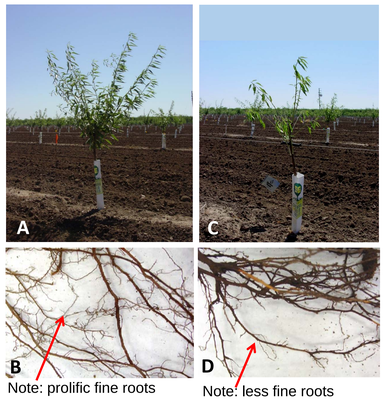


Fig. 1. Symptoms of replant disease. **A** and **B**, healthy tree and roots in soil pre-plant fumigated with chloropicrin; **C** and **D**, tree and roots affected RD in non-fumigated soil. Note there are fewer healthy fine roots in **D**, compared to **B**.

Note: prolific fine roots

Note: less fine roots

Objectives

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

Metagenomics: a culture-independent analysis of microbial communities mediating replant disease (under Obj. 1)

Our previous studies have emphasized culture-based profiling of the communities, but in 2010, we focused on culture-independent metagenomics (Fig. 2), which uses diagnostic DNA amplification and sequencing of genetic material recovered directly from environmental samples, instead of culture-based isolations.

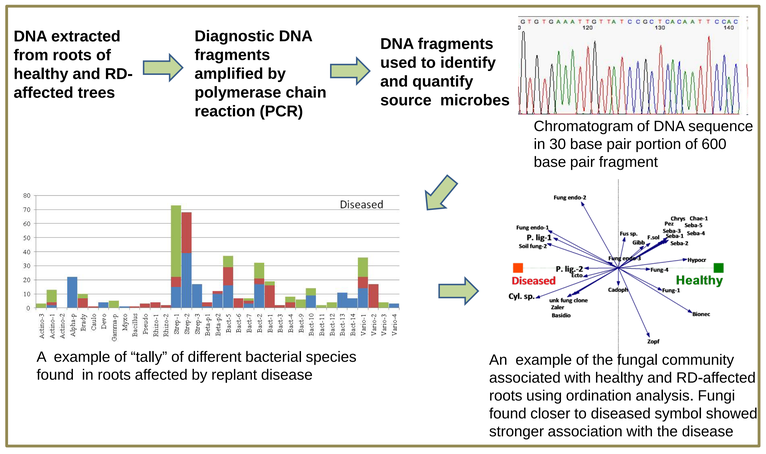
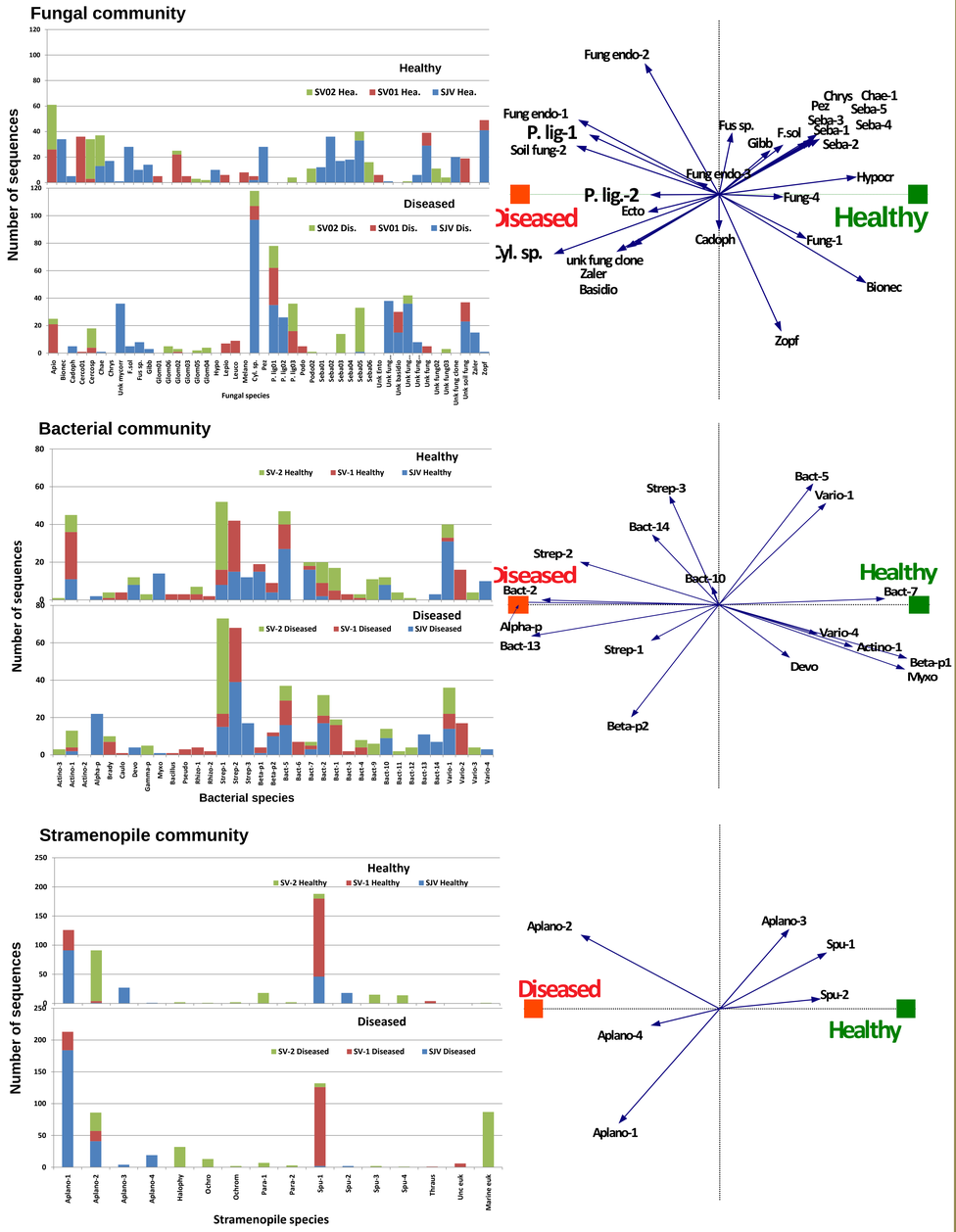


Fig. 2. Metagenomics approach used to characterize microbial populations in roots of healthy and diseased almond trees.

Metagenomics results: Counts in bar graphs, left ; ordination in "vector" diagrams, right)



Discussion, interpreting the data

- Metagenomics analysis: the fungi *Cylindrocarpon* sp. and *Phaeonectriella lignicola* show the strongest association with RD.
- Culture-based analyses also associated *Cylindrocarpon* sp. with RD from same orchards but not *P. lignicola*.
- Culture isolations found species of *Pythium* and *Fusarium* associated with the RD, metagenomics approach did not.
- Both metagenomics and culture-based approaches are needed for complete analysis of the microbial communities.
- Current and planned research includes development of quantitative PCR detection methods for *Cylindrocarpon* sp. and *P. lignicola* (see Table 1) and genetic and pathogenic examination of these fungal populations.

Following leads from metagenomic results, developing and testing qPCR primers (under Obj. 1)

- We designed, tested, and optimized quantitative PCR primers for key organisms associated with RD
- We are using the primers to validate metagenomic results (see Table 1).
- qPCR primers may help to identify orchards at high risk for RD
- qPCR primers were validated for *Cylindrocarpon* spp., *Pythium helicoides* (positively assoc. w/ RD), and *Trichoderma harzianum* (negatively assoc. w/ RD at some sites).
- qPCR primers for *P. lignicola* were designed and tested but need further refinement..

Table 1. Results from testing *Cylindrocarpon* qPCR primers*

Orchard	Type of environmental sample	Concentration of target *Cylindrocarpon* rDNA (ng DNA/g root)
SJV[a]	Roots fr. healthy tree	0
	Roots fr. RD-affected tree	117
SV[b]	Roots fr. healthy tree	16
	Roots fr. RD-affected tree	75
	***P* value, tree status effect:**	***0.0004***
	***P* value, tree status x grower interaction:**	***0.16***

*Data from replicated plots. Note: results confirm association of *Cylindrocarpon* spp. with RD

Testing of non-fumigant treatments for control of RD (under Obj. 2)

Fig. 3. Application of steam and soil amendments to tree sites.

Table 2. First-growing-season results from replant trial testing non-fumigant treatments for management of replant problems*

Trt. number	Components of treatment: No trt. (control)	Telone C35, 540 lb/trt. ac	Tree site augered	BSM 4000 lb/trt. ac	BSM 8000 lb/trt. ac	Steam	Avg. trunk circ. increase, 2010 (mm)	95% Conf. interval: Lower limit	Upper limit
1	+						12.9	10.9	14.8
2		+					21.4	18.5	24.2
3			+				18.8	16.4	21.3
4			+	+			17.3	14.8	19.7
5			+		+		20.1	17.7	22.6
6			+			+	18.0	16.0	20.1
7			+	+		+	17.4	15.4	19.4
8		+	+	+			25.1	21.3	28.9
9		+	+		+		27.0	20.8	33.1

*Data from replicated plots, Delhi sand, Merced Co., with David Doll. Trial planted Jan. 2010. Previous orchard affected by the ring nematode, current orchard probably affected by the nematode and RD.

Testing rootstocks for resistance to RD (under Obj. 2)

- Replicate fumigated and non-fumigated plots were prepared Oct. 2010 at USDA-ARS, Parlier for testing 20 rootstocks for resistance to RD
- The plots will be planted in spring 2011 and the rootstock growth will be monitored

Acknowledgements

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