Developing Improved Management Strategies for Replant Problems

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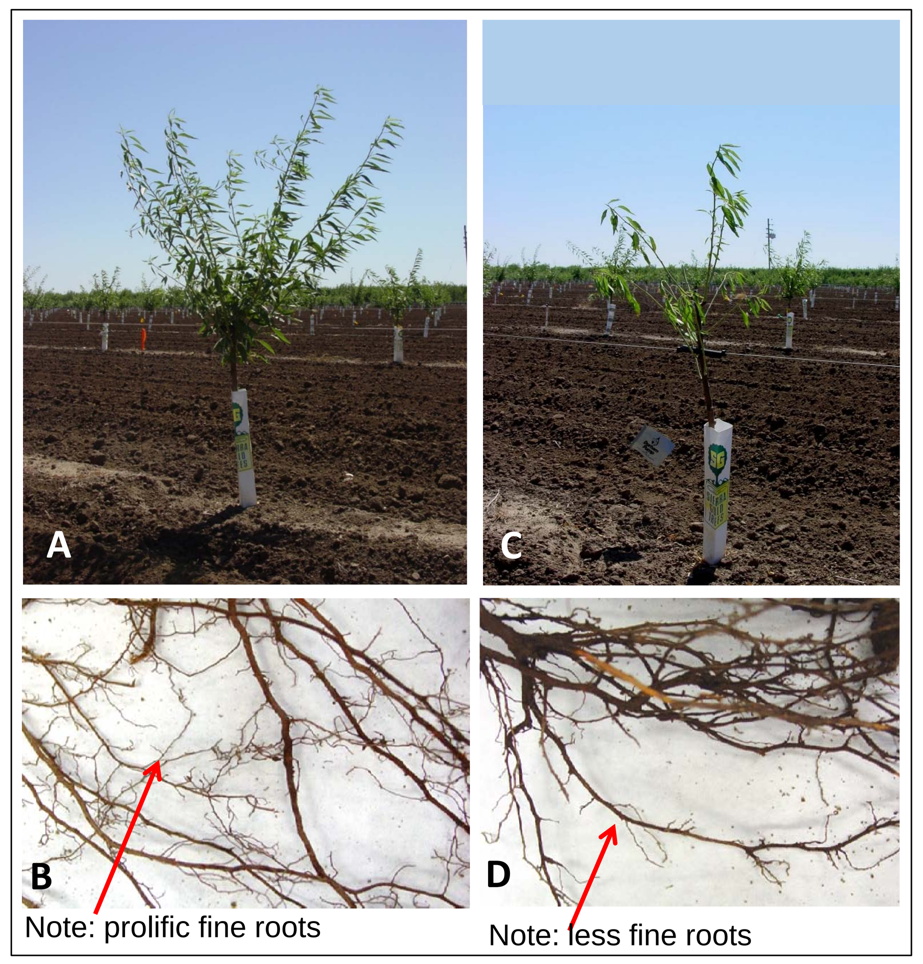
BACKGROUND

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**.



PROJECT OBJECTIVES

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

Rootstock resistance to replant disease (RD) and *Phytophthora* NEW PROJECT EMPHASES

A prime strategy for managing soilborne diseases economically is to use rootstock resistance, but work is required to select or develop the resistance in rootstocks with desirable horticultural characteristics (i.e., appropriate vigor, broad resistance to important soilborne pathogens). As part of our work under Objective 2, we evaluated a diverse set of clonal rootstocks (**Table 1**) for their response to the RD complex and to *Phytophthora* crown and root rot. **Many of the rootstocks we evaluated are appropriate for almond, but some of them are appropriate for stone fruits other than almond**. Results from selected 2010-11 rootstock trials are highlighted here (Figs. 1-3, right).

Table 1. Rootstocks tested for resistance to replant disease complex and *Phytophthora* crown and root rot in 2010-11

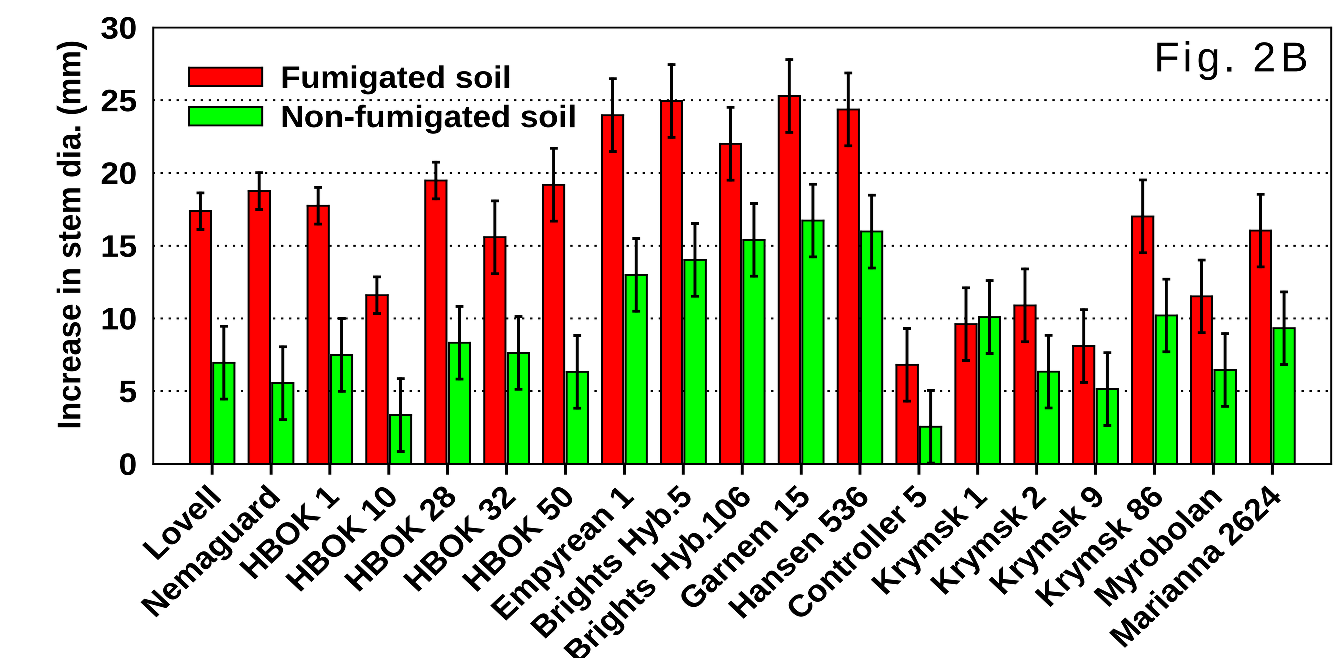
Rootstock	Type	Genetic background
HBOK1	Pe	*HB x OK peach*
HBOK 10 (Controller 8)	Pe	*HB x OK peach*
HBOK 28	Pe	*HB x OK peach*
HBOK 32 (Controller 7)	Pe	*HB x OK peach*
HBOK 50 (Contoller 9.5)	Pe	*HB x OK peach*
Lovell	Pe	*P. persica*
Nemaguard	Pe	*P. persica x P. davidiana*
Empyrean#1 (Barrier 1)	Pe	*P. persica x P. davidiana*
Bright Hybrid-5	Pe x Al	*P. persica x P. dulcis*
Bright Hybrid 106	Pe x Al	*P. persica x P. dulcis*
GxN 15(Garnem)	Pe x Al	*P. dulcis x P. persica (Nemared)*
Hansen 536	Pe x Al	*[Okin.x (P. davidiana x Pe PI 6582)] x alm.*
Controller 5 (=K146-43)	Pl hybrid	*P. salicina x P. persica*
Krymsk #1 (VVA 1)	Pl hybrid	*P. tomentosa x P. cerasifera*
Krymsk 2	Pl hybrid	*P. incana x P. tomentosa*
Krymsk 9	Pl hybrid	*P. armeniaca x P. ceracifera (?)*
Krymsk#86 (Kuban 86)	Pl hybrid	*P. persica x P. cerasifera*
Tempropac	(Pe x Al) x Pe	*(P. dulcis x P. persica) x P. persica*
PAC 9908-02	(Pe x Al) x Pe	*(P. dulcis x P. persica) x P. persica*
Replantpac	Pl hybrid	*P. ceracifera x P. dulcis*
Myrobalan	Pl hybrid	*P. ceracifera?*
Marianna 2624	Pl hybrid	*P.munsoniana x P. cerasifera*

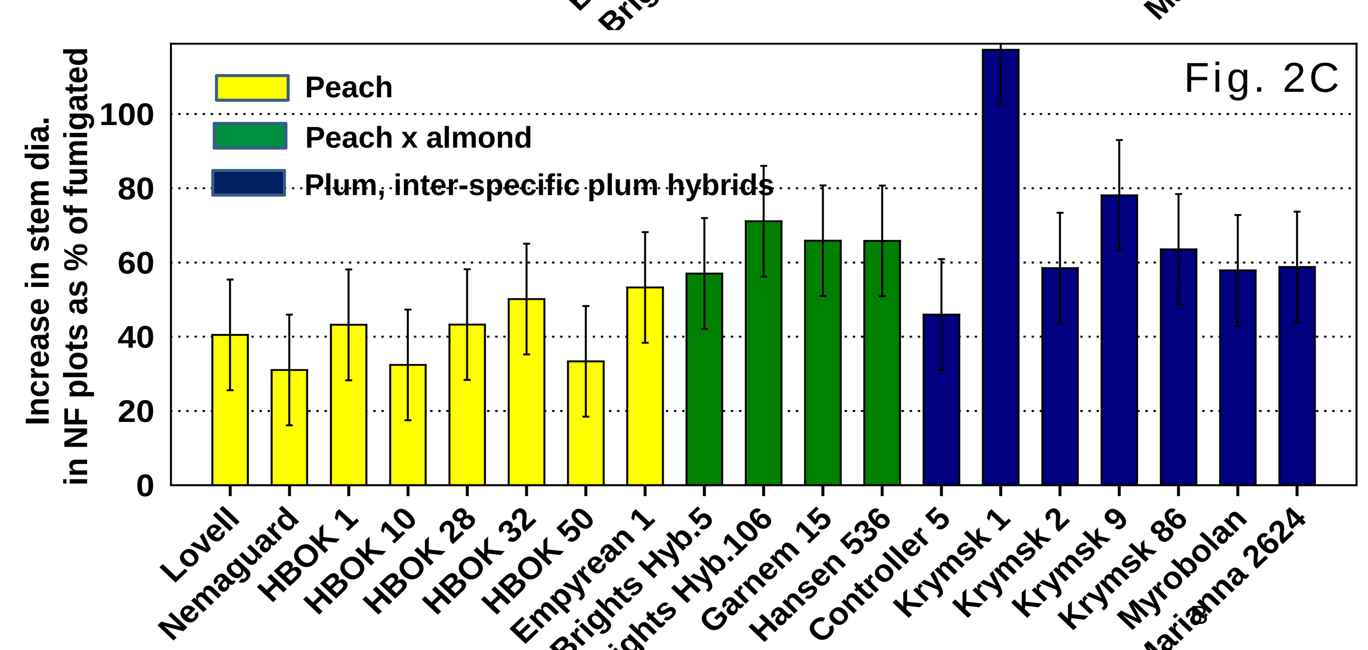
Rootstock resistance to RD, METHODS

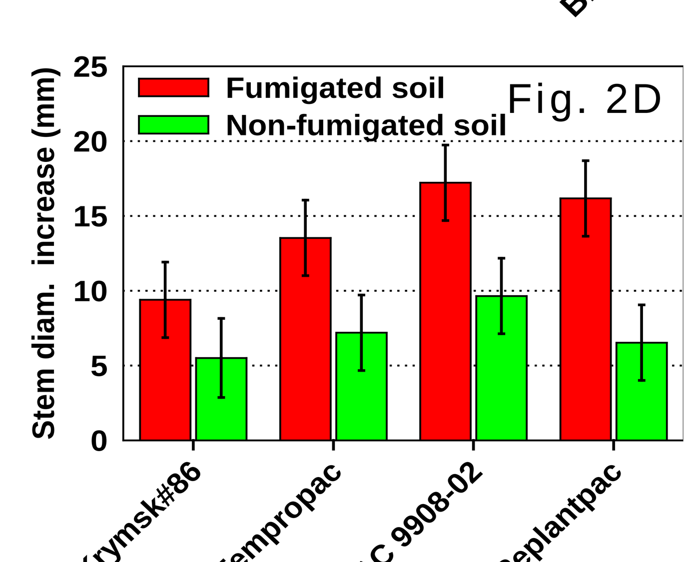
Twenty-two rootstocks, clonally propagated and including Lovell, Nemaguard, and Marianna 2624 as standards, were planted in replicate fumigated (Telone C35) and non-fumigated plots of Hanford Sandy Loam soil near Parlier, CA. The site was known to induce severe RD. Resistance to RD was assessed according to the degree to which rootstock performance in non-fumigated soil matched that in the fumigated soil. Two experiments were completed (expt. 1 and expt. 2) to accommodate rootstocks from two nurseries. .

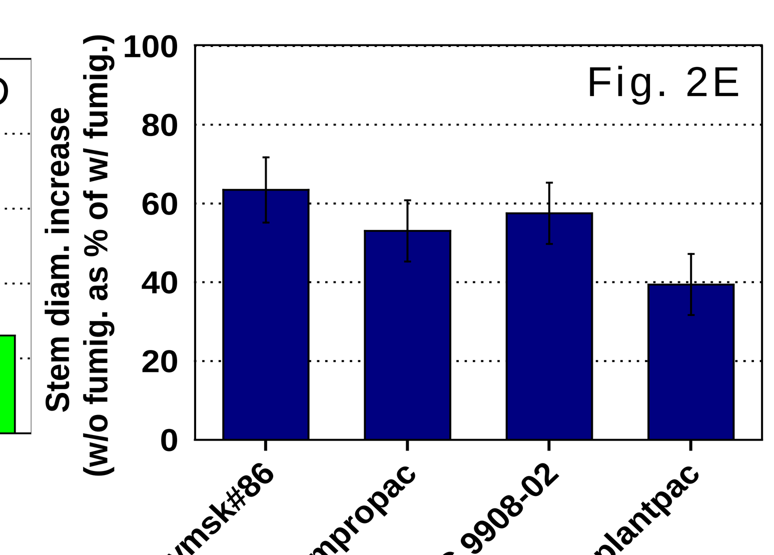
Rootstock resistance to RD, RESULTS

Fig. 2A-E , below: A, severe expression of replant disease occurred in non-fumigated soil (foreground) compared to growth in fumigated soil (background). **B**, expt. 1, rootstock stem diameter growth as a function of soil trt.; **C**, expt. 1, rootstock stem growth in non-fumigated plots expressed as a percentage of growth in fumigated plots; **D**, expt. 2, rootstock stem growth as a function of soil trt.; and **E**, expt. 2, rootstock stem growth in non-fumigated plots expressed as a percentage of growth in fumigated plots.







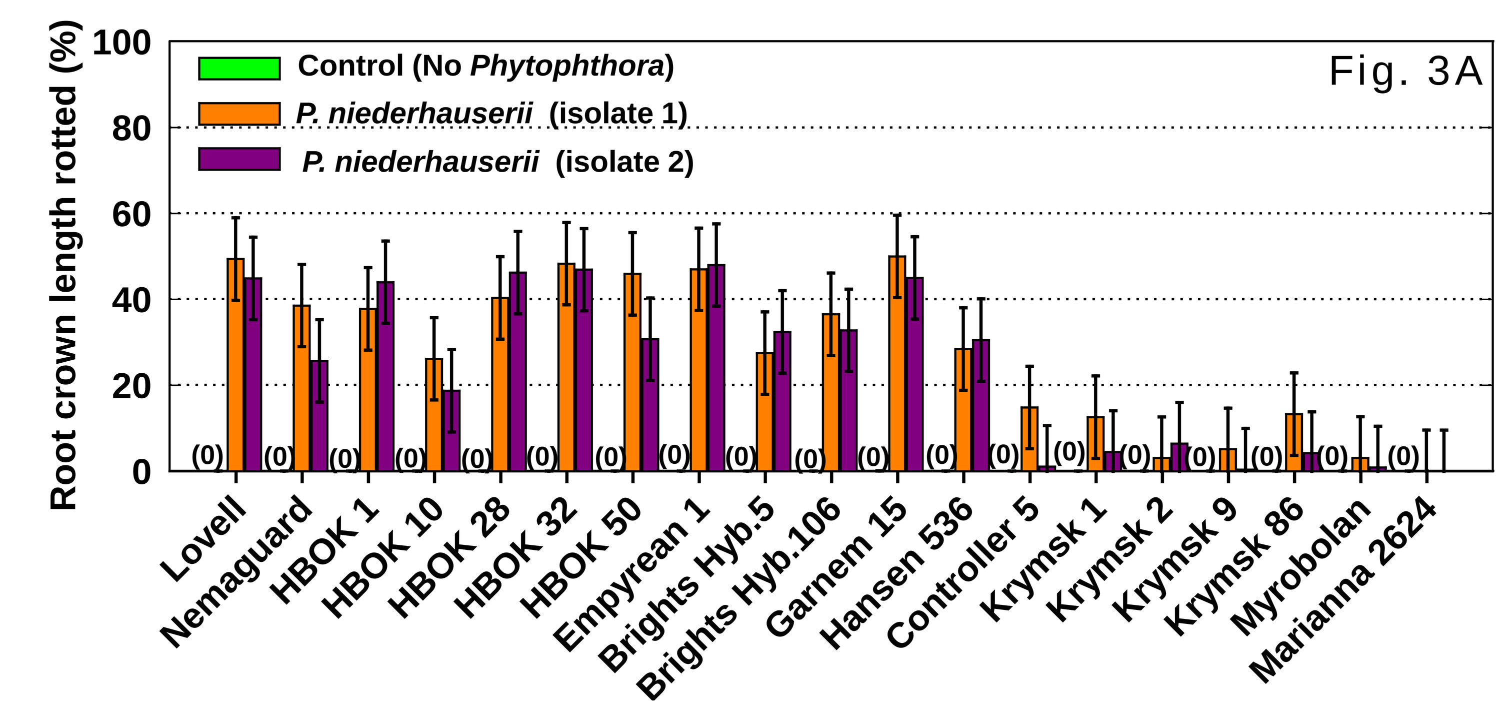


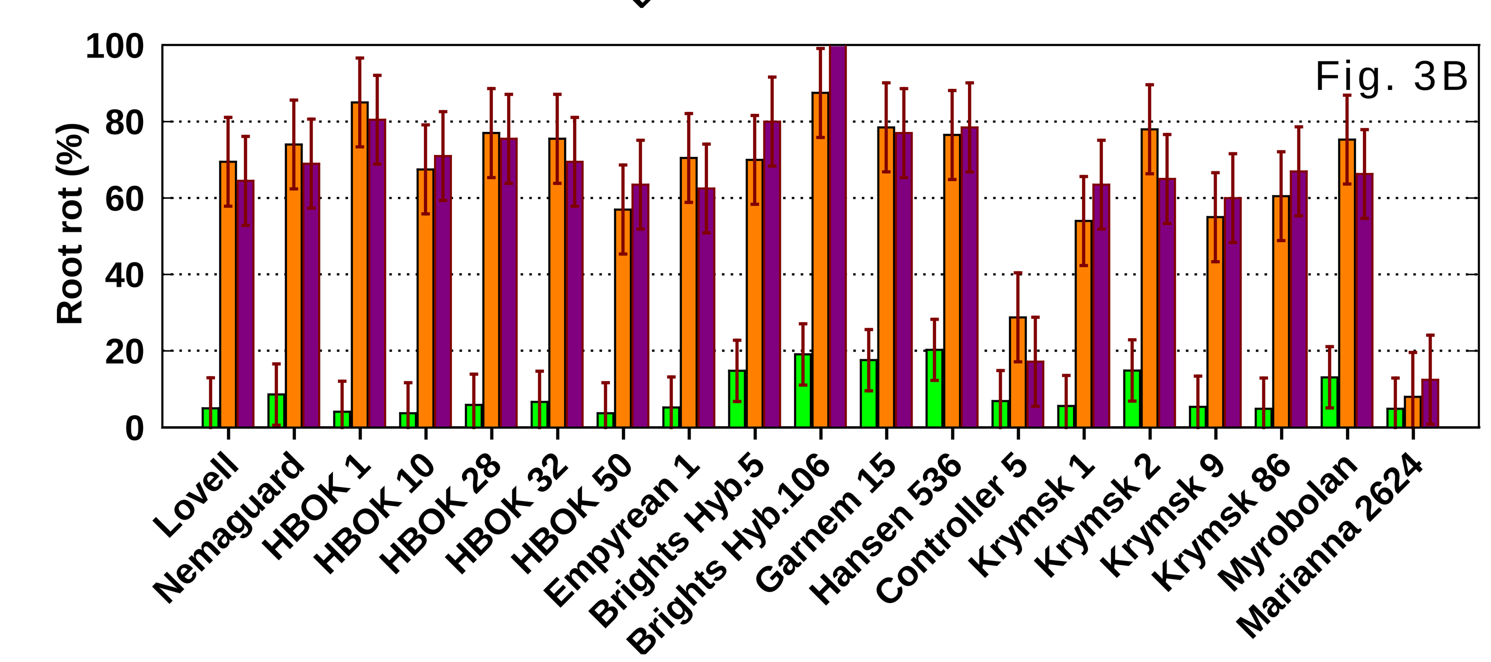
Resistance to *Phytophthora*, METHODS

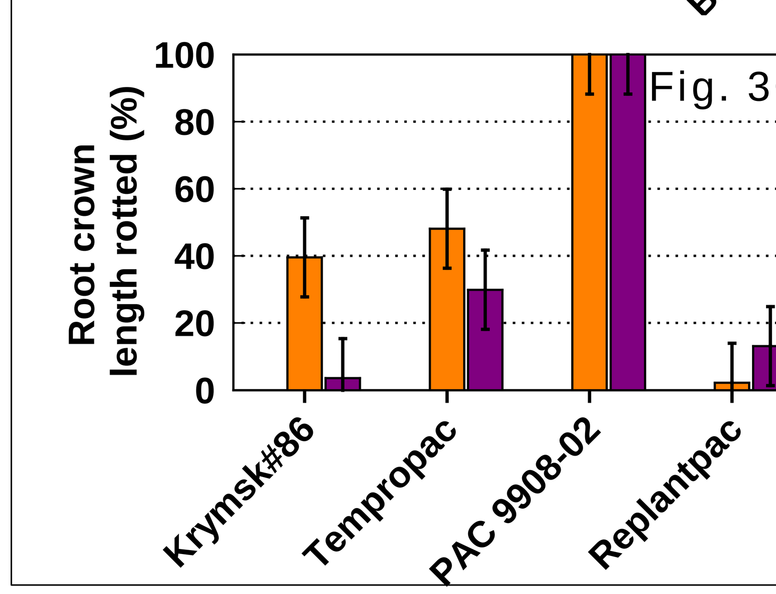
The same rootstocks tested for resistance to RD were tested for resistance to two isolates of *Phytophthora niederhauserii*, isolated from dying almond trees in Fresno and Kern Counties. Each rootstock was grown in a greenhouse in replicated pots of non-infested soil and soil artificially infested with the *Phytophthora* isolates. Bi-weekly 48-hr episodes of soil flooding were imposed to stimulate infection. Resistance to *Phytophthora* was assessed according to the severity of root and crown rot.

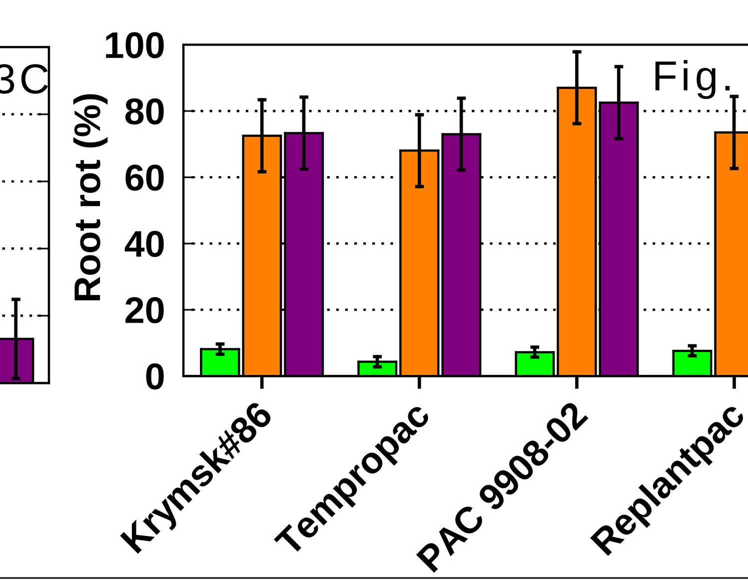
Resistance to *Phytophthora*, RESULTS

Fig. 3A-D, below. Each isolate of *P. niederhauserii* caused: **A**, expt. 1, moderate crown rot in all rootstocks except those including plum parentage (Controller 5, Krymsk selections, Myrobolan, and Marianna 2624. **B**, expt. 1, severe root rot in all rootstocks except Controller 5 and Marianna 2624; **C**, expt 2, moderate crown rot in Krysmsk 86 and Tempropac, severe crown rot in PAC 9808-02, and negligible crown rot in Replantpac; and **D**, expt. 2, severe root rot in all selections in the expt.









SUMMARY, DISCUSSION

- **Regarding resistance to RD:** In rootstock grouping (i.e., peach, peach x almond, and plums / plum hybrids) some clones suffered much less growth suppression than others, indicating that careful rootstock choice, appropriate tree spacing in replanted orchards, and future breeding may permit control of RD without or with minimal soil fumigation.
- **Regarding resistance to *Phytophthora***: Plum parentage, which is not appropriate for all growing regions, appears to offer the strongest resistance to *P. niederhauserii*. Confirmation of these results and rootstock evaluations with additional *Phytophthora* species are needed.

ACKNOWLEDGEMENTS

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Additional Research, Replant Disease and the Pacific Area-Wide Program for Methyl Bromide Alternatives (PAW-MBA)

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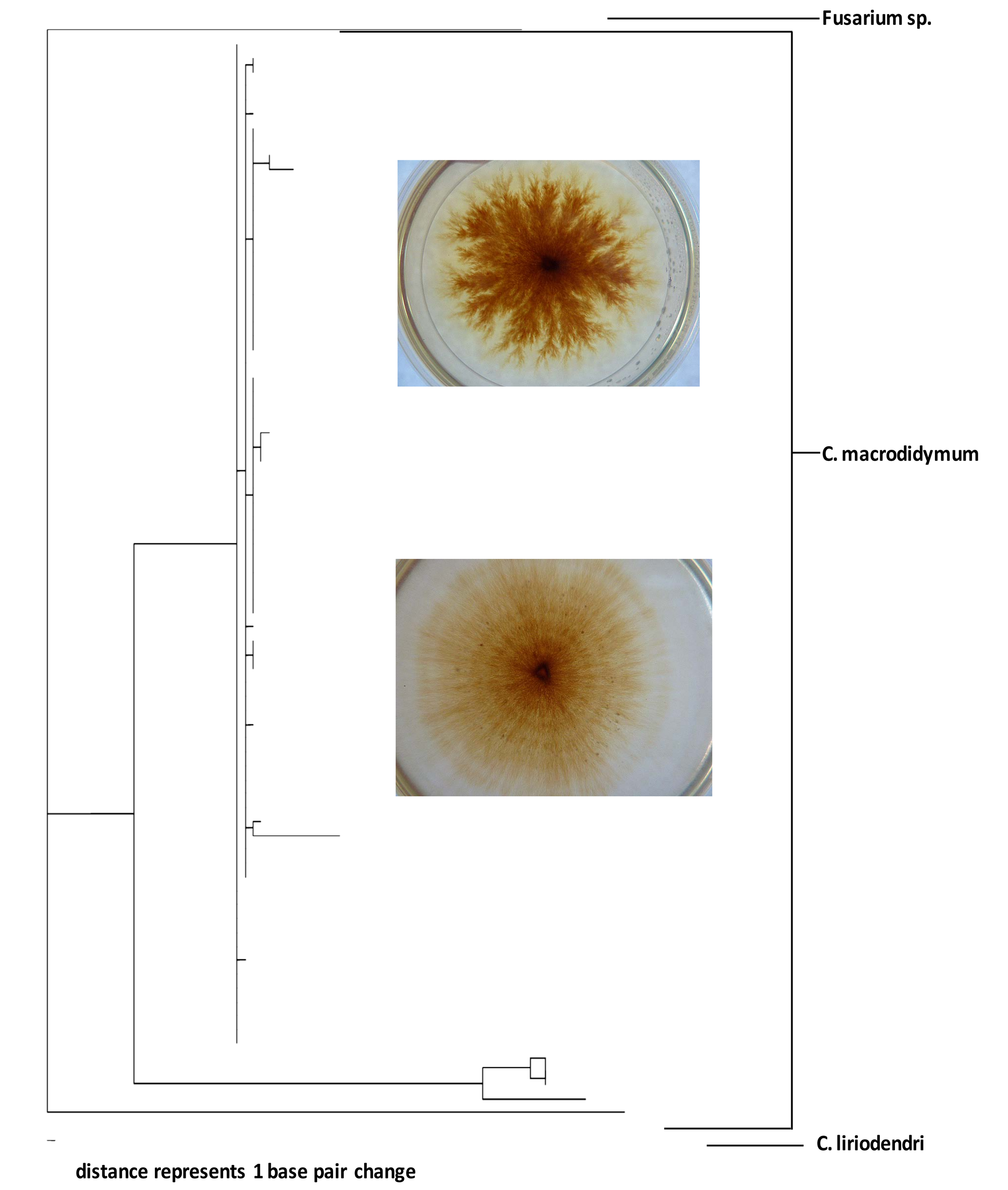
Examining causes of replant disease



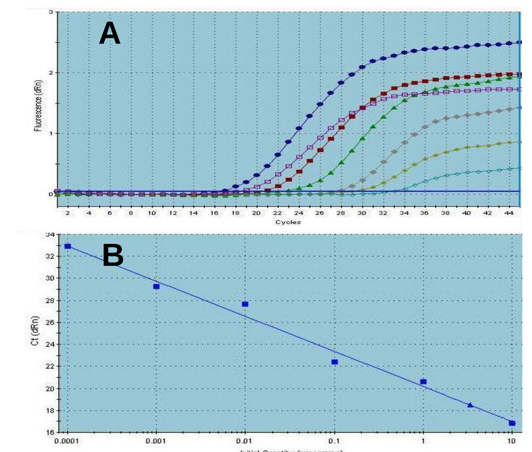
Phylogenetic cluster analysis of 79 isolates of *Cylindrocarpon* from roots of trees in RD-affected orchards. Clustering was based on partial DNA sequences from ITS regions of rDNA, partial beta tubulin, and partial mtSSU rDNA. *C. macrodidymum* predominated; there were: 1 isolate of *Fusarium* sp., 77 isolates of *C. macrodidymum*, and 1 isolate of *C. liriodendri*)

- In replant trials that express replant disease, including the rootstock trial described above, we are continuing to use culture-based and culture independent approaches to examine causes for the growth suppression.
- Organisms that have often exhibited association with the disease include *Cylindrocarpon macrodidymum* (, *Pythium* spp., *Fusarium* spp. and others.
- For organisms associated with the disease, our approach has been: a) definitive identification (see example of phylogenetic analysis of RD-associated *Cylindrocarpon* population, left), b) quantification in healthy and diseased samples (see development of quantitative PCR detection methods for *C. macrodidymum*, right), and c) completion of pathogenicity testing and Koch's postulates (see greenhouse pathogenicity test, above).

Developing PCR detection technology for *Cylindrocarpon macrodydimum*, a suspected contributor to replant disease

ABSTRACT

Prunus replant disease (PRD) is a poorly understood soilborne complex in replanted almond and peach blocks in California. Using culture-dependent and culture-independent approaches, we found *Cylindrocarpon macrodidymum* (Cylmac) among microbes often associated with PRD. Here we report on development and application of a qPCR assay to examine the Cylmac-PRD association. A selective primer pair that amplified a 374-bp rDNA fragment from Cylmac was coupled with a specific hydrolysis probe. The assay was optimized using genomic DNA from the target and >70 non-target microbes and rootstocks. The lower detection limit was 100 fg Cylmac DNA per 25 µL of PCR mix. The assay was used with DNA from root samples of replicated healthy and PRD-affected almond and peach trees (in fumigated and non-fumigated plots, respectively) in five California orchards. All orchards were planted in winter and expressed PRD symptoms by the following summer. Samples were collected on 1 to 5 dates per orchard from Apr-Sept of the year trees were planted. In orchards 1-3, Cylmac levels were significantly higher in PRD-affected than in healthy roots on some dates (7 of 11 sampling dates), but in orchards 4 and 5 (1 sampling date each), Cylmac levels were near the lower detection limit and did not differ in relation to PRD incidence. We conclude that the assay is effective; and pathogenicity tests, seasonal sampling and qPCR are required to further examine the association of Cylmac to PRD.



A typical amplification plot (**A**) and standard curve (**B**) for identification and quantification of *Cylindrocarpon macrodidymum*.

Table 1. Results from testing a pair of qPCR primers and a hydrolysis probe specific for *Cylindrocarpon macrodidymum*

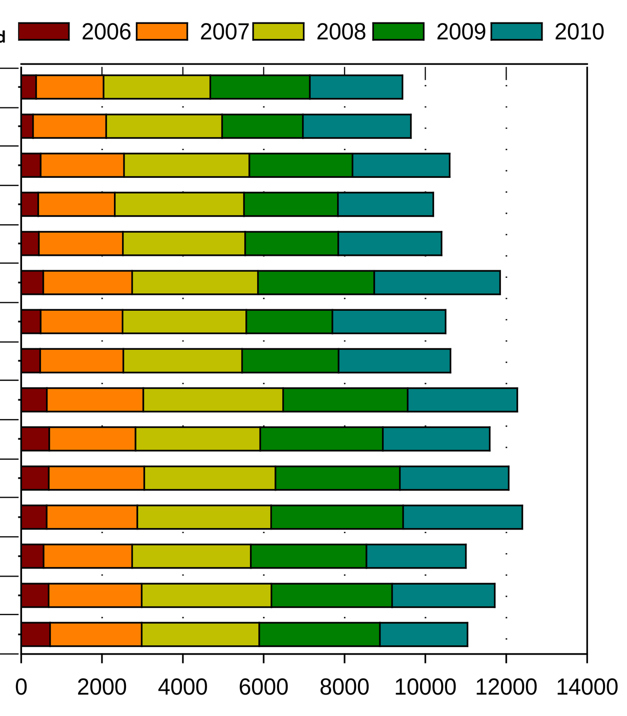
Almond orchard [a]	Total times sampled	Total trees sampled (affected + healthy)	Cylindrocarpon DNA in roots (ng DNA/g root)		*P* value
			PRD-affected tree	Healthy tree	
Orchard 1	5	20 + 20	615.3	168.8	0.0111
Orchard 2	4	16 + 16	606.1	134.6	0.0001
Orchard 3	2	12 + 12	277.2	2.0	0.0001
Orchard 4	1	6 + 6	60.9	20.8	0.1962
Orchard 5	1	5 + 5	35.1	9.6	0.2372

[a] Orchards 1, 2 and 3 were from Sacramento Valley, and Orchards 4 and 5 were from SJV = San Joaquin Valley

Yield updates from selected PAW-MBA replant trials

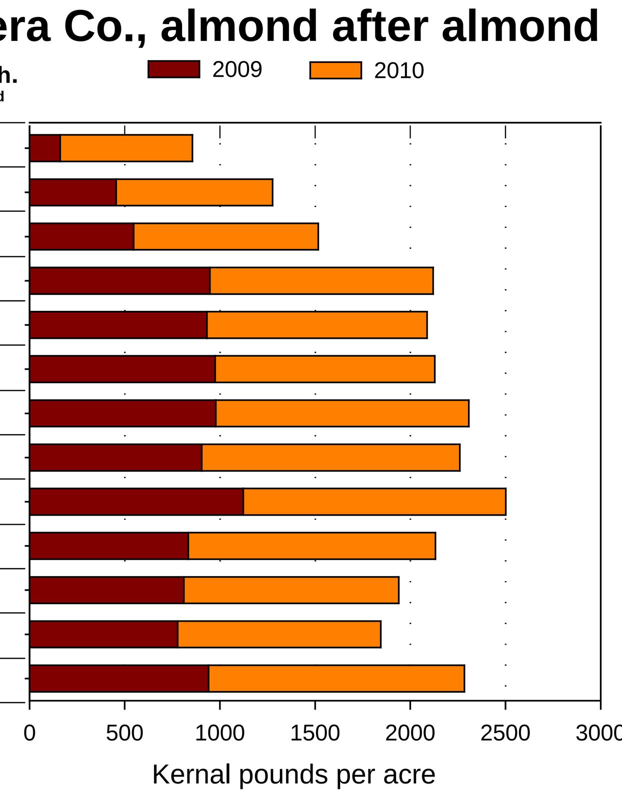
2004 trial, Ave. 7, Madera Co, almond after almond

Fumigant[a]	Treated area[b]	Mulch	lb/treated acre[c]	lb/orchard acre[d]
Control	None	None	0	0
Control	None	VIF	0	0
MB	Br. (100%)	None	400	400
MB	R. strip (38%)	None	400	152
MB	R. strip (38%)	VIF	400	152
Telone II	Br. (100%)	None	340	340
Telone II	R. strip (38%)	None	340	129
Telone II	R. strip (38%)	VIF	340	129
Telone C35	Br. (100%)	None	535	535
Telone C35	R. strip (38%)	None	535	203
Midas	Br. (100%)	None	400	400
Midas	R. strip (38%)	None	400	400
CP	Br. (100%)	None	400	400
CP	R. strip (38%)	None	400	152
CP	R. strip (38%)	VIF	400	152



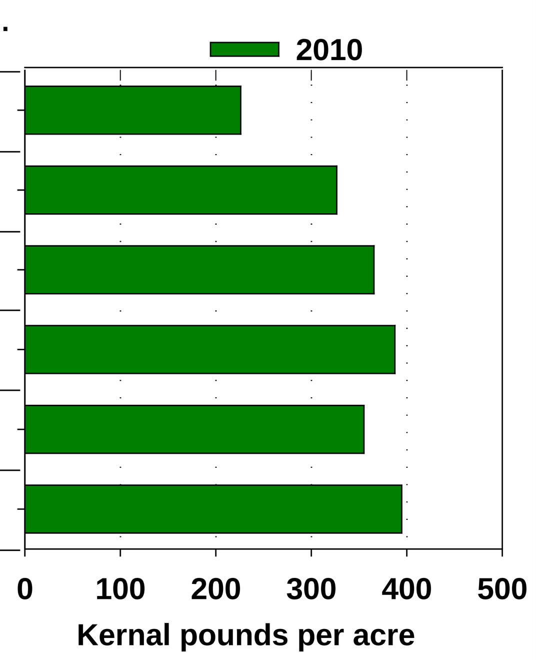
2007 trial, Ave. 7.5 Madera Co., almond after almond

Fumigant[a]	Treated area[b]	lb/treat. acre[c]	lb/orch. acre[d]
Control	None	0	0
MB	Br. (100%)	400	400
Telone II	R. strip (38%)	340	129
Midas	R. strip (38%)	400	400
CP	R. strip (38%)	400	152
CP	R. strip (38%)	300	114
CP	R. strip (38%)	200	76
Telone C35	R. strip (38%)	544	209
Pic-Clor 60	Row strip (38%)	560	209
Pic-Clor 60	Row strip (38%)	400	152
Chloropicrin	Tree square (11%)	400	68
Telone C35	Tree square (11%)	544	93
Telone C35	Broadcast (100%)	544	550



2008 trial, Ave 16, Madera Co. , almond after almond

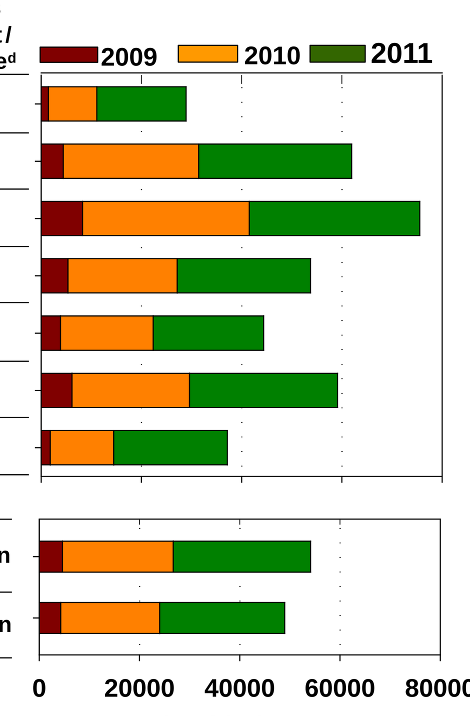
Fumigant[a]	Treated area[b]	lb/treat. acre[c]	lb/orch. acre[d]
Control	Row strip (38%)	0	0
Pic-Clor 60	Row strip (38%)	100	38
Pic-Clor 60	Row strip (38%)	200	76
Pic-Clor 60	Row strip (38%)	300	114
Pic-Clor 60	Row strip (38%)	400	152
Pic-Clor 60	Tree square (11%)	400	44



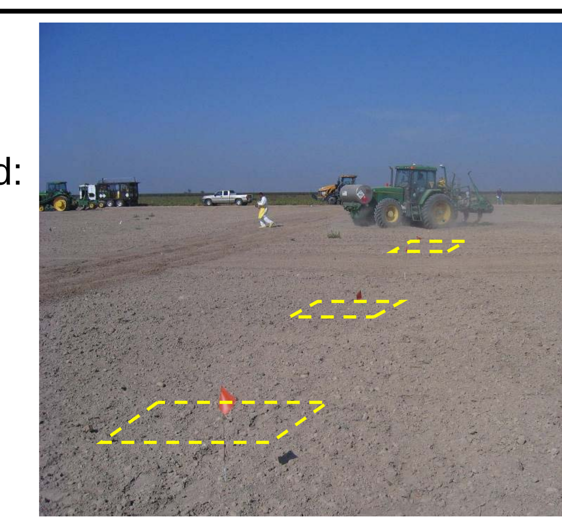
2008 trial, USDA-Parlier, peach planted after plum

Treatment[a]	Treated area[b]	Pounds fumigant / treated acre[c]	Pounds fumigant / orch. acre[d]
Control	None	0	0
MB	R. strip (42%)	400	168
Telone C35	R. strip (42%)	540	227
Telone C35	Tree site (13%)	540	70
Inline	Tree site (5%)	540	28
CP	Tree site (13%)	400	52
Yeast extract	Drench	0	0

Cover crop treatment
No sudan grass rotation
2-month pre-plant sudan grass rotation



Spot treatment methods being tested and refined: left and center GPS-controlled spot fumigation; right, auger injected steam pasteurization



Results summary and future directions

Causes of replant disease

- Field assays and pathogenicity tests indicate that strains of *Cylindrocarpon macrodidymum* and *Pythium* sp. can contribute to RD at some locations and under some conditions
- Work is underway to verify these results and explore contributions of other organisms and develop diagnostic assays for RD causal agents

Control of replant disease

- Fumigants containing chloropicrin (chloropicrin, Telone C35, Pic-Clor 60) are effective for control of RD.
- GPS-controlled spot shank fumigation treatments, which save fumigant and reduce undesirable emissions, are effective and becoming commercially available
- We are attempting to develop a predictive assay for risk of RD. Growers that would like to be part of this work and are scheduled to replant almond after almond in the next 2 years are invited to contact G. Browne (gtbrowne@ucdavis.edu) for more information.
- Field trials examining efficacy of spot treatments with steam, fungicides, Brassica seed meals, and have been established with D. Doll and B. Hanson.
- We will continue to emphasize non-fumigant strategies, including use of rootstocks, for management of replant disease.

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