Biology and Management of Almond Replant Disease
2006 Comprehensive Report to the Almond Board of California

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Interpretive Summary:

This report is an expanded version of the 2006 interim report to the Almond Board and includes results of soil microbial community characterizations not found in the interim report. This project is dedicated to development of improved approaches for managing replant disease (RD) and other replant problems that affect almond. It includes orchard and microplot testing of alternative fumigants, fumigant application practices, and short-term crop rotations to manage the replant problem complex. The project also is determining the fundamental cause(s) of RD by characterizing shifts in microbial populations associated with the disease and testing the roles of individual microbes in development of the disease. Growth and yield data collected from team trials near Madera and Parlier indicate that chloropicrin and fumigant mixtures including chloropicrin (i.e., Telone C35, Midas [iodomethane:chloropicrin 50:50]) are superior to MB and Telone II for control of RD. Focused narrow row strip and spot treatments offer potential as effective, economical, and environmentally acceptable preplant fumigation treatments for improved RD management, and they are being explored more fully and optimized in ongoing research. We are working with S. Upadhyaya and TriCal, Inc. to test efficacy of GPS-directed spot treatments, which may facilitate valuable fumigant use reductions without sacrificing treatment efficacy. Microplot trials were completed and confirmed that 1-year crop rotations can help to remediate almond RD. We now are working with B. Hanson, USDA-ARS Parlier, to advance tests of selected crop rotations to orchard replant settings. Our work towards determining RD causes has expanded to include culture-independent, DNA-based examination of microbial populations shifts associated with the disease. Multiple lines of evidence suggest potential contributions of certain fungi and bacteria to RD.

Objectives:

1. Develop improved management strategies for replant disease (RD) on California almonds.
2. Determine the unknown causes of RD.

Materials and Methods:

Objective 1, improved management strategies for RD.

Fumigation trials. Almond replant trials were established in three commercial orchards, two near Madera and one near Parlier, CA, to test alternative pre-plant fumigation treatments for management of RD and other replant problems. The Madera trials are conducted with Brent Holtz and other members of a USDA CSREES-supported team project (Bruce Lampinen et. al), and the Parlier trial was organized and led by Tom Trout (USDA, ARS). The trials are evaluating alternative fumigants and fumigant application strategies (spot, strip, and broadcast treatments; with and without tarp). Results of the trials will guide development of optimized treatments that minimize fumigant emissions and maximize economic benefit.

The treatments in Madera trials were applied in October and November 2003 and included methyl bromide (MB, 400 lb/acre), Telone II (1,3-dichloropropene [1,3-D], 340 lb/acre), chloropicrin (CP, 400 lb/acre), Telone C35 (1,3-D:CP 61:35, 535 lb/acre), and Midas (iodomethane [IM]:CP 50:50, 400 lb/acre). The fumigants were applied with shanks spaced 20" apart through nozzles spaced 10" apart at a soil depth 18", with the exception that a hand-held probe was used to apply tree site spot fumigation treatments at 20" depth in one experiment. Some of the fumigants were applied alternatively as broadcast, row-strip, and spot treatments, and some of the row-strip treatments were applied with and without virtually impermeable film (VIF), a tri-layer plastic that can dramatically reduce fumigant emissions and increase fumigant retention near the soil surface. One of the Madera trials occurred on loam soil previously devoted to almond on Nemaguard rootstock, and the other occurred on loam soil previously devoted to grape. All of the treatments were compared to non-fumigated control treatments with and without the VIF. There were four replicate plots per treatment, and each plot eventually was planted to 3 rows of 9 to 10 trees. Data have been collected from the center rows, which are planted to Nonpareil. Tree trunk circumference has been measured annually since planting in winter 2004. Nut yields were measured after the first harvest in 2006.

Treatments in the Parlier trial were applied in October 2005. The soil was Hanford Sandy Loam that had been devoted to peach production. We evaluated effectiveness of hand-probe and drip-delivered spot treatments, compared with shank and drip applications of row strip treatments and a control managed by T. Trout. The hand probe spot treatment involved applying Telone C35 (1,3-D:CP, 61:35) to tree sites that had been prepared for injection with an 18-inch-diameter auger; 0.8 lb of the fumigant was injected at 20-inch depth at each tree site. The drip spot treatment applied Inline (1,3-D:CP, 61:33) to tree sites; 0.2 lb of fumigant was applied at each tree site over a 24-h period through one 2 gph emitter buried at 20" soil depth. The area wetted by each emitter at the soil surface was approximately 4 feet in diameter. The row strip treatments included a standard of shank-applied MB (325 lb/treated acre) and shank- and subsurface-drip-applied treatments with the following: 1,3D (338 to 360 lb/acre), 1,3-D:CP (61:35 or 61:33, 540 to 560 lb/acre), and Midas (IM:CP, 33:67, 300 lb/acre). All of the treatments were compared to a non-fumigated control. There were four replicate plots per

treatment. The plots were planted to peach on Nemaguard rootstock in winter 2006, and we measured tree heights on 5 October 2006.

Crop rotation trials. A microplot trial was established to broaden previous evaluations of short-term rotations with cover crops for cultural remediation of RD. Previously, we found that single-season rotations with Piper sudan grass suppress RD and approach the benefit provided by preplant fumigation with MB:CP (50:50, 400 lb/acre). Single rotations with corn or wheat were not as consistently effective as the sudan rotation (2005 Interim Report to the Almond Board). In the trial reported here we evaluated crop rotation with Caliente 119 blend mustard.

The trial occurred in 24-in.-diameter by 4-ft.-deep microplots filled with Hanford Sandy Loam that had been used to grow crops on Nemaguard rootstock continuously for many years. The treatments included: 1) no fallowing or crop rotation (i.e., almond on Nemaguard was not removed from plots until the fall before replanting) and no fumigation; 2) no fallowing or crop rotation, but plots were fumigated (MB:CP 50:50, 400 lb/acre, fall before replanting); 3) fallowing for one growing season and no fumigation; 4) fallowing for one growing season, followed by preplant fumigation (MB:CP 50:50, 400 lb/acre, fall before replanting); 5) a single rotation with Penewawa wheat; 6) a double rotation with Penewawa wheat; 7) a single rotation with Caliente 119 blend mustard, and 8) a single rotation with Piper Sudan grass. None of the crop rotations treatments were followed by preplant fumigation. Efficacy of the preplant treatments was evaluated by replanting the plots with Nemaguard peach seedlings and monitoring their growth in summer 2006.

Objective 2, determining causes of RD.

Examining microbial community shifts in the rhizosphere. We are examining shifts in bacterial and fungal populations that may contribute to RD. Previously, S. Schneider and N. Goodell conducted repeated assays of soil and roots from our RD trials for plant parasitic nematodes, and no significant numbers of these pests were found, with the exception of pin nematode in Parlier microplots. We demonstrated that severe RD occurred in absence of the pin nematode in the Parlier microplots and therefore discounted its role and that of other nematodes in the disease. As discussed previously, RD is a replant problem distinct from nematode parasitism.

For examination of the fungal and bacterial populations associated with RD in our previous microplot trials at Parlier and commercial orchard trials near Durham, we used samples of feeder roots obtained from the RD-affected and healthy trees in non-fumigated and fumigated plots of the field trials, respectively. Many of the roots were used for isolations of bacteria, fungi, and oomycetes in Petri dish cultures as documented previously (2005 Comprehensive Report to the Almond Board). Individual isolates of cultured bacteria and fungi were saved for traditional and rDNA-sequence-based identification and for pathogenicity tests.

In addition, because many soil microbes are not readily isolated in culture, samples of the roots and adhering rhizosphere soil from the diseased and healthy trees were used for "culture-independent" microbial examinations. These root samples were placed on dry ice immediately after collection in the field and stored at -80°C until DNA was extracted from them for culture-independent characterization of the microbial communities on and in them. Standard DNA extraction and purification procedures were modified as needed to permit polymerase chain reaction (PCR) amplification of diagnostic rDNA fragments from bacteria and fungi that were present in the samples. We used described bacterial and fungal primers that are known to

amplify rDNA universally from most bacterial and fungal organisms, respectively (Table 1). Because the fragments of rDNA were amplified from mixtures that contained DNA from many organisms, it was necessary to purify and separate the fragments from each other by "cloning" before they could be properly sequenced and used to identify source organisms. The cloned rDNA fragments, each potentially from a different soil microbe, were then "sequenced" (i.e., the sequence of nucleotide bases A, G, C, T composing them was determined) with an automated DNA sequencer. The rDNA fragment sequence is a genetic "fingerprint" of the organism from which it came. Using what is called "Basic Local Alignment Search Tool" (BLAST) searches among catalogued rDNA sequences online at the National Center for Biotechnology Information (NCBI), the fingerprints were used to obtain putative genus- to species-level identifications of microbes present in the root samples. As a more precise alternative approach to categorizing the microbes, we also are using statistical clustering techniques that group rDNA fragment sequences into rigorously defined operational taxonomic units (OTUs) that can be used to cross check and refine microbe identifications based on the BLAST searches. The relative abundance of the different microbes associated with RD-affected and healthy trees is being statistically examined for shifts associated with the disease.

Using responses to semi-selective soil treatments to examine RD etiology. In a microplot experiment at Parlier, we applied semi-selective chemicals and nutritional treatments to the soil to gain additional insight into causes of RD. The treatments included Cannonball (a.i. fludioxonil, a general fungicide; 5 lb formulation per acre), Folicur (a.i. tebuconazole, a general fungicide, 2 qts formulation per acre), Ridomil Gold (a.i. mefenoxam, an oomycete fungicide, 2 quarts formulation per acre), Lorsban (a.i. chlorpyriphos, an insecticide, 150 qts formulation per acre), and two levels of a commercial yeast extract (270 and 2700 lb of formulation per acre). Each of the treatments was applied by removing the surface foot of soil in the microplot, placing the soil in a cement mixer, and a spraying solution or suspension the appropriate chemical or amendment on the soil as it was mixed. The treated soil was returned to the microplot. Preplant fumigation with CP (400 lb per acre, injected 2 months before applying the other chemical treatments) and a non-treated control were included; the surface foot of soil in the CP-treated and control plots was subjected to excavation and mixing as were the other treatments, but water was sprayed on the soil in the mixer instead of a chemical or amendment. Each of the microplots was planted with three Nemaguard rootstock seedlings and irrigated by a drip emitter. Growth of the seedlings was used as an indication of effects of the preplant treatments on RD.

Results and Discussion

Objective 1, improved management strategies for RD.

Fumigation trials. In the Madera County fumigation trials, results differed at the two locations. At the field previously cropped to almond, row-strip or broadcast preplant fumigation with Telone C35, IM:CP, or CP improved trunk circumference growth through the first two growing seasons (by 16 to 30% in 2004, 12 to 19% in 2005) and resulted in proportional yield increases in 2006 (Table 2, Experiment 1-A). In contrast, the treatments with MB and Telone II did not improve growth and yield (Table 2 Experiment 1-A). There was no apparent advantage of broadcast treatments over the row strip treatments, and use of VIF did not improve tree performance. Compared to the effective row strip and broadcast treatments, the tree site treatments with MB, CP and Telone II were detrimental. The latter treatments apparently were applied too late in the fall and resulted in phytotoxicity in the newly planted trees in 2004. Most of the trees recovered (a few were replaced), however, and the spot-treated plots yielded as well

as the controls and row strip treatments with MB and Telone II (Table 2 Experiment 1-A,B). At the field previously cropped to grape, none of the fumigation treatments increased or decreased yields compared to the controls (Table 2, Experiment 2).

To date, the results of the Madera trials initiated in 2003 are consistent with previous results in our microplots. In the microplot studies, as in the trials, CP was more effective than MB for stimulating tree growth. Also, in previous microplot studies as in the trials, there was no evidence for RD in soil previously cropped to grape. Despite the agreement between the microplot and orchard studies to date, it will be important to monitor the orchard responses, as parasitic nematode populations may build over time. Furthermore, additional field trials in diverse soils posing different replant problems are needed to thoroughly test the treatments used in the Madera trials.

We will continue monitoring yields in the Madera trials so that comprehensive economic assessments of the treatments are possible. It appears that growth of the trees among all treatments is slowly but steadily equalizing, but it is uncertain what the long-term effects on yield will be. Parasitic nematode populations remained low in both of the experiments in 2005, but we will continue to monitor them.

In the orchard replant trial including spot and strip preplant fumigation treatments near Parlier in 2005, all of the treatments, except drip-applied Telone II EC significantly increased tree heights attained by October 2006 (Table 3). Based on the height measurements, the spot treatments with 1,3-D:CP were as effective as the shank and drip row strip treatments with 1,3-D:CP. Both of the Midas treatments and the spot probe treatment with 1,3-D:CP resulted in greater tree height than the Telone II EC treatment applied by drip. We will continue monitoring this orchard to evaluate long-term effects of the preplant treatments.

It appears that spot treatments, once optimized for efficient application, offer a promising approach for management of RD. Spot treatments have potential to reduce fumigant costs and emissions, compared to strip and broadcast treatments. We are working in collaboration with Shrini Upadhyaya, TriCal, and UCCE advisors to evaluate GPS-controlled spot treatments in commercial almond orchards. More trials are needed and planned to examine the long-term economics of spot treatments in commercial settings. One commercial trial using S. Upadhyaya's GPS spot treatment rig has been established with Brent Holtz this fall; replicate plots of 5'x5' and 8'x8' spot treatments were included with additional row strip and broadcast treatments.

Crop rotation trials. In the microplot trial examining preplant fallowing, fumigation, and crop rotation, fumigation with MB:CP resulted in the best growth of replanted peach seedlings, whether or not the fumigation was preceded by fallowing or continuous culture of almond on Nemaguard peach rootstock (Table 4). The only preplant crop rotation that significantly improved height growth of Nemaguard rootstock was the double rotation with wheat, although growth of Nemaguard after the other rotations (i.e., single crops of wheat or sudan or two successive plantings of mustard) was not significantly less than that after the double rotation with wheat. Fallowing alone for 1 year provided no apparent benefit.

It appears that some short-term crop rotations are worthy of testing in commercial orchards. In collaboration with Brad Hanson, USDA ARS, Parlier, we have initiated such testing in a peach orchard at the USDA-ARS San Joaquin Agricultural Sciences Center, Parlier, CA. We are looking for commercial almond growers who may wish to test preplant crop rotations in their orchards.

Objective 2, determining causes of RD.

Examining microbial community shifts in the rhizosphere. The ongoing culture-based and culture-independent examinations of microbial populations in root samples from RD-affected and healthy trees revealed some differences between populations from the healthy and diseased trees, but the differences were not striking, and additional work is needed before conclusions are justified. Below, we summarize the current status of our microbial examinations.

In culture-based bacterial isolations from Parlier, both in 2003 and 2004, incidence of isolation of *Rhizobium* and *Sinorhizobium* was greater from RD-affected Nemaguard seedlings than from healthy ones (Table 5). Some *Rhizobium* species are host-specific symbionts with legumes and fix nitrogen in root nodules that they induce. However, *Rhizobium* species are closely related to *Agrobacterium* species, and there are reports of *Rhizobium* inducing chlorosis in some plants. In the coming months we will confirm the identity of the isolates of *Rhizobium* and determine whether they negatively impact Nemaguard peach rootstock in greenhouse tests. In culture-based bacterial isolations from Durham samples, there was no apparent suggestion of shifts in bacterial communities associated with RD, at least at the genus level identifications based on BLAST searches (Table 6).

The culture-independent characterizations of bacterial communities associated with RD-affected and healthy plants at Durham and Chico revealed more diversity in the populations than was detected using the culture-based approach, but there were not major shifts in the culture-independent populations associated with the disease, neither from Parlier (Table 7) nor Durham samples (Table 8).

The culture-independent examination of fungi from roots of healthy and diseased Nemaguard peach seedlings indicated elevated incidence of a fungus that matched with *Coniothyrium palmarum* in the diseased plants (Table 9). Also, *Fusarium* sp. and several other genera, although low in incidence, were detected only in RD-symptomatic plants.

This report has summarized microbial identifications based on BLAST searches, but we also are assigning the microbes to operational taxonomic units (OTUs) which, compared to BLAST identifications, are more rigorously defined and reliable. Grouping into the OTUs involves computer-intensive alignments and cluster analysis of the rDNA sequences. We currently are catching up our culture-independent fungal community characterizations to those for the bacterial communities, but we need to increase the numbers of individuals identified, both for bacteria and fungi, to facilitate statistical analysis of the populations.

Using responses to semi-selective soil treatments to examine RD etiology. In the microplot trial evaluating effects of semi-selective and nutritional preplant treatments on severity of RD, the general fungicide fludioxonil and the oomycete fungicide mefenoxam improved growth of Nemaguard peach in the soil conducive to RD (Table 10). In contrast, the general fungicide tebuconazole was detrimental to growth of peach; the appearance of the plants, deep green but stunted, suggested that the dose of fungicide selected was excessive, resulting in phytotoxicity. Lorsban, the insecticide, had no apparent effect on growth, compared to the control. Soil amendment with yeast extract, at either rate, increased growth of the peach seedlings, compared to the control.

The most likely mechanism of growth stimulation by fludioxonil and mefenoxam is suppression of true fungi and oomycete fungi, respectively. In our 2005 Comprehensive Almond Board Report we presented results of culture-based assessments of microbial populations associated with RD in the Hanford Sandy Loam at Parlier. We reported that *Fusarium* spp., *Rhizoctonia* sp. (true fungi), and *Pythium* sp. (an oomycete) were isolated more frequently from roots of Nemaguard peach affected by RD than from roots of healthy Nemaguard. The responses

to the fungicides and the isolation results suggest these fungi and oomycetes have at least a partial role in RD. The lack of effect of the Lorsban treatments provided no evidence for involvement of root feeding insects or other arthropods in RD.

We chose to test the yeast extract treatments to explore nutritional aspects of RD. Some symptoms of RD in leaves resemble nutrient deficiencies, and although the symptoms may result from inadequate root function, we applied the yeast extract as a balanced source of nutrients that may alleviate RD. The yeast extract contains only negligible numbers of living yeast cells and is derived from autolysed and heated yeast cultures. At the low rate, our yeast treatment included about 30 pounds of nitrogen per acre. We consider it unlikely that the applied nitrogen, alone, stimulated plant growth, but it, as well as the other nutritional ingredients may have been a factor. It should be kept in mind that soil amendments can influence plants and microbial communities directly, by providing needed nutrients, and indirectly, through effects on the soil microbe populations. We will repeat variations of the soil treatment experiment to examine repeatability of the treatment effects and whether there are practical applications of them in management of replant problems.

Table 1. PCR primer pairs used in analysis of microbial communities associated with replant disease

Primer pair	Use	Reference
63F and 1401R	Identification of bacteria isolated in culture	Marchesi, J.R., T. Sato, A.J. Weightman, T.A. Martin, J.C. Fry, S.J. Hiom, and W.G. Wade. 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl. Envir. Microbiol.* 64:795-799.
341F (2) and 1401R (1)	Identification of bacteria detected by culture-independent amplification of rDNA	(1) Marchesi, J.R., T. Sato, A.J. Weightman, T.A. Martin, J.C. Fry, S.J. Hiom, and W.G. Wade. 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl. Envir. Microbiol.* 64:795-799. (2) Muyzer, G., S. Hottenträger, A. Teske, and C. Wawer. 1996. Denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA—a new molecular approach to analyse the genetic diversity of mixed microbial communities, p. 1-23. *In* A. D. L. Akkermans, J. D. van Elsas, and F. J. de Bruijn (ed.), Molecular microbial ecology, manual 3.4.4. Kluwer Academic Publishers, Dordrecht, The Netherlands.
Fungal small-subunit rDNA primer 463 and 464	Identification of fungi detected by culture-independent amplification of rDNA	Valinsky et al. 2002. Oligonucleotide fingerprinting of rRNA genes for analysis of fungal community composition. Appl Environ. Microbiol 68: 5999-6004

Table 2. Growth and yield responses of almond trees to preplant fumigation treatments on ground previously devoted to almond (Experiment 1-A,B) and grape (Experiment 1-B). Data from USDA CSREES team trial, Lampinen, Browne, Holtz, and Schneider

Experi-ment	Fumigant, rate	Plot area treated	Mulch system	Trunk circ. increase (% of control)		2006 Gross Nut Yield (kg/tree)
				2004	2005	
1-A[a]	Control	None	None	0	0	4.09 de
	Control	None	VIF	-6	-2	3.04 e
	MB, 400 lb/a	Br. (100%)	None	4	3	5.07 bcd
	MB, 400 lb/a	R. strip (38%)	None	-4	1	4.60 cde
	MB, 400 lb/a	R. strip (38%)	VIF	-2	-3	4.52 cde
	Telone II, 340 lb/a	Br. (100%)	None	11	9	5.68 abcd
	Telone II, 340 lb/a	R. strip (38%)	None	6	4	5.01 bcd
	Telone II, 340 lb/a	R. strip (38%)	VIF	0	0	5.01 bcd
	Telone C35, 535 lb/a	Br. (100%)	None	16	17	6.97 a
	Telone C35, 535 lb/a	R. strip (38%)	None	27	16	6.73 a
	IM:CP (50:50), 400 lb/a	Br. (100%)	None	29	18	7.19 a
	IM:CP (50:50), 400 lb/a	R. strip (38%)	None	19	19	6.37 ab
	CP 400 lb/a	Br. (100%)	None	17	12	5.92 abc
	CP 400 lb/a	R. strip (38%)	None	30	19	6.37 ab
	CP 400 lb/a	R. strip (38%)	VIF	28	17	7.05 a
1-B[b]	Control	None	None	0	0	4.09 de
	MB, 1 lb / tree site	Tree site	None	0	0	5.05 bcd
	CP, 1 lb / tree site	Tree site	None	-13	0	4.41 cde
	Telone II, 1 lb / tree site	Tree site	None	-11	-7	4.57 cde
2[c]	Control	None	None	0	0	5.96 abc
	Control	None	VIF	-3	-2	5.32 bcd
	MB, 400 lb/a	Br. (100%)	None	-5	2	6.72 ab
	MB, 400 lb/a	R. strip (38%)	None	-9	-5	5.65 abcd
	MB, 400 lb/a	R. strip (23%)	None	-9	-3	5.77 abc
	MB, 400 lb/a	R. strip (38%)	VIF	-10	-4	5.67 abcd
	Telone II, 340 lb/a	Br. (100%)	None	-5	-3	4.29 cd
	Telone II, 340 lb/a	R. strip (38%)	None	-4	-2	5.10 bcd
	Telone II, 340 lb/a	R. strip (38%)	VIF	-8	-4	4.02 d
	Telone C35, 535 lb/a	R. strip (38%)	None	-12	-5	5.57 bcd
	Telone C35, 535 lb/a	R. strip (38%)	VIF	-10	-4	5.17 bcd
	IM:CP (50:50), 400 lb/a	Br. (100%)	None	-4	-2	7.31 a
	IM:CP (50:50), 400 lb/a	R. strip (38%)	None	-7	-3	6.12 ab
	CP 400 lb/a	R. strip (38%)	None	-5	-3	5.33 bcd
	CP 400 lb/a	R. strip (23%)	None	-3	-1	5.49 bcd
	CP 400 lb/a	R. strip (38%)	VIF	-13	-7	5.96 abc

[a]Fumigants applied 27 October 2003
[b]Fumigants applied 10 November 2003
[c]Fumigants applied 11 November 2003

Table 3. Effects of pre-plant shank and drip applied strip and spot treatments, team trial led by Tom Trout[a]

Formulation	Fumigant	Application method	Rate	Tree height 10-5-06 (meters)
None	Control	None	0	1.07 c
MB	MB	Shank, 11-ft strip	350 lb/a	1.51 ab
Telone II	1,3-D	Shank, 12-ft strip	338 lb/a	1.50 ab
Telone II EC	1,3-D	Subdrip, 10-ft strip	360 lb/a	1.29 bc
Telone C35	1,3-D:CP (61:35)	Spot probe	0.8 lb/tree site	1.67 a
Inline	1,3-D:CP (61:35)	Spot drip	0.2 lb/tree site	1.54 ab
Telone C35	1,3-D:CP (61:35)	Shank, 12-ft strip	540 lb/a	1.59 ab
Inline	1,3-D:CP (61:35)	Subdrip, 10-ft strip	560 lb/a	1.52 ab
Midas	IM:CP (33:67)	Shank, 11-ft strip	300 lb/a	1.64 a
Midas EC	IM:CP (33:67)	Subdrip, 10-ft strip	300 lb/a	1.66 a

[a]Fumigation treatments applied October 27, 2005. Previous crop was Nectarine on Nemaguard peach rootstock removed August 2005. Planted to Sweet O'Henry Peach on Nemaguard rootstock

Table 4. Effects of pre-plant cropping history, microplots, Parlier[a]

Treatment name	Preplant Cropping sequence								Post plant growth; height of Nemaguard (cm)
	2004	2005				2006			
	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	
Continuous almond	Ald/NG	Ald/NG	Ald/NG	Ald/NG	Ald/NG	Fallow	Fallow	NG	32 c
Continuous almond + fumigation.	Ald/NG	Ald/NG	Ald/NG	Ald/NG	Ald/NG fb MB:CP	Fallow	Fallow	NG	55 a
Fallow	Fallow	Fallow	Fallow	Fallow	Fallow	Fallow	Fallow	NG	35 c
Fallow + fumigation	Fallow	Fallow	Fallow	Fallow	Fallow fb. MB:CP	Fallow	Fallow	NG	55 a
Wheat, 1x	Wheat	Wheat	Wheat	Fallow	Fallow	Fallow	Fallow	NG	39 bc
Wheat, 2x	Wheat	Wheat	Wheat	Fallow	Wheat	Wheat	Wheat	NG	45 b
Mustard, 2x	Fallow	Fallow	Mustard	Mustard	Fallow	Mustard	Mustard	NG	41 bc
Sudan, 1x	Fallow	Fallow	Fallow	Sudan	Sudan	Fallow	Fallow	NG	38 bc

[a]All plots were regularly hand weeded. Fallow plots were not irrigated during fallow. Fumigation was MB:CP (50:50) 400 lb/a. There were five replicate four-plant microplots per treatment. Post-plant growth of Nemaguard peach was measured from planting on 28 Jun 2006 to 5 Oct 2006. "fb" indicates followed by.

Table 5. Incidence of bacteria isolated from roots of RD-affected and healthy Nemaguard peach seedlings in non-fumigated and non-fumigated soils, respectively, of Parlier trials

	Year of sampling, preplant treatment, and incidence (%) among isolates (n=total)					
	2003			**2004**		
Bacterium	**Control (n=78)**	**MB (n=90)**	**CP (n=115)**	**Control (n=151)**	**MB (n=134)**	**CP (n=142)**
Arthrobacter	2.6	14.4	1.7	1.3	3.0	2.1
Aurebacterium	0.0	0.0	1.7	0.0	0.0	0.0
Citricoccus	0.0	0.0	1.7	0.0	0.0	0.0
Curtobacterium	0.0	0.0	0.9	0.0	0.0	0.0
Microbacterium	0.0	5.6	2.6	1.3	3.0	3.5
Micrococcus	0.0	0.0	0.9	0.0	0.0	0.0
Nocardioides	0.0	0.0	0.0	0.0	2.2	4.9
Promicronospora	0.0	0.0	0.0	0.7	2.2	0.0
Rarobacter	0.0	0.0	0.9	0.0	0.0	0.0
Rhodococcus	0.0	0.0	0.9	0.7	0.0	0.0
Streptomyces	0.0	0.0	0.0	1.3	0.0	0.0
Unk., Actinomycetales	0.0	0.0	0.0	0.0	2.2	0.0
Brevundimonas	0.0	0.0	0.0	1.3	0.0	0.0
Caulobacter	1.3	0.0	0.0	0.0	0.0	0.0
Devosia	0.0	0.0	0.0	0.0	0.0	2.1
Novosphingobium	0.0	0.0	0.9	0.0	0.7	0.0
Phyllobacterium	0.0	0.0	0.0	0.0	2.2	0.0
Rhizobium	28.2	4.4	11.3	34.4	23.1	7.7
Rhodobium	0.0	0.0	0.9	0.0	0.0	0.0
Sinorhizobium	7.7	0.0	0.0	12.6	1.5	1.4
Sphingomonas	1.3	0.0	0.0	0.0	0.7	0.0
Paenbacillus	0.0	0.0	0.9	0.7	1.5	2.8
Bacillus	10.3	8.9	7.0	7.9	6.7	5.6
Brevibacillus	0.0	0.0	0.0	0.0	0.0	1.4
Sporosarcina	0.0	0.0	0.0	0.0	0.7	0.0
Acidovorax	0.0	0.0	0.0	0.7	3.0	0.0
Bordetella	0.0	0.0	0.0	0.0	0.0	0.7
Hydrogenophaga	0.0	0.0	0.0	0.0	0.7	0.7
Janthinobacterium	0.0	0.0	1.7	0.0	0.0	0.0
Oxalobacteriaceae	1.3	0.0	0.0	0.0	0.0	0.0
Ralstonia	1.3	0.0	0.0	0.0	0.0	0.0
Variovorax	7.7	6.7	17.4	16.6	27.6	47.2
Flavobacteria	5.1	0.0	6.1	0.0	0.0	0.0
Porphyromonas	0.0	0.0	0.0	0.0	0.7	0.0
Acinetobacter	0.0	0.0	0.9	0.0	0.0	0.0
Enterobacter	0.0	1.1	2.6	0.0	0.0	0.0
Lysobacter	0.0	0.0	0.0	2.0	0.0	0.0
Microbulbifer	0.0	0.0	0.9	0.0	0.0	0.0
Neptunomonas	0.0	0.0	0.9	0.0	0.0	0.0
Oceanspiralles	0.0	0.0	2.6	0.0	0.0	0.0
Pantoea	0.0	0.0	1.7	0.0	0.0	0.0
Pseudomonas	30.8	58.9	32.2	18.5	16.4	19.0
Stenotrophomonas	0.0	0.0	0.9	0.0	0.7	0.7
Deinococcus	0.0	0.0	0.0	0.0	0.7	0.0
Unk., Spirochaete	2.6	0.0	0.0	0.0	0.0	0.0

Table 6. Incidence of bacteria cultured and isolated from roots of RD-affected and healthy almond trees on Marianna 2624 rootstock in non-fumigated and non-fumigated soils, respectively, in commercial trials near Durham, CA

Bacterium	Year of sampling, preplant treatment, and incidence (%) among isolates (n=total)			
	2003		2004	
	Control (n=114)	CP (n=94)	Control (n=140)	CP (n=135)
Agrococcus	0.0	0.0	1.4	0.0
Agromyces	0.0	0.0	0.7	0.0
Arthrobacter	0.9	0.0	1.4	2.2
Couchiplanes	0.0	0.0	0.7	0.0
Kocuria	0.0	0.0	0.0	0.7
Microbacterium	0.0	0.0	2.9	2.2
Micrococcus	0.0	0.0	0.7	0.0
Mycetocola	0.0	0.0	0.7	0.0
Rhodococcus	0.0	0.0	0.7	0.0
Streptomyces	0.0	0.0	2.1	2.2
Blastomonas	0.0	0.0	0.7	0.0
Brevundimonas	0.0	0.0	0.7	0.0
Caulobacter	0.0	0.0	5.7	0.7
Novosphingobium	1.8	1.1	0.7	1.5
Rhizobium	0.9	2.1	7.1	4.4
Paenbacillus	0.0	0.0	0.0	0.7
Bacillus	0.0	3.2	2.9	0.7
Burkholderia	0.0	0.0	3.6	0.0
Diaphorobacter	0.0	0.0	0.7	0.0
Duganella	0.0	0.0	2.1	0.0
Janthinobacterium	0.0	2.1	0.0	0.0
Massilia	0.0	0.0	0.7	0.0
Rhodoferax	0.9	0.0	0.0	0.0
Variovorax	0.9	0.0	22.9	28.9
Chryseobacterium	0.0	0.0	0.0	0.7
Flavobacteria	17.5	24.5	12.1	14.8
Acinetobacter	0.0	0.0	0.7	0.0
Lysobacter	0.0	0.0	0.0	0.7
Microbulbifer	0.0	0.0	0.0	0.7
Pseudomonas	77.2	62.8	27.1	34.8
Pseudoxanthomonas	0.0	1.1	0.0	0.0
Stenotrophomonas	0.0	3.2	0.0	0.7
Xanthomonas	0.0	0.0	0.0	0.7
Pedobacter	0.0	0.0	0.7	1.5
Desulfocella	0.0	0.0	0.0	0.7

Table 7. Incidence of rDNA of bacteria detected by culture-independent PCR from root samples collected from RD-affected and healthy trees of Nemaguard rootstock in non-fumigated and non-fumigated soils, respectively, in a 2003 microplot trial near Parlier, CA

Bacterium or classification	Year of sampling, preplant treatment, and incidence (%) among isolates (n=total)		
	Control (n=157)	MB (n=175)	CP (n=166)
Unclassified Bacteria	11.5	3.4	1.2
Unclassified Proteobacteria	22.3	12.0	15.1
Unclassified Alphaproteobacteria	3.8	0.0	0.0
Unclassified Caulobacteraceae	0.0	1.1	4.8
Brevundimonas	0.0	0.6	0.0
Phenylbacterium	0.0	1.7	0.6
Unclassified Rhizobiales	2.5	3.4	5.4
Unclassified Rhizobiaceae	0.0	1.7	1.8
Rhizobium	3.8	2.9	4.2
Sinorhizobium	1.3	0.0	0.6
Unclassified Bradyrhizobiaceae	2.5	0.0	1.2
Devosia	1.9	0.6	0.6
Unclassified Betaproteobacteria	0.0	1.7	4.8
Unclassified Burkholderiales	5.7	1.1	0.0
Burkholderia	0.0	0.6	0.0
Unclassified Comamonadaceae	0.6	3.4	2.4
Acidovorax	0.6	1.7	1.8
Variovorax	1.3	1.1	0.6
Delftia	0.0	0.6	0.0
Methylophilus	0.6	4.0	0.6
Unclassified Incertae Sedis 5	2.5	0.6	0.0
Deltaproteobacteria	0.0	0.0	2.4
Unclassified *Gammaproteobacteria*	4.5	9.1	6.0
Unclassified *Xanthomonadaceae*	0.0	2.3	1.8
Pseudomonas	0.0	6.9	1.2
Unclassified Oxalbacteraceae	0.6	1.1	1.2
Unclassified Actinomycetales	0.6	0.0	0.0
Unclassified Microbacteriaceae	0.0	2.9	5.4
Unclassified Micromonosporaceae	0.0	2.9	0.6
Streptomyces	10.2	1.1	8.4
Arthrobacter	0.6	0.6	0.0
Flavobacterium	1.3	2.9	1.2
Gemmatimonas	0.0	1.7	3.6
Unclassified Verrucomicrobiales	0.6	0.6	1.2
Unclassified TM7	4.5	1.1	4.2
Haliangium	0.0	1.1	2.4
Unclassified Chlamydiales	0.6	0.0	0.0
Unclassified Rhodobacteraceae	0.0	1.7	0.0
Chondromyces	1.3	0.0	0.0
Unclassified Spriochaetaceae	2.5	0.0	0.6
Roseiflexus	0.6	0.0	0.0
Opititus	1.3	1.1	0.0

Table 7. (continued)

Bacterium or classification	Year of sampling, preplant treatment, and incidence (%) among isolates (n=total)		
	Control (n=157)	MB (n=175)	CP (n=166)
Pedobacter	0.0	0.0	1.2
Bdellovibro	1.3	0.6	0.0
Unclassified Rhodospiralles	2.5	0.6	0.6
Unclassified Acetobacteraceae	0.0	1.1	0.0
Actinoplanes	0.0	3.4	1.8
Hydrogenphaga	1.3	2.9	4.2
Nocardioides	0.6	0.6	1.2
Bacillus	0.0	0.6	0.6
Unclassified Flexibacteraceae	0.0	0.6	3.0
Unclassified Chloroflexaceae	0.6	0.0	0.0
Nitrospira	2.5	0.0	0.0
Lentzea	0.0	0.0	0.6
Polarmonas	0.6	1.1	0.0
Aminobacter	0.0	0.0	0.6
Unclassified Rubrobacteraceae	0.0	0.6	0.0
Unclassified Rhodocylclaceae	0.0	5.1	0.0
Azoarcus	0.0	0.6	0.0
Ferribacterium	0.0	2.3	0.0
Unclassified Geobacteraceae	0.0	0.6	0.0

Table 8. Incidence of rDNA of bacteria detected by culture-independent PCR from root samples collected from RD-affected and healthy trees of almond on Marianna 2624 rootstock in non-fumigated and non-fumigated soils, respectively, in commercial trials near Durham, CA

Bacterium or classification	Year of sampling, preplant treatment, and incidence (%) among isolates (n=total)			
	2003		2004	
	Control (n=162)	CP (n=160)	Control (n=136)	CP (n=137)
Unclassified Bacteria	3.7	1.3	8.8	10.2
Unclassified Proteobacteria	1.2	1.9	8.1	6.6
Unclassified Alphaproteobacteria	1.2	0.0	5.9	0.0
UnclassifiedCaulobacteraceae	0.6	1.9	2.2	1.5
Brevundimonas	3.7	1.9	0.0	0.0
Phenylbacterium	0.0	1.3	0.0	0.0
Unclassified Rhizobiales	0.0	1.9	5.1	2.2
Unclassified Rhizobiaceae	25.9	29.4	0.7	0.7
Rhizobium	8.6	9.4	0.7	0.7
Sinorhizobium	0.6	0.6	0.7	0.0
Unclassified Bradyrhizobiaceae	0.6	0.6	0.7	3.6
Devosia	0.0	0.6	0.7	0.7
Unclassified Betaproteobacteria	3.1	0.0	0.7	0.7
Unclassified Burkholderiales	3.1	3.8	3.7	0.0
Burkholderia	0.6	0.6	2.2	0.0
Unclassified Comamonadaceae	1.9	2.5	2.9	1.5
Acidovorax	0.0	0.6	0.7	1.5
Variovorax	0.0	1.9	0.7	2.9

Table 8. (continued)

Bacterium or classification	Year of sampling, preplant treatment, and incidence (%) among isolates (n=total)			
	2003		2004	
	Control (n=162)	CP (n=160)	Control (n=136)	CP (n=137)
Delftia	4.3	1.9	0.0	0.0
Methylophilus	1.2	1.3	0.7	8.0
Unclassified Incertae Sedis 5	0.6	1.3	1.5	0.0
Deltaproteobacteria	0.0	0.6	0.0	0.0
Unclassified Gammaproteobacteria	2.5	4.4	7.4	14.6
Unclassified Xanthomonadaceae	0.6	0.0	0.7	0.7
Lysobacter	0.0	0.6	0.0	0.0
Serratia	0.0	0.6	0.0	0.0
Pseudomonas	0.6	1.3	2.9	3.6
Unclassified Oxalbacteraceae	3.7	0.0	0.7	0.0
Achromobacter	2.5	1.3	0.0	0.7
Unclassified Actinomycetales	0.0	0.6	0.0	0.0
Unclassified Microbacteriaceae	0.6	1.3	0.0	0.0
Unclassified Micromonosporaceae	0.6	0.6	0.7	3.6
Streptomyces	16.0	15.0	19.1	8.8
Arthrobacter	0.0	0.6	0.0	0.7
Anaerolinea	0.6	0.0	0.7	3.6
Flavobacterium	3.7	5.6	4.4	9.5
Gemmatimonas	1.9	0.6	0.0	0.0
Unclassified Verrucomicrobiales	0.6	0.6	1.5	1.5
Unclassified TM7	1.9	1.3	1.5	2.2
Unclassified Cystobacteraceae	0.6	0.0	0.7	0.0
Haliangium	0.6	0.0	1.5	0.7
Paenbacillus	0.6	0.0	0.0	0.0
Unclassified Chlamydiales	1.2	0.0	0.0	0.0
Unclassified Rhodobacteraceae	0.0	0.0	0.7	0.0
Chondromyces	0.0	0.0	0.7	0.0
Pseudonocardia	0.0	0.0	0.7	0.0
Unclassified Nocardioides	0.0	0.0	0.0	1.5
Unclassified Spriochaetaceae	0.0	0.0	1.5	0.0
Roseiflexus	0.0	0.0	1.5	0.0
Opititus	0.0	0.0	1.5	5.8
Nonomurea	0.0	0.0	0.7	0.0
Unclassified Coxiellaceae	0.0	0.0	0.7	0.0
Pedobacter	0.0	0.0	0.7	0.0
Bdellovibro	0.0	0.0	0.0	1.5
Unclassified Rhodospiralles	0.0	0.0	0.7	0.0
Unclassified Acetobacteraceae	0.0	0.0	0.7	0.0
Hyphomicrobium	*0.0*	*0.0*	*0.7*	*0.0*
Actinoplanes	*0.0*	*0.6*	*0.0*	*0.0*

Table 9. Incidence of rDNA of fungi detected by culture-independent PCR from root samples collected from RD-affected and healthy trees of almond on Marianna 2624 rootstock in non-fumigated and non-fumigated soils, respectively, in a 2004 commercial trial near Durham, CA

	Year of sampling, preplant treatment, and incidence (%) among isolates (n=total)	
Fungus	**Control (n=135)**	**CP (n=157)**
*Alternaria longissima**	3.0	1.9
*Anguillospora sp.**	0.7	0.0
*Calcarisporium arbuscula**	17.8	19.1
Capronia sp.	0.7	0.0
*Chaetomium globosum**	2.2	1.9
*Chlorophyllum_agaricoides**	0.7	0.0
Claviceps_purpurea	1.5	4.5
*Colletotrichum sp.**	0.7	0.0
Coniothyrium palmarum	18.5	3.2
Conocybe lacteal	0.7	0.0
Cryptococcus sp.	0.0	1.3
Phoma sp.	0.7	1.3
*Engyodontium album**	0.7	0.0
*Helicodendron sp.**	0.0	1.3
Exidia_uvapsassa	0.7	0.0
*Fusarium sp.**	5.2	2.5
Geomyces_panorum	0.7	0.0
Gibberella pulicaris	0.7	0.0
Glomus_intraradices	0.0	3.2
Halosarpheia_heteroguttula	0.7	0.0
Helminthosporium_solani	0.0	1.3
Herpotrichia sp.	0.7	0.0
Hymenocyphus ericae	1.5	0.0
Leptosphaeria sp.	0.0	14.0
Madurella mycetomas	0.7	10.8
Mariannaea_elegans	0.7	0.6
Meliniomyces_variabilis	0.0	0.6
Mrakia_frigida	0.7	0.0
Myrothecium sp.	0.0	0.6
Nais_inornata	5.9	0.0
*Nectria lgdunensis**	2.2	0.0
Paraphaeosphaeria_sp.	0.0	0.6
Plectosphaerella_cucumerina	0.7	0.0
Pleurotus_ostreatus	1.5	0.0
Psathyrella_candolleana	11.1	16.6
*Sebacina sp.**	0.7	0.0
Tetracladium marchalianum	0.7	0.0
Tolypocladium/Cordyceps	0.0	0.6
Tolypocladium_inflatum	0.0	0.6
Tremellodendron_sp	0.0	3.2
*Tricladium sp.**	2.2	0.0
Truncatella_angustata	0.0	1.3
Uncultured clone af504753	0.7	0.0
Uncultured_ascomycetes	8.8	1.3
uncultured_eukaryotes	2.9	0.0
uncultured_zygomycete	0.0	0.6

Table 10. Effects of semi-selective and nutritional soil treatments on severity of replant disease in microplots[a]

Treatment (and active ingredient)	Rate	Expected effect	Application method	Height of NG. peach rootstock 2 months after planting (cm)
Control	0	None	None	25 d
Chloropicrin	400 lb/a	General biocide	Injected 2 months preplant	35 a
Cannonball (fludioxonil)	5 lb/a	General fungicide	Sprayed into soil at planting	31 bc
Folicur (tebuconazole)	2 qts/a	General fungicide	Sprayed into soil at planting	15 e
Ridomil (mefenoxam)	2 qts/a	Oomycete fungicide	Sprayed into soil at planting	29 bc
Lorsban (chloropyriphos)	150 qts/a	Insecticide	Sprayed into soil at planting	27 cd
Commercial yeast extract	270 lb/a	Nutrient source	Sprayed into soil at planting	31 b
Commercial yeast extract	2700 lb/a	Nutrient source	Sprayed into soil at planting	29 bc

[a]The soil treatments were added by excavating the surface foot of soil in each microplot and mixing a solution or suspension of the chemical or nutrient into the soil with a cement mixer. In the same manner, water alone was mixed into the control soil and the soil that had been treated with chloropicrin 2 months earlier.

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Almond Replant Disease and Its Management with Alternative Pre-Plant Soil Fumigation Treatments and Rootstocks

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ABSTRACT

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Trials were conducted in orchards near Chico, CA and microplots near Parlier, CA to examine symptoms and control measures for a replant disease (RD) on almond (*Prunus dulcis*). In the orchard trials, areas with a recent history of severe RD were cleared, given soil fumigation treatments in the fall, and replanted with almond trees on various rootstocks the following winter. The replants in nonfumigated soil developed severe RD (stunting, wilting, chlorosis, defoliation) by the following summer, while those in most fumigated treatments remained healthy. Trees in nonfumigated soil developed smaller trunk diameters and fewer healthy roots ≤1 mm diameter, compared with the healthy trees. Almond developed RD on all rootstocks evaluated (Marianna 2624, Lovell, and Nemaguard), but the trees on Marianna 2624 were the most severely affected. Pre-plant tree-site (spot) fumigation treatments with methyl bromide (MB), chloropicrin (CP), 1,3-dichloropropene (1,3-D), 1,3-D + CP, iodomethane, and iodomethane + CP all prevented severe RD. Broadcast soil fumigation with CP also was effective, but broadcast MB and 1,3-D were ineffective. In microplots filled with RD-conducive soil, CP was more potent than MB for prevention of RD on Nemaguard peach. There was no association between nematodes and RD in orchard or microplot trials. The RD apparently was mediated by a biological agent(s) other than nematodes and can be prevented by appropriate fumigation with CP or other MB alternatives.

Additional keywords: *Prunus persica*, stone fruit replant disorder

Young trees of stone fruits (i.e., species of *Prunus*) often suffer from diverse replant problems that cause them to grow suboptimally or, in severe cases, die when planted after other crops. Some replant problems result in part or primarily from abiotic causes. For example, nutrient deficiencies and toxicities, improper soil pH, and soil compaction associated with previous crop production can impede development of replanted orchards (15). Most replant problems, however, have strong microbial components. For example, populations of plant-parasitic nematodes, fungi, and *Phytophthora* spp. can increase in orchards and singly or collectively cause disease on replanted stone fruits (15). Several species of endo- and ectoparasitic nematodes attack the roots of fruit and nut crops in California (27,28), and pre-plant soil fumigation treatments typically are directed at these pests. Even in the absence of known pests and pathogens, however, young trees of *Prunus* spp. tend to lag in growth and productivity when planted after a previous generation(s) of the same crop (15). We advocate using the terms "replant disorder" and "replant disease" in reference to replant problems resulting primarily from abiotic and biotic causes, respectively.

In the northern part of California's Central Valley (i.e., Butte County), we have repeatedly observed poor vegetative growth and high incidences of tree mortality (≥50%) in young almond (*Prunus dulcis*) orchards planted on land with a long-term history (i.e., more than 10 years) of almond production (J. H. Connell and G. T. Browne, *unpublished*). The replant failures were not apparently associated with known pests or pathogens or substandard horticultural practices. Although the disease is not always apparent where almond is planted after almond, we have not observed it where almond is planted on sites devoted to herbaceous crops for many years. Symptoms and circumstances of the disease bear similarity to a previously described peach replant problem in California (5,23–25), but we are aware of no formal characterization of replant disease (RD) on almond.

Previous research has illustrated the etiological complexity of RD in Rosaceous and other plants. In 1941, a "peach replant problem" not associated with plant-parasitic nematodes or other known root pathogens was reported in California (25); peach after peach was affected, but not peach after apple (24). Application of macro and micro nutrients failed to alleviate the problem. In soils infested with *Pratylenchus penetrans* and collected from apple, cherry, and pear orchards, RD on apple, cherry, and pear seedlings was partially controlled by pre-plant fumigation with dichloropropene-dichloropropane, but fumigating the soils with chloropicrin (CP) or autoclaving them before planting was more effective (16). The RD was attributed to parasitism by the nematode and other unknown biological agent(s). Union mild etch, a disorder of young almond trees on Marianna 2624 rootstock, has interfered with the development of young orchards in Northern California (30). Evidence has been presented for a role of toxigenic peach root residues in peach replant problems (7,22,25), but the reports have not found consistent support (10,14). In the state of Washington, apple replant disease was shown to result primarily from root infection by *Cylindrocarpon destructans*, *Phytophthora cactorum*, *Pythium* spp., and *Rhizoctonia solani* (18). For many plant species, depressed growth or yield has been associated with deleterious rhizosphere microorganisms that negatively affect plants without parasitizing them (1,26).

Improved management strategies are needed for RD on almond. Land area planted to the crop has roughly quadrupled in the last 30 years in California, and the risk of almond replant problems is expected to increase as the growing districts age. Pre-plant soil fumigation with methyl bromide (MB) has been used to prevent replant problems in deciduous tree plantings, but the fumigant is being phased out due to its ozone depleting potential. Alternative fumigants are available, but research is needed to test their efficacy and optimize their application for management of replant problems. All fumigants may face increased regulatory constraints in the future, and research is needed to develop cultural and biological approaches for managing replant problems. Little is known concerning tolerance of different almond rootstocks to RD.

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The objectives of the research reported here were to characterize effects of RD on almond tree growth and development and to develop effective control measures for the disease. A portion of this work was reported previously (3).

MATERIALS AND METHODS

Orchard trials. Replanting trials were conducted from 2000 to 2005 in three commercial orchards within 20 km of Chico, CA (orchards 1, 2, and 3; details in sections below). Depending on the orchard, the soils were Nord loam (taxonomic class: Coarse-loamy, mixed, superactive, thermic Cumulic Haploxerolls), pH 7.4; or Farwell loam (Fine-loamy, mixed, superactive, thermic Fluventic Haploxerepts), pH 7.2 to 7.4. The orchards had been in commercial almond production for at least 15 years before the experiments. In the year before the trials, the growers had cleared and replanted the orchards with almond trees. The new orchards suffered a high incidence of severe RD (i.e., failure of >50% of replanted trees in land areas covering >2 ha) in the first year after planting. The affected areas were cleared again, and replant trials involving different preplant fumigation treatments and almond rootstocks were established where disease incidence and severity had been greatest.

Microplot trials. Additional replant trials were conducted from 2000 to 2004 in microplots at the USDA-ARS San Joaquin Valley Agricultural Science Center, near Parlier, CA (details in sections below). The microplots were open-ended 0.5-m-diameter × 1.2-m-long sections of concrete pipe inserted lengthwise into holes in the ground and filled with soil. The soil, Hanford fine sandy loam (Coarse-loamy, mixed, superactive, nonacid, thermic Typic Xerorthents), pH 7.6 to 7.8, was collected from 0- to 0.2-m depth in an adjacent peach orchard. Replant trials involving Nemaguard peach seedlings (a common rootstock for almond) and different preplant fumigation treatments were established in a new set of the microplots each trial year.

Pre-plant fumigation. In the orchard trials, depending on the year and experiment, pre-plant fumigation occurred from 21 October to 1 November. Within an experiment, all fumigation treatments were applied on the same day. The soil at 10 to 60 cm depth was 14 to 20°C and had moisture contents from 0.14 to 0.31 kg per kg of oven-dry soil.

Broadcast soil fumigation treatments were applied in one orchard trial (experiment 1, described below). The soil had been prepared by deep cultivation followed by harrowing to smooth the soil surface. Fumigants were injected into the soil at a depth of 40 to 50 cm through tractor-pulled shanks spaced 50 cm apart. A roller attached to the back of the fumigation rig compressed the soil surface immediately after the fumigants were injected to prevent premature escape of the gas.

Planting-site spot fumigation treatments were applied in several orchard trials (experiments 2 to 5 and 9 to 11 described below). Planting sites were prepared for fumigation with a tractor-powered auger, which removed the soil from 50- to 60-cm-deep × 60-cm-diameter holes. The loose soil was pushed back into and mounded above the holes. Fumigation treatments were injected through a 1-cm-diameter hollow metal probe that was inserted to a depth of 45 to 50 cm in the center of the soil-filled holes. At its upper end, the probe was connected to a pressurized supply of fumigant. After fumigation, the soil surface was compressed over the injection hole to prevent premature escape of the fumigant.

In microplot trials (experiments 6 to 8, described below), pre-plant soil fumigation treatments were applied, depending on experiment, on 30 April 2002, 20 November 2002, or 19 November 2003. The soil at 10 to 45 cm depth was 11 to 30°C and had moisture contents from 0.07 to 0.13 kg per kg of oven-dry soil. Before fumigation, the soil was cultivated with a hand shovel to a depth of 0.4 m and tamped moderately at the surface. Fumigants were injected into the soil at a depth of 30 cm near the center of each microplot through an 8-mm-diameter hollow metal probe. The probe was connected by flexible tubing to a frame-mounted gas-tight syringe (Hamilton Company, Reno, NV) that was used in a valve-controlled closed supply system to deliver fumigation treatments. After fumigation, the soil surface was compressed over the injection hole, and virtually impermeable film (VIF) mulch (Bromostop, Bruno Riminni, Ltd., London) was used to seal the top openings of the microplots. Control microplots were cultivated, tamped, and sealed with VIF, but they received no fumigant.

Planting and cultural practices. The orchard trials involved planting conventionally grown, dormant, bare-root almond trees into plots that had received a pre-plant fumigation or control treatment (details below). Depending on the year and experiment, trees were planted from the last week of January to the first week of March, 3 to 4 months after fumigation. Immediately after planting, the tree stems were trimmed off at 0.6 m above the soil surface, lateral shoots were trimmed to stubs that retained one to two buds, and a plastic-impregnated white paper tube (10 cm diameter, 0.4 m high) was slipped over each tree stem for protection from sun and herbicides. The trees were irrigated by high-impact sprinklers; up to one irrigation per week was applied to meet crop evapotranspiration needs.

The microplot trials involved planting 2- to 3-month-old Nemaguard peach seedlings into soil that had received pre-plant fumigation or control treatments (details below). The peach seeds were stratified for 2 months (8), planted and grown in a greenhouse for 2 to 3 months in trays of 2 × 2 × 4 cm cells filled with UC potting mix (17), trimmed to a main stem height of 10 cm, and transplanted into the microplots. Depending on the experiment, transplanting occurred on 3 June 2002, 9 April 2003, or 14 April 2004. The microplots were irrigated daily with 0.4 to 2.0 liters of water per microplot through a drip system and, starting 1 month after planting, fertilized monthly with $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ (28 to 56 kg N/ha per fertilization). Irrigation amounts were increased and decreased according to soil moisture level, which was kept near field capacity. Weeds were controlled by regular hand pulling.

Effects of alternative fumigation treatments (experiments 1 to 8). Effects of pre-plant soil fumigation treatments on incidence and severity of RD were examined in five orchard trials (experiments 1 to 5, in orchards 1, 2, and 3 introduced above) and three microplot trials (experiments 6 to 8, in the microplots near Parlier). Experiment 1 compared broadcast applications of MB (98:2 formulation, included 2% CP, TriCal, Inc., Hollister, CA), CP (Tri-clor, TriCal, Inc.), and 1,3 dichloropropene (1,3-D) (Telone II, Dow Agrosciences, Indianapolis, IN), all at 400 kg/ha, and a nonfumigated control in orchard 1. Each treatment was applied to four replicate 19 × 22 m plots in randomized complete blocks. Each plot was planted with three rows of six almond trees on Marianna 2624 rootstock; trees were 6.4 and 3.6 m apart between and within rows, respectively.

Experiments 2 to 5 evaluated pre-plant spot fumigation treatments applied to sites where planting holes were to be dug (i.e., planting sites) in orchards 1, 2, and 3. Depending on the orchard, planting sites were 1.8 to 2.9 m apart within rows and 6.4 m apart between rows. Depending on the experiment, treatments included MB at 0.5 kg per planting site and two or more rates of iodomethane (IM), IM:CP (50:50, wt/wt, both referred to as formulations of Midas, Arysta LifeScience North America Corporation, Cary, NC), CP, 1,3-D, and 1,3-D:CP (61:35, Telone C35, DowAgrosciences, Indianapolis, IN), and a nonfumigated control. Each planting site received one Carmel almond tree on Marianna 2624 rootstock. In experiments 2 to 4, there were 12 or 18 trees per treatment arranged in six randomized complete blocks. In experiment 5, there were five trees per treatment in a completely randomized design.

Experiments 6 to 8 examined effects of CP and MB (each at 425 and 3,040 kg/ha) and a nonfumigated control in the microplots. The treatments were arranged in 12 randomized complete blocks; each block had one replicate microplot per

treatment. Three Nemaguard peach seedlings were planted per microplot.

Effects of different rootstocks (experiments 9 to 11). Experiments 9, 10, and 11 were conducted in orchards 1, 2, and 3 near Chico, respectively. In experiment 9, Carmel almond trees on rootstocks of Marianna 2624 and Lovell peach were planted in sites that had been spot fumigated with MB:CP (75:25, wt/wt, 0.5 kg per site) or CP (0.5 kg per site) or left nonfumigated; there were 18 trees per factorial treatment combination, allocated evenly among six randomized complete blocks. Experiments 10 and 11 were similar to experiment 9, except that Nemaguard peach rootstock also was included, and there were 12 trees per treatment allocated evenly among six randomized complete blocks.

Disease assessment. In the orchard trials, effects of treatments on RD were assessed by measuring increases in trunk diameter and assigning disease ratings. The trunk diameters were measured at planting and in late summer or after completion of the growing season, during tree dormancy. Disease ratings were assigned in late August to mid-October using the following scale: 0 = tree healthy aboveground (length of shoot growth normal for healthy replanted trees in region, no wilting, leaf discoloration, or defoliation); 1 = trees slightly stunted (i.e., shoots 20 to 30% shorter than normal), but otherwise appear healthy; 2 = trees moderately stunted (i.e., shoots 40 to 50% shorter), exhibiting little or no wilting, leaf discoloration, or defoliation; 3 = trees severely stunted (i.e., shoots ≥60% shorter) and/or exhibiting moderate wilting, defoliation, or leaf discoloration; 4 = trees dying (i.e., regardless of size, tree severely wilted and defoliated and starting to dehydrate); 5 = tree dead, i.e., all leaves that remain are necrotic, shoot epidermis wrinkled from dehydration. Near the end of the growing season, trees with shoots that reached a height of at least 1.2 m above the soil surface and had disease ratings of 0 to 2 were considered commercially acceptable; those that were shorter or had higher disease ratings were considered unacceptable.

To examine effects of RD on root length density, Carmel almond trees on Marianna 2624 and Lovell rootstocks were planted in February 2004 in CP-fumigated (0.5 kg per planting site, applied in fall 2003) and nonfumigated control sites in orchards 2 and 3, adjacent to areas used for experiments 3, 4, 10, and 11. The fumigation treatments were randomized in blocks containing one (orchard 3) or two (orchard 2) tree planting sites per fumigation treatment. The trees on Marianna 2624 and Lovell rootstocks were considered to be in separate experiments because their treatment blocks were grouped separately. On 20 October 2004, root systems were sampled from three randomly selected trees on each rootstock in each of the orchards. Each sample included the roots within a 60-cm-diameter × 45-cm-deep cylinder of soil centered around one almond tree's trunk. The roots and adhering soil were excavated with shovels, collected in plastic bags, and stored at 4°C. The roots were gently washed free from the soil while being supported on a 2-mm mesh screen and blotted to remove free water. Roots that washed through the screen were collected and included in length analyses. An Epson 1640 XL scanner optimized for root system analyses by Regent Instruments, Inc. (Ste-Foy, QC, Canada) and WinRHIZO v.2004b software were used to determine the root length density in each sample. The "Regents simple scanner interface" was used with 800 dpi grayscale images specified, and the roots were spread on the scanner glass so that there was seldom overlap among them. Exclusion regions were defined to eliminate contributions of debris.

For experiments in microplots, effects on RD were assessed by weighing plant tops (i.e., stems and shoots) twice during the growing season. On each date, the tops from four randomly selected blocks were weighed. Effects of RD on root length densities of Nemaguard peach plants were determined in the 2004 microplots (experiment 8). The first week of November 2004, 13-cm-diameter × 30-cm-deep cores of soil and the enclosed roots were collected from four randomly selected blocks. Each soil-root core was centered around the stem of one peach plant, and two cores were collected per microplot. The root samples were processed and analyzed as described above.

Examining plant-parasitic nematode populations. Samples of soil and roots were collected periodically from trees in the orchard and microplot trials and assayed for plant-parasitic nematodes. In orchard 1, the samples were collected on 11 November 2002 from the trees in three randomly selected blocks of experiment 9; a 500-cm^3 sample of soil and roots was collected from depths of 5 to 45 cm below the soil surface within 30 cm of each tree's trunk and stored at 5°C for nematode extraction. A sieving/sugar flotation/centrifugation protocol with a 500-mesh sieve (25 μm opening) was used to extract nematodes from the samples (12). The extracted nematodes were identified and counted under a microscope. In orchards 2 and 3, samples were collected in October 2003 by excavating almond trees on Marianna 2624 rootstock; four trees were sampled per treatment in randomized complete blocks of nonfumigated and CP-fumigated (0.5 kg per planting site) plots. The plots had been established solely for sampling purposes and were adjacent to areas used for experiments 3, 4, 10, and 11. At least 20 g of fine roots (diameter ≤3 mm) and 500 cm^3 of adjacent soil (5 to 45 cm soil depth, ≤30 cm from the tree trunk) were collected from each of the trees and stored at 5°C. The soil was assayed for plant-parasitic nematodes as described above, and the roots were assayed using the mist chamber protocol (11). In orchard 3, the sampling and assay procedures used in 2003 were repeated in 2004 using an additional four blocks of single-tree CP-fumigated and control plots. The plots had been planted with almond trees on Marianna 2624 rootstock in March 2004 after pre-plant fumigation in November 2003.

Each microplot trial was sampled for nematodes on one or two occasions (i.e., 13 August and 25 September for the 2002 trial, 14 August for 2003, and 24 October for 2004). On each occasion, the microplots in four randomly selected replicate blocks were sampled. A soil sampling tube (2 × 45 cm) was used to collect multiple cores of soil and roots, totaling 500 cm^3 per microplot, from 0 to 45 cm soil depth within 20 cm of the experimental peach plants. Nematodes were extracted from the soil by flotation and counted as described above. In addition, 20 g of roots were collected from each microplot sampled on 24 October 2004 and processed by the mist chamber protocol.

Data analyses. All plant growth and health data were subjected to analysis of variance (ANOVA) using PROC MIXED of SAS software (SAS, Release 9.1, Cary, NC). Data from subsample trees (i.e., those given the same treatment within a microplot or block) were averaged and disease ratings were transformed to square root values before ANOVA. Block was specified as a random effect in experiments with randomized complete block designs. Confidence intervals (95%) were generated to facilitate mean separation. Although nontransformed disease rating means are presented, the associated mean separations are based on 95% confidence intervals determined from the square-root-transformed data. For all tree performance data, the Levine's test option of PROC ANOVA (SAS, Release 9.1) was used to test for homogeneity of variance. To accommodate variance heterogeneity, the "vargrp" option of PROC MIXED was used to calculate variances and 95% confidence intervals separately among treatment groups with dissimilar variance.

RESULTS

Foliar symptoms of RD and effects of alternative fumigation treatments (experiments 1 to 8). The onset and progress of aboveground symptoms of RD were qualitatively similar in each orchard trial with almond trees on Marianna 2624 rootstock (experiments 1 to 5). Until April, regardless of experiment, the trees in nonfumigated plots generally grew well, appeared healthy, and were not clearly distinguishable from those in fumigated plots. However, in May or June, the trees in con-

trol plots suffered a marked decline in the rate of shoot elongation, and many of them exhibited chlorosis, wilting, and defoliation. The trees in plots treated with effective fumigants remained healthy and continued rapid shoot growth.

Some of the pre-plant fumigation treatments had significant positive impacts on tree trunk diameters, disease ratings, and tree height in each of the orchard fumigation trials with almond on Marianna 2624 rootstock (experiments 1 to 5; Tables 1 and 2; Fig. 1) ($P < 0.0001$ to 0.0012). In experiment 1, which involved broadcast shank applications of MB, CP, and 1,3-D at 400 kg/ha in orchard 1, only the CP treatment was effective (Table 1). In the first growing season after planting, trees in the control plots increased little in trunk diameter (mean increase 1 mm) or tree height (mean tree height 1.0 m). The control trees developed high disease ratings (mean 3.4), and only 3% of them were commercially acceptable. The MB treatment slightly decreased the disease ratings and slightly increased trunk growth (Table 1), but only 42% of the trees were commercially acceptable. The CP treatment increased trunk diameter growth and decreased disease ratings by a factor of approximately 10 compared with the control, and 96% of the trees in CP plots were commercially acceptable. The 1,3-D treatment did not significantly improve tree growth or health (Table 1), and only 8% of the trees were acceptable.

In experiment 2, which involved spot fumigation of tree planting sites in orchard 1, greatest tree growth and lowest disease ratings occurred following CP at 0.2 to 0.5 kg per planting site, but CP at 0.9 kg per planting site caused phytotoxicity (Table 2). Pre-plant spot fumigation with MB and 1,3-D at 0.5 and 0.8 kg per tree site, respectively, resulted in trunk diameter

Table 1. Effects of broadcast pre-plant soil fumigation treatments applied through tractor-mounted shanks on growth of almond trees on Marianna 2624 rootstock in experiment 1, orchard 1, near Chico, CA

Pre-plant treatment[y]		Tree performance in first growing season[z]		
Fumigant	Rate of application (kg/ha)	Tree height (m)	Increase in trunk diameter (mm)	Disease rating
Control	None	1.0 a	1 a	3.4 a
MB	400	1.2 a	4 b	2.1 b
CP	400	1.7 b	10 c	0.3 c
1,3-D	400	1.1 a	2 a	2.9 a

[y] All fumigants, methyl bromide (MB), chloropicrin (CP), and 1,3-dichloropropene (1,3-D), were injected into soil by tractor-mounted shanks with nozzles spaced 50 cm apart at a soil depth of 40 to 50 cm. MB included 2% CP.

[z] Trees planted 22 January 2001. Tree height and disease ratings determined 13 August 2001. Increase in trunk diameter measured from time of planting to 13 August 2001. Disease rating based on a scale of 0 = healthy tree, 5 = dead tree, and 1, 2, 3, and 4 were progressive increments of disease within the extremes. Means within a column and without letters in common are significantly different according to 95% confidence intervals.

Table 2. Effects of pre-plant soil fumigation treatments applied to planting sites through a hand-held probe on growth of almond trees on Marianna 2624 rootstock near Chico, CA

Experiment no.	Pre-plant treatment[y]		Tree growth and health parameters at end of indicated growing season[z]						
			Height (m)	Increase in trunk diameter (mm)			Disease rating		
	Fumigant	kg/site	First	First	Second	Third	First	Second	Third
2	Control	0.0	1.4 a	8 a	--	--	2.0 a	--	--
	MB	0.5	1.8 ab	12 ab	--	--	1.0 ab	--	--
	1,3-D	0.8	1.8 ab	12 ab	--	--	1.0 ab	--	--
	CP	0.2	2.0 b	17 b	--	--	0.4 b	--	--
	CP	0.5	2.0 b	17 b	--	--	0.4 b	--	--
	CP	0.9	1.6 ab	12 ab	--	--	1.7 ab	--	--
3	Control	0.0	1.0 a	6 a	16 a	31 a	3.3 a	2.1 a	2.0 a
	MB	0.5	1.7 bc	18 bc	47 b	63 ab	1.0 b	0.0 b	0.4 b
	CP	0.2	2.0 c	25 d	54 b	78 c	0.3 b	0.0 b	0.0 b
	CP	0.5	1.9 bc	23 bcd	56 b	80 c	0.4 b	0.0 b	0.0 b
	IM:CP	0.2	1.9 bc	22 bcd	55 b	77 c	0.3 b	0.1 b	0.0 b
	IM:CP	0.5	1.9 bc	21 bcd	47 b	75 bc	0.7 b	0.0 b	0.3 b
	1,3-D	0.2	1.6 b	17 b	45 b	70 bc	1.2 b	0.0 b	0.0 b
	1,3-D	0.5	1.7 bc	20 bcd	50 b	74 bc	0.7 b	0.0 b	0.0 b
	1,3-D:CP	0.2	1.7 bc	20 bcd	51 b	71 bc	0.9 b	0.0 b	0.0 b
	1,3-D:CP	0.5	1.9 bc	24 cd	53 b	76 c	0.3 b	0.0 b	0.0 b
4	Control	0.0	1.2 a	3 a	19 a	40 a	3.5 a	1.0 a	0.0 a
	MB	0.5	1.6 b	11 b	34 bc	60 bc	0.8 bc	0.0 b	0.0 a
	CP	0.2	2.0 d	17 e	39 bc	63 bc	0.1 d	0.0 b	0.0 a
	CP	0.5	2.0 d	17 e	36 bc	66 c	0.3 cd	0.5 b	0.0 a
	IM	0.2	2.0 d	12 bc	36 bc	63 bc	0.1 d	0.1 b	0.0 a
	IM	0.5	2.0 d	14 bcde	36 bc	62 bc	0.2 cd	0.0 b	0.0 a
	IM:CP	0.2	1.8 bcd	16 cde	40 c	65 c	0.8 bcd	0.0 b	0.0 a
	IM:CP	0.5	1.9 bcd	16 de	39 bc	65 c	0.4 bcd	0.0 b	0.0 a
	1,3-D	0.2	1.7 bc	13 bcd	32 b	55 b	0.8 b	0.4 b	0.0 a
	1,3-D	0.5	2.0 d	15 cde	37 bc	62 bc	0.3 bcd	0.0 b	0.0 a
	1,3-D:CP	0.2	1.9 cd	14 bcde	36 bc	60 bc	0.3 bcd	0.3 b	0.0 a
	1,3-D:CP	0.5	1.9 cd	15 cde	38 bc	64 c	0.3 bcd	0.0 b	0.0 a

[y] All fumigants, methyl bromide (MB), chloropicrin (CP), iodomethane (IM), IM:CP (50:50 wt/wt), 1,3-dichloropropene (1,3-D), and 1,3-D:CP (61:35 wt/wt, Telone C35), were injected by a hand-held probe at one point at a soil depth of 45 to 50 cm in the center of sites where trees were to be planted.

[z] Tree height was measured at the end of the growing season (mid-October to early December, depending on experiment). Increases in trunk diameter were determined by measuring tree trunks near the end of the indicated growing seasons (late October to early February, depending on experiment) and calculating the net increase in diameter from time of planting. Disease ratings were made near the end of the indicated growing seasons (late August to mid-October) and based on a scale of 0 = healthy tree, 5 = dead tree, and 1, 2, 3, and 4 were progressive increments of disease within the extremes. Means within a column and experiment and without letters in common are significantly different according to 95% confidence intervals.

growth and disease ratings intermediate between the effective CP treatments and the control. In the control and phytotoxic CP treatments, 70 and 65% of the trees were acceptable, respectively, whereas 85 to 93% of the trees were acceptable in the other fumigated plots.

In experiments 3 and 4, which involved spot fumigation of planting sites in orchards 2 and 3, respectively, the control trees in nonfumigated soil grew little and developed high disease ratings in the first growing season (Table 2). The control trees showed only partial recovery in the second and third growing seasons after planting. Pre-plant fumigation with MB at 0.5 kg per planting site significantly increased tree trunk diameters and tree heights and lowered disease ratings in the first two growing seasons compared with the control. All the other pre-plant fumigation treatments (i.e., CP, IM, IM:CP, 1,3-D, 1,3-D:CP; 0.2 to 0.5 kg per tree site) improved tree performance to similar or greater extents through the third year after planting compared with the MB treatment.

In experiment 5, all rates of CP (0.12, 0.24, 0.45, and 0.9 kg per planting site) resulted in large and equivalent increases in tree trunk growth compared with the control (Fig. 1A). The treatment benefits persisted and were manifested in 10-fold increases in marketable nut kernel yields in the first harvest, which occurred in the fall of the third growing season (Fig. 1A and B).

Symptoms of RD developed on Nemaguard peach rootstock in the nonfumigated microplots within 2 months after planting (experiments 6 to 8). The control seedlings developed relatively small, chlorotic leaves with tip necrosis and boat-shaped deformation. Some of the control plants suffered partial defoliation, but few died. Effects of the pre-plant fumigation treatments were highly significant in each microplot experiment ($P < 0.0001$; Fig. 2A to C). The low rate of MB (425 kg/ha) had no significant effect on the plant top weights, whereas the same rate of CP increased shoot weights by 2.5 to 15.5× compared with the control, depending on the experiment and date of measurement. Both fumigants were effective at the high rate (3,040 kg/ha) and increased shoot weights by 3.5 to 21× compared with the control (Fig. 2). The favorable growth responses to fumigation became evident by early summer and persisted through the growing season.

Effects of alternative rootstocks. In experiment 9, first-season tree height, trunk diameter growth, and disease ratings were affected significantly by rootstock ($P = 0.0003$ to 0.007) and fumigation treatments ($P < 0.0001$), but there was no significant interaction between the factors ($P = 0.18$ to 0.31) (Table 3). In nonfumigated plots, almond trees on Lovell rootstock grew taller than those on Marianna 2624 rootstock (Table 3). Nevertheless, without pre-plant fumigation, tree performance was suboptimal on either rootstock. Pre-plant fumigation with either MB:CP or CP (0.5 kg per planting site) resulted in large increases in trunk diameters and tree heights for both rootstocks.

Results of experiments 10 and 11 were combined due to lack of significant experiment × rootstock and experiment × rootstock × fumigation interaction ($P = 0.13$ to 0.92) for all variables except disease ratings in the third growing season (Table 3; experiments 10 and 11). The ANOVA could not be completed with the latter variable due to a large number of zero disease ratings. Rootstock and fumigation had significant main effects for all variables in the first and second growing seasons ($P < 0.0001$ to 0.03). Interaction of rootstock × fumigation was significant only for trunk diameter increases and disease ratings in the second growing season ($P < 0.0001$ to 0.0003). In nonfumigated soil, almond trees on Lovell or Nemaguard peach rootstocks grew taller than those on Marianna 2624 rootstock for the first season (Table 3). Similarly, in the first and second growing seasons, trees on the peach rootstocks generally produced greater trunk diameter increases than those on Marianna 2624 roots. Nevertheless, without pre-plant fumigation, trees grew suboptimally regardless of rootstock. Pre-plant fumigation with either MB:CP or CP (0.5 kg per planting site) resulted in large, significant increases in tree trunk diameters and heights for all rootstocks. The benefit to trunk diameters from pre-plant soil fumigation persisted through the duration of experiments.

Effects of pre-plant fumigation on root length density. Pre-plant soil fumigation with CP increased length density of fine roots (≤1 mm diameter) on almond trees in orchards 2 and 3 (Fig. 3). For the trees on Lovell rootstock, results from the two orchards were combined due to lack of significant interaction of orchard location with other factors ($P = 0.10$, Fig. 3A). On Lovell, the length densities were affected by significant interaction between pre-plant fumigation treatment and root diameter class ($P < 0.0001$). The greatest length density occurred in roots ≤0.5 mm, and pre-plant fumigation with CP more than doubled the length density in this category. For the trees on Marianna 2624 rootstock, there was a significant three-way interaction among fumigation treatment, orchard location, and root diameter class ($P < 0.0001$); therefore the results are presented by orchard (Fig. 3B and C). In orchard 2, most root length occurred in roots ≤0.5

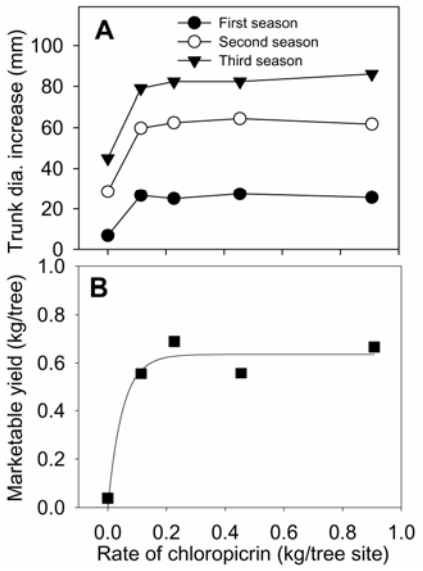

Fig. 1. Effect of different pre-plant doses of chloropicrin, injected at planting sites at soil depth of 45 to 50 cm, on growth of almond trees on Marianna 2624 rootstock in experiment 5. **A,** increases in trunk diameter by the end of indicated growing seasons after planting; and **B,** marketable nut (kernel) yield from the first harvest at the end of the third growing season. For each line, mean growth increases and yields were significantly greater in fumigated plots than in nonfumigated plots (based on 95% confidence intervals). Nut yield means were fit to the line described by $y = 0.63(1 - e^{-19.67})$ with an r^2 value of 0.95.

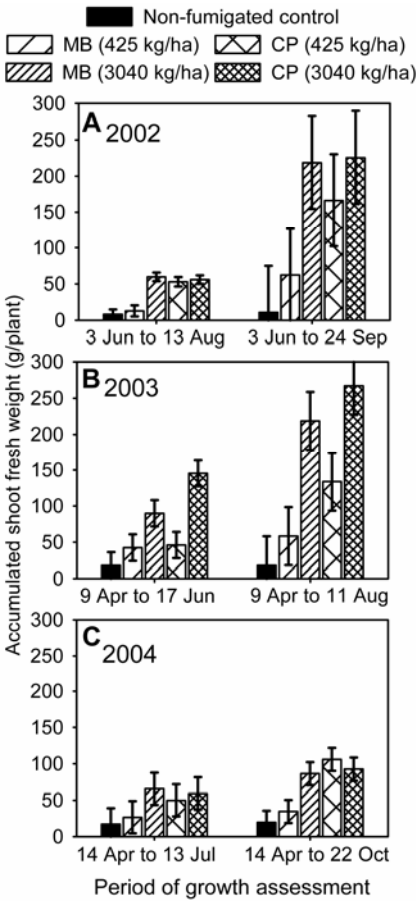

Fig. 2. Effect of pre-plant doses of methyl bromide (MB) and chloropicrin (CP), injected at soil depth of 30 cm, on growth of Nemaguard peach seedlings in microplots near Parlier, CA. Vertical bars are 95% confidence intervals.

mm diameter, and trees in CP plots had an average of approximately seven times more total root length compared with the control (Fig. 3B). In orchard 3, the results were similar, except CP only significantly increased the length density of roots ≤0.5 mm diameter, and the trees in fumigated soil had approximately twice the root length density of trees in nonfumigated soil (Fig. 3C).

In Nemaguard peach root samples from microplots, both of the CP treatments (425 and 3,040 kg/ha) significantly increased root length densities in the 0 to 0.5 mm diameter class compared with the other treatments. Samples from both the MB treatments had root length densities equal to or smaller than the control (Fig. 4).

Lack of significant populations of plant-parasitic nematodes. In orchard 1, experiment 9, the sugar flotation method extracted 0 to 1 lesion nematode (*Pratylenchus* sp.) per 250 cm^3 of soil, regardless of pre-plant fumigation treatment. No other plant-parasitic nematodes were detected. Similarly, no plant-parasitic nematodes were detected by sugar flotation or mist chamber extraction from soil and root samples collected from orchard 2 in 2003 and 2004 and orchard 3 in 2003.

In the 2002 microplots, numerically significant populations of the pin nematode (*Paratylenchus* sp.) were detected in samples from the nonfumigated treatment, but there was no clear association between the populations and incidence or severity of RD. An average of 424 and 122 pin nematodes per 250 cm^3 were extracted by sugar flotation from nonfumigated plot samples collected on 14 August and 24 September, respectively. Fewer than 8 pin nematodes per 250 cm^3 were detected in the other treatments in 2002, and none were in plots fumigated with MB at 425 kg/ha. The lesion nematode (*Pratylenchus* sp.) was detected in only one control plot (2 per 250 cm^3; 14 August 2002).

Similarly, in 2003 and 2004 microplots, there was no evidence for contributions of plant-parasitic nematodes to RD. In 2003, lesion nematode was not detected, and a mean of three pin nematodes per 250 cm^3 was extracted by sugar flotation from the controls. No other plant-parasitic nematodes were detected. In 2004, one of the four nonfumigated plots had six lesion nematodes per 20 g roots and 10 lesion nematodes per 250 cm^3 soil, but no other plant-parasitic nematodes were detected.

DISCUSSION

We have characterized symptoms of RD on almond in the Central Valley of California and determined that the disease is not associated with nematode infestation, that it seriously impacts almond on three important rootstocks, and that it can be prevented by pre-plant fumigation with several MB alternatives. The results are important to California peach production as well as almond production because the crops have their most prevalent rootstocks in common (i.e., Nemaguard and Lovell peach) (8) and are grown in overlapping areas of the state. At sites severely affected by RD, broadcast and spot fumigation treatments with CP consistently prevented the disease and often were more effective than comparable treatments with MB. Also, spot fumigation with 1,3-D, IM, or combinations of them with CP, prevented the disease and matched or exceeded the efficacy of spot treatments with MB. To our knowledge, this is the first detailed report of RD on almond and relative effectiveness of the tested fumigants for managing the disease.

The RD seriously compromised tree performance on all three rootstocks tested (Marianna 2624, and Lovell and Nemaguard peach). For some of the performance variables, the disease impact was less on the peach rootstocks than on Marianna 2624, but our results indicate that use of peach rootstock, without effective pre-plant fumigation, is not an adequate RD control strategy for the disease on almond. Nevertheless, further investigation of almond rootstock tolerance to RD is warranted. In an Italian study, peach and nectarine trees were less affected by a replant disease on rootstocks of *Prunus persica* × *P. dulcis* or *P. persica* × *P. davidiana* than on rootstocks of *P. persica* or *P. domestica* (6). Almond cultivars are compatible with rootstocks of *P. persica* × *P. dulcis* (13).

The effectiveness of the spot fumigation treatments at planting sites demonstrated that RD can be prevented without applying fumigants to entire areas or wide strips of land. This is important because a reduction of treated area potentially reduces environmental impact and fumigant costs. In repeated experiments, 0.2 and 0.5 kg CP per planting site were equally effective and consistently prevented RD, and in experiment 5, 0.12 kg per planting site was also effective. At commercial planting densities of 200 to 350 trees per ha, the use of 0.2 kg of CP per planting site requires 40 to 70

Table 3. Effects of rootstocks and pre-plant soil fumigation treatments applied to planting sites through a hand-held probe on growth of almond trees near Chico, CA

Experiment no.	Rootstock	Pre-plant treatment[y]		Tree growth and health parameters at end of indicated growing seasons						
				Height (m)	Increase in trunk diameter (mm)[z]			Disease rating		
		Fumigant	kg/site	First	First	Second	Third	First	Second	Third
9	Marianna 2624	Control	0	1.1 a	4 a	--	--	2.9 a	--	--
		MB:CP	0.5	1.9 cd	15 b	--	--	0.4 b	--	--
		CP	0.5	1.9 cd	14 b	--	--	0.6 b	--	--
	Lovell	Control	0	1.6 b	7 a	--	--	1.7 a	--	--
		MB:CP	0.5	2.1 cd	15 b	--	--	0.3 b	--	--
		CP	0.5	2.3 d	17 b	--	--	0.0 b	--	--
10, 11	Marianna 2624	Control	0	1.1 a	4 a	23 a	39 a	3.4 a	1.3 a	0.8 a
		MB:CP	0.5	1.9 c	20 cd	47 c	68 b	0.6 c	0.0 b	0.3 a
		CP	0.5	2.0 cd	22 cd	52 c	76 b	0.3 c	0.0 b	0.0 a
	Lovell	Control	0	1.5 b	9 b	33 b	57 a	2.1 b	0.1 b	0.0 a
		MB:CP	0.5	2.3 d	21 cd	48 c	73 b	0.1 c	0.0 b	0.0 a
		CP	0.5	2.2 d	22 d	51 c	76 b	0.2 c	0.0 b	0.0 a
	Nemaguard	Control	0	1.4 b	7 a	30 b	54 a	2.6 ab	0.3 b	0.0 a
		MB:CP	0.5	2.0 cd	18 c	46 c	71 b	0.3 c	0.0 b	0.0 a
		CP	0.5	2.2 d	20 cd	46 c	71 b	0.2 c	0.0 b	0.0 a

[y] Methyl bromide + chloropicrin (MB:CP, 75% MB 25% CP) and chloropicrin (CP) were injected by a hand-held probe at a soil depth of 45 to 50 cm in the center of sites where trees were to be planted.

[z] Tree height was measured at the end of the growing season (mid-October to early December, depending on experiment). Increases in trunk diameter were determined by measuring tree trunks near the end of the indicated growing seasons (late October to early February, depending on experiment) and calculating the net increase in diameter from time of planting. Disease ratings were made near the end of the indicated growing seasons (late August to mid-October) based on a scale of 0 = healthy tree, 5 = dead tree, and 1, 2, 3, and 4 were progressive increments of disease within the extremes. Means within a column and experiment group (i.e., experiment 9 or experiments 10, 11) and without letters in common are significantly different according to 95% confidence intervals.

kg/ha (orchard basis), which is substantially less than the amount required for a typical broadcast treatment with CP (approximately 336 kg/ha).

Using the 60-cm-diameter tractor-powered auger to loosen the soil centered around future planting holes was essential for optimal fumigation of the tree sites with the probe. In exploratory experiments, poor results were obtained when an 8-cm-diameter auger was used to loosen the soil. Use of the larger auger facilitated penetration of the fumigation probe and probably facilitated diffusion of the fumigant through the soil at the planting sites. Concentrating a relatively large fumigant dose in a small volume of soil almost certainly contributed to the efficacy of planting site spot fumigation treatments. For example, under the reasonable assumption that a 0.2-kg dose of fumigant applied to a planting site is retained within a 0.7-m radius of a single injection point, the average application rate in the circumscribed area would be 1,300 kg/ha, about four times that of conventional broadcast rates.

Tree performance data were collected for one to three growing seasons, depending on experiment. This time span was sufficient for evaluating treatment effects during the most critical period. We have not observed severe cases of RD (i.e., high incidence of tree death or failure to grow) after successful tree establishment in the first growing season. Nevertheless, long-term research (i.e., >3 years) is needed and underway to comparatively assess effects and economics of broadcast, strip, and spot pre-plant fumigation treatments for orchards in different replant scenarios (i.e., in orchards at risk for RD, nematode parasitism, or both). It is possible that, in the long term, restricting the proportion of the orchard area fumigated will shorten the period of benefit from pre-plant fumigation as the tree roots explore the soil. In apparent contrast to RD, plant-parasitic nematodes such as *Meloidogyne* spp., *Mesocriconema xenoplax*, and *Pratylenchus vulnus* can cause progressive decline of trees for the life of an orchard, and *M. xenoplax* can predispose stone fruits to bacterial canker disease for up to 8 years after planting (9,28,31). Therefore, tree-site spot fumigation treatments may be less effective for management of nematode parasitism than for management of RD. Our findings apply only to almond RD in absence of other replant problems.

Tree-site spot fumigation treatments, as applied in our study, were relatively labor intensive, mainly because of the site preparation requirements. Such spot treatments, which involve a hand-held probe, may involve greater risk of worker exposure to fumigant than applications using drip systems or tractor-mounted shanks. We are involved in developing potentially safer and more efficient methods for pre-plant spot fumigation.

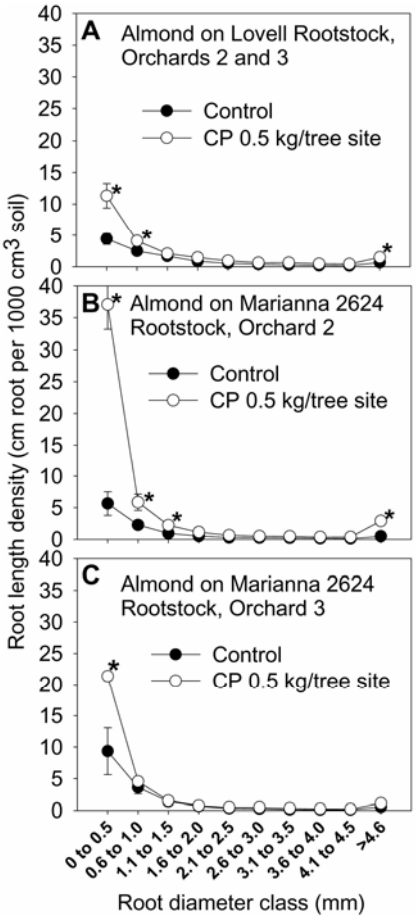

Fig. 3. Effect of pre-plant fumigation with chloropicrin (CP), injected at planting sites at soil depth of 45 to 50 cm, on root length density of almond trees on Lovell peach and Marianna 2624 rootstocks in commercial orchards affected by replant disease near Chico, CA. Trees were planted in February 2004. On 20 October 2004, root system samples were collected from known volumes of soil around three randomly selected trees for each combination of rootstock, fumigation treatment, and orchard. Vertical bars are 95% confidence intervals; asterisks indicate means greater than the control.

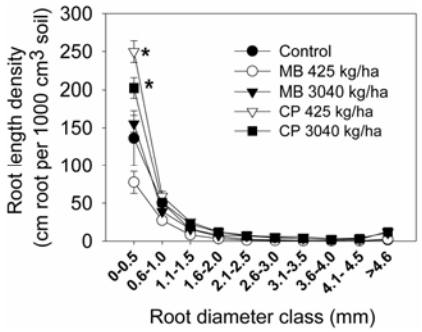

Fig. 4. Effect of pre-plant doses of methyl bromide (MB) and chloropicrin (CP), injected at soil depth of 30 cm, on root length densities on Nemaguard peach seedlings in microplots near Parlier, CA. Seedlings were planted on 14 April 2004, and root length samples were collected the first week of November 2004. Vertical bars are 95% confidence intervals; asterisks indicate means greater than the control.

Although the orchard and microplot trials involved different locations and procedures (i.e., the Sacramento and San Joaquin valleys; use of orchard plots and microplots, and almond trees and peach rootstock seedlings, etc.), there were important similarities in the results. At all locations, the experimental trees grew satisfactorily for several weeks, but within the first growing season those planted in nonfumigated soil exhibited varying degrees of stunting, chlorosis, wilting, and defoliation. At all locations, CP was generally more effective than MB for prevention of RD. These results suggest that RD has widespread geographical significance for almond and peach production in California and that CP is widely effective in preventing the disease.

The efficacy of the diverse fumigants in repeated orchard and microplot trials is evidence for biological mediation of RD. Although fumigants vary in toxicity to various pests and pathogens, CP, MB, IM, and 1,3-D are all broad-spectrum biocides (4,21). The repeated negative results from nematode sampling indicated that plant-parasitic nematodes did not play an important role in RD at the test locations. Significant populations of the pin nematode were detected in control plots containing RD-affected peach seedlings in the 2002 microplot experiment, but the association between RD and the nematode did not hold; in plots treated with MB at 425 kg per ha, RD, but not the nematode, was present. The pin nematode is parasitic on *Prunus* spp. (2), but it is not regarded as an economic pest on these crops (19). It is interesting to note that CP, which was highly effective for prevention of RD, is 8.5% N and is relatively toxic to nitrifying bacteria (29). In a previous report, soil fumigation with either CP or 1,3-D resulted in a net increase in N availability, despite an accompanying decrease in the rate of nitrogen transformations (i.e., mineralization, nitrification) (20). Similarly, soil sterilization with steam or soil fumigation with CP resulted in greater ammonium accumulation and nitrogen availability for several months compared with soil fumigation with MB (29). These reports suggest that improved nitrogen availability may have contributed to the positive growth responses to CP in our trials, although this was not investigated. It is unlikely that the relatively small amounts of nitrogen contained in the CP dose used for broadcast fumigation (experiment 1, 34 kg N/ha) and the low rate of CP in the microplots (experiments 6 to 8, 36 kg N/ha) were solely responsible for much of the positive plant responses to those treatments.

In orchards 2 and 3, decreased root length density was associated with RD incidence. The relationship was less clear in the 2004 microplots. The orchard results suggest that the disease may be initiated on fine roots, whereas the microplot results

suggest that the root sampling protocol for peach plants was inadequate. We have preliminary evidence for contributions of culturable fungi to etiology of RD (3). Research is clearly needed and underway on microbial contributions to RD etiology.

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Biology and Management of Almond Replant Disease

Project No.: 06-PATH3-Browne/Kluepfel **Interim Report**

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Interpretive Summary:

The two objectives for this project in 2006/07 were 1) to continue development of improved approaches for managing replant disease (RD) and other replant problems that affect almond and 2) to determine the specific cause(s) of RD of almond. This report presents a summary update of new and ongoing field trials and laboratory research dedicated to these objectives. We established a new field trial in 2006 near Firebaugh that includes evaluations of GPS-controlled spot fumigation treatments and reduced and standard rates of alternative fumigants. To date, several low application rates have afforded good tree growth in this trial. The Firebaugh trial also has provided new bacterial and fungal isolates useful for our work on etiology of replant disease. In collaboration with Brad Hanson, USDA-ARS, Parlier, we completed crop rotations for a factorial fumigation-crop rotation trial in an orchard setting. The trial includes rotations with wheat, sudan grass, and mustard as well as a fallow control. Each of these treatments will be planted to almond in 2008 with and without pre-plant fumigation. We have continued our examinations of microbial communities associated with RD-affected and healthy trees of almond. New sampling efforts for fungi, bacteria, and nematodes were completed and will be continued. Graphical ordinations were used extensively to examine the disease-associated shifts in bacterial and fungal communities. Both for fungi and for bacteria, the ordinations and supplemental statistical summaries identified organisms positively associated with replant disease (i.e., *Rhizobium* and *Streptomyces* species among bacteria; *Fusarium*, *Cylindrocarpon*, *Rhizoctonia*, and *Pythium* species among fungi) and negatively associated with the disease (i.e., certain *Pseudomonas* species among bacteria, *Trichoderma* and *Gliocladium* among the fungi). In the next year we will renew assessments of these organisms in pathogenicity and disease suppression tests using them as individual isolates and in mixed populations. We also

will continue sampling selected fungal, bacterial, and nematode communities in upcoming field trials.

Objectives:

1. Develop improved management strategies for replant disease (RD) on California almonds.

2. Determine the unknown causes of RD.

Materials and Methods:

Objective 1: improved management strategies for RD.

Background. Under this objective in 2006/07 we have: 1) continued assessment of alternative strip and broadcast fumigation treatments that were applied in two Madera County almond replant trials in 2003; 2) completed first-year assessment of strip, broadcast, and GPS-controlled spot fumigation treatments that were applied in a Madera County almond replant trial in 2006; and 3) completed pre-plant crop rotations in preparation for a 2008 fumigation-crop rotation replant trial at the USDA station near Parlier. All of the Madera trials are being conducted as a team effort with Brent Holtz and Bruce Lampinen, and the Parlier trial is being managed with leadership from Brad Hanson (USDA, ARS). The trials were designed to foster development of optimized pre-plant treatment strategies with minimal dependence on fumigants, minimal fumigant emissions, and maximal economic benefit.

Details, continued assessment pre-plant fumigation treatments applied in 2003 in Madera County. The treatments were applied in October and November 2003 with TriCal, Inc. and included methyl bromide (MB, 400 lb/acre), Telone II (1,3-dichloropropene [1,3-D], 340 lb/acre), chloropicrin (CP, 400 lb/acre), Telone C35 (1,3-D:CP 61:35, 535 lb/acre), and Midas (iodomethane [IM]:CP 50:50, 400 lb/acre). The fumigants were applied with shanks spaced 20" apart through nozzles spaced 10" apart at a soil depth 18", with the exception that a hand-held probe was used to apply tree site spot fumigation treatments at 20" depth in one experiment. Some of the fumigants were applied alternatively as broadcast, row-strip, and spot treatments, and some of the row-strip treatments were applied with and without virtually impermeable film (VIF), a tri-layer plastic that, under ideal conditions, can dramatically reduce fumigant emissions and increase fumigant retention near the soil surface. One of the Madera trials occurred on loam soil previously devoted to almond on Nemaguard rootstock, and the other occurred on loam soil previously devoted to grape. All of the treatments were compared to non-fumigated controls with and without the VIF plastic mulch. There were four replicate plots per treatment, and each plot eventually was planted to 3 rows of 9 to 10 trees. Data have been collected from the center rows, which are planted to Nonpareil. Tree trunk circumference has been measured annually since planting in winter 2004.

Gross nut yields were measured in 2006 and 2007 (harvests led by Lampinen and Metcalf).

First-year assessment of pre-plant fumigation treatments applied in 2006 in Madera County. Pre-plant treatments similar to those applied for the 2003 trials were applied in 2006 near Firebaugh, CA. A new element of the 2006 trial, however, is the first orchard test of GPS-controlled spot fumigation treatments. The Firebaugh trial is on loam soil, and its almond trees were replanted within a year of removal of the previous almond orchard on Nemaguard rootstock. We measured trunk circumferences after planting in the spring and repeat the measurements annually. In June and August 2007 we visually rated the trees for severity of RD symptoms using a scale in which 0= no disease, tree vigorously growing; 1=trees stunted, approximately 70% of the size of healthy vigorous trees, but otherwise healthy; 2=trees severely stunted, approximately 50% of the size of healthy vigorous trees, most shoot growing points not active, foliage otherwise healthy; 3= tree size similar to that for score of 2, but foliage exhibiting additional disease symptoms (chlorosis, leaf burning); 4=tree wilting and dying; 5= tree dead.

Completion of pre-plant crop rotations for fumigation-crop rotation trial in Fresno County. Previously, in microplot trials, we found that single-season rotations with Piper sudan grass, Penewawa wheat, or mustards could suppress severity of RD (Almond Board reports 2004-2006). For the last year we have been preparing an orchard trial designed to test selected crop rotations in a commercial setting. At the USDA-ARS station near Parlier, Brad Hanson managed four alternative pre-plant cropping/fallowing treatments during 2007 at the site of a former peach orchard destined to be replanted to almond in Winter 2008 (**Table 1**). As shown in the table the treatments include: a non-fallowed control, i.e., continuous peach until the fall before replanting with almond (Treatments 1,2); a bare-fallowed control (Treatments 3,4); a rotation of mustard (Treatments 5,6); and a rotation of wheat followed by sudan grass (Treatments 7,8). The odd-numbered treatments will serve as non-fumigated controls, whereas the even-numbered treatments will be followed by pre-plant fumigation with Pic-clor 60 at 400 lb/a. Effects of the factorial treatments will be assessed by monitoring the growth and productivity of Nonpareil and Monterey almond trees that will be planted in winter 2008.

Objective 2: determining causes of RD.

Background. We have continued to conduct sampling experiments in orchards affected by RD but apparently free of significant populations of plant parasitic nematodes to identify other soil microbes that may contribute to the disease. Our sampling began in 2003 with multiple orchard and microplot trials (near Durham and Parlier, respectively) and has continued through 2007 with sampling from the 2006 Madera replant trial described above. In each trial we sampled feeder roots (roots $\leq$ 1 mm diameter) from RD-affected and healthy trees in non-fumigated and fumigated plots of the field trials, respectively. The root samples were used for isolations of bacteria, fungi, and oomycetes in Petri dish cultures as documented previously (2005 Comprehensive Report to the Almond Board). Individual isolates of cultured bacteria

and fungi were saved for traditional and rDNA-sequence-based identification and for pathogenicity tests.

In addition, because many soil microbes are not readily isolated in culture, samples of the roots and adhering rhizosphere soil from the diseased and healthy trees were used for "culture-independent" microbial examinations as described here: The root samples were placed on dry ice immediately after collection in the field and stored at -80°C until DNA was extracted from them for culture-independent characterization of the microbial communities on and in them. Standard DNA extraction and purification procedures were modified through trial and error as needed to permit polymerase chain reaction (PCR) amplification of diagnostic rDNA fragments from bacteria and fungi that were present in the samples. We used described bacterial and fungal primers that amplify rDNA universally from most bacterial and fungal organisms, respectively (**Table 2**). Because the fragments of rDNA were amplified from mixtures that contained DNA from many organisms, it was necessary to purify and separate the fragments from each other by "cloning" (i.e., inserting them into plasmids for replication in pure cultures of surrogate bacteria, one fragment of rDNA per culture). The individually cloned rDNA fragments, each potentially from a different soil microbe, were then "sequenced" (i.e., the sequence of nucleotide bases A, G, C, T composing the fragments was determined) with an automated DNA sequencer. The rDNA fragment sequence is a genetic "fingerprint" of the organism from which it came. Using what is called "Basic Local Alignment Search Tool" (BLAST) searches among catalogued rDNA sequences online at the National Center for Biotechnology Information (NCBI), the fingerprints were used to obtain putative genus- to species-level identifications of microbes present in the root samples.

Evaluating bacterial and fungal population shifts associated with RD. In the last year we have focused on applying statistical methods that go beyond the initial putative identifications, incidence calculations, and preliminary stepwise discrimination methods we used previously (Almond Board reports 2005-06). One of the new methods we employed is analysis of molecular variance (AMOVA), which, among other things, can be used to determine whether DNA sequence variation associated with treatments or environmental factors is statistically significant or may be due to chance alone. We applied AMOVA to rDNA sequences from bacteria associated with the roots of healthy and RD-affected trees in our field trials (2003 and 2004 orchard replant trials near Durham, 2003 and 2004 microplot trials near Parlier). Using squared Euclidean distances based on DNA sequence differences, AMOVA was used to partition the sequence variance into components attributable to random variation and to the pre-plant soil fumigation treatments (and health status of the almond trees). This allowed us to determine whether the rDNA sequence variations which represented changes in the root-associated bacterial communities were significantly linked to pre-plant soil fumigation treatments and therefore with occurrence of RD. Below, we report results from AMOVA only for our bacterial populations (the analyses have not been completed for our fungal populations). In addition to AMOVA, we are using ordination methods (primarily redundancy analysis, RDA), which allow one to graphically and quantitatively examine community shifts associated with treatments or environmental factors. In

preparation for the ordinations, we classified the microbes (and rDNA sequences representing microbes) into operational taxonomic units (OTUs) that represent genus-to-species-level groupings. Depending on the source of microbes, the groupings were based on morphological identifications made with a microscope (this was the case for fungi we isolated in culture) or rDNA sequences (this was the case for all bacteria and for fungi detected by PCR of rDNA). The latter groupings were made with the software DOTUR (distance based OTU and richness determination). Bacterial OTUs were grouped into clusters with 92% genetic similarity (sequence homology), and fungal OTUs were grouped into clusters with 97% genetic similarity.

Interpreting ordination diagrams. Below, several figures (**Figs. 1 to 7**) will present graphical representations of bacterial and fungal population shifts associated with occurrence of RD. This paragraph is intended to aid the reader's interpretation of the ordinations. On each ordination diagram, arrows indicate the directions of greatest increase in incidence for each genus or OTU displayed. The star symbols indicate treatments included in the study (i.e., the non-fumigated control, fumigation treatments, and whether roots pieces were merely rinsed in sterile water or surface sterilized with bleach before placement in culture plates) or whether the treatments resulted in healthy or RD-affected trees; additional symbol details given in the figure legends. The degree of alignment and length of an arrow towards a treatment symbol is an indication of the abundance of the arrow's organism (i.e., the genus or OTU) in the treatment(s) represented by the symbol. Similarly, the degree to which any two arrows are aligned is an indication of the degree to which incidence of the two organisms is correlated; angles <90° between arrows indicate positive correlations between organisms, 90° angles between organism arrows indicate no correlation between organisms, and angles greater than 90° between the arrows indicate negative correlations.

Results and Discussion

Objective 1: improved management strategies for RD.

Continued assessment of 2003 fumigation trials, Madera County. In the 2003 Madera trial with almond replanted after almond, by 2006 the replanted trees had developed trunk circumferences that were 11 to 17% larger in plots treated with fumigants containing chloropicrin or mixtures of it with IM or 1,3-D, compared to the circumferences in non-fumigated control plots (**Table 3, Experiment 1-A**, the non-mulched control was used). Gross nut yields in 2007 were significantly increased by pre-plant fumigation with Telone C35 (535 lb/a, broadcast), IM:CP (50:50, 400 lb/a broadcast or row strip), CP 400 lb/a row strip no tarp, and CP 400 lb/a row strip with VIF tarp; increases resulting from the other treatments in 2007 were not statistically significant. There were no consistent growth or yield responses to use of VIF film. Yields in plots given the spot treatments were not improved relative to the control, and, as described previously (Almond Board reports 2004-2006), this was due at least in part to phytotoxicity resulting from the hand-probe treatment (Table 3, Experiment 1-B). The growth and yield benefit due to the effective strip and broadcast fumigation treatments is

gradually diminishing as the trees age, and it will be important to conduct overall economic assessments of their value. The economic assessments will be completed in a related USDA-ARS Area-Wide Project.

In the 2003 Madera trial with almond planted after grape, there was no practical benefit from pre-plant fumigation with or without VIF mulch; trees in all treatments have been vigorous and productive in this trial (**Table 3, Experiment 2**). As has been discussed previously, the cropping history of a vineyard and the apparent host specificity of many replant diseases is a logical explanation for the lack of response to fumigation in this orchard.

First-year assessment of 2006 fumigation trial, Madera County. In the Firebaugh trial, the trees planted 2007 in non-fumigated plots exhibited pronounced stunting, and many of them produced little or no new shoot growth from May to August (**Table 4,** disease severity rating scale). Pre-plant fumigation in row strips with either MB (400 lb/a) or Telone II (350 lb/a) improved growth slightly compared to the control. The other pre-plant fumigation treatments, which included CP, Midas, Telone C35, and Pic-clor 60 at various rates in strip, spot, and broadcast treatments, were more stimulatory to tree growth than the strip treatments with Telone II or MB. Although it is too early to judge the economic significance of treatments in this trial, it is encouraging that some of them involving relatively low rates of fumigant per orchard acre have afforded good tree performance in the first growing season. In particular, the GPS-directed spot treatments with Telone C35 and CP look promising. Concerning the spot treatments, it is likely that their performance can be improved as Upadhyaya et al. refine the software and hardware system that controls the application equipment. In our orchard testing of the GPS-controlled prototype in 2006, Upadhyaya determined a need for better precision in the system and is working to achieve it.

Objective 2: determining causes of RD.

Overview of bacterial populations sampled from roots. An overview of the sources, sample sizes, and diversity of the bacterial populations examined is presented in **Table 5**. In this table, the column of Chao 1 diversity estimates gives approximations of the maximum number of OTUs that could be detected with unlimited use of the given sampling method (i.e., culture-dependent or culture-independent, depending on the position in the table). The Shannon Index values provide a measure of the diversity in the sampled population categories; the greater the number of OTUs, and the more evenly they are represented in the population, the higher the index value. The values in the table indicate that the numbers of OTUs represented in our sample collections are generally minimal; this suggests that our samples will only allow us to assess shifts among dominant members of the bacterial communities. Also, it can be seen that the culture-independent sampling detects greater diversity than the culture-dependent sampling.

Using culture-based isolations from roots, the dominant OTUs detected in 2003 from the orchard trials near Durham (and their percentages, overall, of the population) were

Flavobacterium sp. (43%) and *Pseudomonas* sp. (45%), while those in 2004 were *Flavobacterium* sp. (14%), *Pseudomonas* sp. (30%), and *Variovorax* sp. (37%). Similarly, the dominant OTUs detected by culturing from the 2003 microplots (and their percentages, overall, in the population) were *Pseudomonas* sp. (38%), *Variovorax* sp. (12%), and members of the family Rhizobiaceae (18%), while those in 2004 were *Pseudomonas* sp. (16%), *Variovorax* sp. (30%) and members of the Rhizobiaceae (30%).

Using culture-independent detection methods, the dominant taxonomic groups detected in 2003 from the orchard trials were an unclassified member of Rhizobiaceae (21.5% of the samples), *Agrobacterium* sp. (13.0%), and *Streptomyces* sp. (10.8%), while those detected in 2004 were *Streptomyces* sp. (8.8%) and *Flavobacterium* sp. (4.2%). Culture independent sampling from the microplots near Parlier in 2003 revealed the following dominant genera in 2003: *Hydrogencarbonipaga* sp. (5.1%), *Streptomyces* sp. (2.9%), and *Pseudmonas* sp. (3.4%). culture-independent sampling was not undertaken in 2004 for the microplots.

Using AMOVA to examine shifts in culture-sampled bacterial populations associated with RD. Among bacterial collections cultured from roots in the orchard trials, AMOVA did not consistently reveal 16S rDNA sequence variation attributable to pre-plant soil fumigation treatments. In 2003 samples, non-surface-sterilized roots had significant bacterial sequence variation attributable to pre-plant fumigation treatments (**Table 6, Orchard**, 10.44% of the variation, P=0.02). However, in 2004 samples from non-surface-sterilized roots and in 2003 and 2004 samples from surface-sterilized roots, the soil treatments did not significantly affect genetic variation among subpopulations (**Table 6, Orchard**, *P*=0.18 to 0.51). In all AMOVAs for CD sampling, most of the variation in rDNA sequences (70.9 to 97.1%) was attributable to variation among individual isolates within populations (Tables 9,10).

In contrast to the AMOVA results from orchard trials, those for isolates cultured from the microplot trials consistently revealed significant sequence variation associated with pre-plant soil fumigation treatments. Among the isolates cultured from surface-disinfested roots, 23.17 and 5.86 % of the rDNA sequence variation was attributable to pre-plant fumigation treatments in 2003 and 2004, respectively (P=0.005 and 0.026, respectively) (**Table 6, Microplots**). Among isolates from the rinsed roots, there was no significant rDNA sequence variation attributable to pre-plant soil fumigation treatments in either 2003 or 2004 (P=0.36 to 0.11, respectively). As with the bacteria sampled from the orchard trials, most of the genetic variation (71.09 to 91.25%) was attributable to individual isolates within the populations.

Using AMOVA to examine shifts in PCR-sampled bacterial populations associated with RD. The rDNA sampled using culture-independent PCR did not reveal genetic variation consistently associated with incidence of PRD (**Table 7**). Among rDNA sequences obtained from the orchard, rDNA sequence variation attributable to preplant soil fumigation treatments was insignificant in 2003 (P=0.28) and significant in 2004 (P=0.03). Only 2.92 % of sequence variation was attributable to the soil treatments in

2004. Among rDNA sequences obtained by culture-independent sampling from the microplots (available only from 2003), no significant sequence variation was attributable to pre-plant soil fumigation treatments (P=0.297) (**Table 7**).

Using ordination analyses to examine shifts in culture-sampled bacterial populations associated with RD. A summary of the bacterial population ordination analyses is presented in **Table 8**. Ordinations of cultured bacterial populations from the orchard trials near Durham were not consistently significant (**Table 8, culture-dependent method, orchard**). In 2003, no significant ordination was possible; neither axis 1 nor the combination of axes 1-3 resulted in a statistically significant ordination of OTU occurrence (P=0.5). In contrast, in 2004 the three canonical axes had low but significant eigenvalues ($P<.006$ to 0.008), and together accounted for 28.5% of the variance in OTU occurrence. The 2004 ordination scored *Caulobacter* ribotype 1 (Caulo-1) with the greatest tendency for occurrence in roots from non-fumigated control plots, while *Pseudomonas* ribotype 2 (Pseud-2) exhibited the greatest association with roots from the fumigated treated plots (**Fig. 1**).

In the microplot trials, RDA revealed consistent OTU shifts in response to the pre-plant soil fumigation treatments and root surface sterilization treatments. Eigenvalues for ordinations of the microplot data from 2003 and 2004 were relatively low (0.12 to 0.18, respectively, for axis 1; 0.007 to 0.49 for axes 2-3), but the ordinations were highly significant (P=0.002 to 0.01) and cumulatively accounted for 25.3 to 31.4 % of variance in OTU counts (**Table 8, culture dependent, microplots; Figs. 2, 3**). In both 2003 and 2004, *Rhizobium* ribotype 1 (Rhiz-1), unclassified *Rhizobiaceae* ribotype 1 (RhizA-1), and unclassified *Rhizobiaceae* ribotype 2 (RhizA-2) scored closely to treatment centroids for surface-sterilized roots from non-fumigated control plots (**Figs. 2, 3**). On the other hand, some of the other OTUs, including *Arthrobacter* ribotype 2 (Arthro-2), *Pseudomonas* ribotype 3 (Pseud-3), and *Pseudomonas* ribotypes 1 and 2) were scored towards centroids of the fumigated treatments, but these relationships were not strong or consistent between years.

Using ordination analyses to examine shifts in PCR-sampled bacterial populations associated with RD. No highly significant ordinations resulted from RDA of the culture-independent data from the orchard or microplot trials (P=0.07 to 0.25), although the two ordinations of the orchard data approached significance (P=0.12 to 0.07) (**Table 8, culture-independent**; ordinations not shown).

Two-population proportions tests, culture-dependent sampling. Among some of the subpopulations cultured from roots of healthy and RD-affected trees in orchard plots and microplots, *Pseudomonas* ribotypes (Pseud 1 and/or Pseud 2) had greater incidence in the communities from healthy trees, compared to those from PRD-affected trees (**Table 9**, P= 0.002 to 0.02). This did not occur consistently among the orchard subpopulations, but it did so among the microplot subpopulations from surface sterilized roots. In addition, among the subpopulations cultured from surface-sterilized roots in the microplots, Rhiz-1 and RhizA-1 consistently were isolated in a higher proportion

from diseased trees (**Table 9**, P=0.001 for both 2003 and 2004). No other OTU shift was consistent for a location over the 2 years.

Two-population proportions tests, culture-independent sampling. Among the subpopulations obtained by culture-independent methods from roots in the orchard trials, the *Streptomyces* OTU designated "Strept-2" had higher incidence in samples from RD-affected trees, compared to those from healthy trees, both in 2003 and 2004 (P=0.001, and P=0.012, respectively) (**Table 9**). No other significant plant-health-related OTU shifts occurred consistently over the 2 years for culture-independent sampling from the orchard plots or in the single year of culture-independent sampling from the microplots.

Overview of fungal populations sampled from roots. We have not completed rigorous assessments of genetic diversity or AMOVA among the fungal populations we have sampled, but an overview of the populations is presented in **Tables 10-13**. To simplify presentation, we have intentionally restricted the tables' contents to fungal species representing $\geq$2 % of the cultured populations and $\geq$ 0.6% of the PCR-detected populations.

Among the fungi detected by culturing roots, it can be seen among the results tables that *Alternaria*, *Cylindrocarpon*, various *Fusarium* species, *Gliocladium*, *Mortierella*, *Pythium*, *Rhizoctonia*, *Trichoderma*, and some unknowns are represented (**Tables 10-12**). Among, the fungal species detected by culture-independent PCR, eight OTUs (9, 18, 31, 3, 19, 7, 21, and 4, in order of decreasing prevalence) were detected commonly by PCR of rDNA extracted from the root samples of Marianna 2624 collected from plots near Durham in 2003-2004 (**Table 13**). Determining the identities of these fungi based on BLAST searches is complicated by the fact that many fungi have names for perfect (sexual) and imperfect (asexual) stages (**Table 13**). This complication applies to the two largest OTUs, 9 and 18, which BLAST searching linked to a "*Nectria-Gibberella-Fusarium*" complex; the former two genera include perfect states of several species of *Fusarium* and *Cylindrocarpon*. Another complication is that, although it is expanding rapidly, the NCBI sequence rDNA database is less developed for fungi than for bacteria. It is interesting to note that OTUs 45 and 36 represented *Tylencholaimus*, a free-living nematode.

Using ordination analysis to examine fungal population shifts associated with RD. All of the ordinations based on data from culture-dependent detection of fungi from roots (i.e., detection from roots placed in Petri dishes of culture media) were statistically significant. This means that it is highly unlikely that the spatial relationships generated in 2-dimensional scatter diagrams could be explained by chance alone; the P values (the probabilities that random chance explain the ordinations) ranged from 0.002 to 0.004 (**Figs. 4-6**).

It can be seen that the ordinations for the culture-based detections of fungi from the Durham orchard trials (**Fig. 4**), the Parlier microplot trials (**Fig. 5**), and the Firebaugh orchard trial (**Fig. 6**) all indicate gradients of increasing incidences of *Fusarium* species

(indicated by arrows labeled with "*Fus*", followed by numbers or letters designating morphological types) and *Cylindrocarpon* sp. (indicated by "*Cyl*") in RD-affected roots from non-fumigated control plots. The letters indicating *Fusarium* subtypes location-specific, e.g., a subtype label from Durham trials does not apply to Parlier trials. Additional fungi occurring less consistently across locations, yet still exhibiting gradients oriented towards roots from the diseased trees in non-fumigated plots included *Mortierella* sp. ("*Mort*" or "*Mor*"), *Pythium* sp. ("Pyth"), and *Rhizoctonia* sp. ("*Rhizoc*") (**Figs. 4,5**). Fungi with increasing incidence gradients among roots from healthy trees in fumigated plots included *Gliocladium* ("*Glio*") and *Trichoderma* ("*Tricho*"). Results in the ordination diagrams suggest that our ability to detect some of the fungi was strongly influenced by whether the roots were merely rinsed in sterile water (labels for this treatment's symbols include the word "Rinsed") or surface sterilized in bleach before culturing (labels for this treatment's symbols include the word "Bleached"). For example, *Cylindrocarpon* was sometimes difficult to detect without first bleaching the roots, whereas some *Fusarium* species, *Mortierella*, and *Pythium* often were favored by the rinsing alone.

The ordinations for culture-independent detections of fungi from the Durham trials (2003-2004) were not statistically significant (P= 0.09 to 0.59) (**Fig. 7**). It can be seen that incidences for OTUs 9 and 18, the two largest groups in the populations and those with affinity to the genus *Fusarium*, were positively correlated. It is unknown why the culture-independent approach discriminated less treatment-associated variation in the fungal populations compared with results of the culture-dependent approach described above. It is conceivable that the fact we did not subject the roots used for culture-independent work to water rinse or bleach treatments before DNA extraction lessened the discrimination power of the method by focusing on rhizosphere fungi more than fungi inside the roots, but this has not been explored.

Concluding comments. The work described above has indicated potentially important shifts in bacterial and fungal communities associated with replant disease of almond. Both for fungi and for bacteria, our ordinations and other statistical summaries have suggested organisms positively associated with replant disease (i.e., *Rhizobium* and *Streptomyces* species among bacteria; *Fusarium*, *Cylindrocarpon*, *Rhizoctonia*, and *Pythium* species among fungi) and negatively associated with the disease (i.e., certain *Pseudomonas* species among bacteria, *Trichoderma* and *Gliocladium* among the fungi). In the next year we will renew assessments of these organisms, as individual isolates and in mixed populations, for their ability to incite and suppress replant disease. We also will continue sampling selected fungal, bacterial, and nematode communities in upcoming field trials.

Table 1. Design of trial with Brad Hanson examining factorial combinations of pre-plant fallowing, crop rotation, and fumigation on performance of almond trees planted after removal of a peach orchard[a]

Treatment	Preplant Cropping sequence					
		2007				
	1999-Oct. 2006	Winter	Spring	Summer	Fall	October
1	Peach on Nemaguard	Peach on Nemaguard	Peach on Nemaguard	Peach on Nemaguard	Fallow	No fumigation
2	Peach on Nemaguard	Peach on Nemaguard	Peach on Nemaguard	Peach on Nemaguard	Fallow	Fumigation with CP:1,3-D[b]
3	Peach on Nemaguard	Fallow	Fallow	Fallow	Fallow	No fumigation
4	Peach on Nemaguard	Fallow	Fallow	Fallow	Fallow	Fumigation with CP:1,3-D
5	Peach on Nemaguard	Mustard	Mustard	Fallow	Fallow	No fumigation
6	Peach on Nemaguard	Mustard	Mustard	Fallow	Fallow	Fumigation with CP:1,3-D
7	Peach on Nemaguard	Penewawa wheat	Piper sudan	Piper sudan	Fallow	No fumigation
8	Peach on Nemaguard	Penewawa wheat	Piper sudan	Piper sudan	Fallow	Fumigation with CP:1,3-D

[a]Nonpareil and Monterey almond trees on Nemaguard rootstock will be planted in 2008.
[b]Chloropicrin:1,3-dichloropropene, 60:35 (Pic-clor 60), 400 lb/a.

Table 2. PCR primer pairs used in culture-independent analysis of microbial communities associated with replant disease

Primer pair	Use	Reference
63F and 1401R	Identification of bacteria isolated in culture	Marchesi, J.R., T. Sato, A.J. Weightman, T.A. Martin, J.C. Fry, S.J. Hiom, and W.G. Wade. 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl. Envir. Microbiol.* 64:795-799.
341F (2) and 1401R (1)	Identification of bacteria detected by culture-independent amplification of rDNA	(1) Marchesi, J.R., T. Sato, A.J. Weightman, T.A. Martin, J.C. Fry, S.J. Hiom, and W.G. Wade. 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl. Envir. Microbiol.* 64:795-799. (2) Muyzer, G., S. Hottenträger, A. Teske, and C. Wawer. 1996. Denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA—a new molecular approach to analyse the genetic diversity of mixed microbial communities, p. 1-23. *In* A. D. L. Akkermans, J. D. van Elsas, and F. J. de Bruijn (ed.), Molecular microbial ecology, manual 3.4.4. Kluwer Academic Publishers, Dordrecht, The Netherlands.
Fungal small-subunit rDNA primer 463 and 464	Identification of fungi detected by culture-independent amplification of rDNA	Valinsky et al. 2002. Oligonucleotide fingerprinting of rRNA genes for analysis of fungal community composition. Appl Environ. Microbiol 68: 5999-6004

Table 3. Growth and yield responses of almond trees to preplant fumigation treatments on ground previously devoted to almond (Experiment 1-A,B) and grape (Experiment 1-B). Data from USDA CSREES team trial, Lampinen, Browne, Holtz, and Schneider

Expt.	Fumigant, rate	Plot area treated	Mulch system	Cumulative response of tree trunk circumference (% increase or decrease relative to control mean)			Gross Nut Yield (lb/tree) [d]	
				2004	2005	2006	2006	2007
1-A[a]	Control	None	None	0	0	0	9.0 de	46.5 de
	Control	None	VIF	-6	-2	1	6.7 e	45.3 e
	MB, 400 lb/a	Br. (100%)	None	4	3	4	11.2 bcd	50.6 bcde
	MB, 400 lb/a	R. strip (38%)	None	-4	1	2	10.1 cde	47.9 cde
	MB, 400 lb/a	R. strip (38%)	VIF	-2	-3	1	10.0 cde	52.0 bcde
	Telone II, 340 lb/a	Br. (100%)	None	11	9	9	12.5 abcd	54.8 abcd
	Telone II, 340 lb/a	R. strip (38%)	None	6	4	4	11.0 bcd	50.6 bcde
	Telone II, 340 lb/a	R. strip (38%)	VIF	0	0	1	11.0 bcd	51.1 bcde
	Telone C35, 535 lb/a	Br. (100%)	None	16	17	13	15.4 a	59.2 ab
	Telone C35, 535 lb/a	R. strip (38%)	None	27	16	13	14.8 a	55.1 abcd
	IM:CP (50:50), 400 lb/a	Br. (100%)	None	29	18	14	15.8 a	61.8 a
	IM:CP (50:50), 400 lb/a	R. strip (38%)	None	19	19	17	14.0 ab	56.8 abc
	CP 400 lb/a	Br. (100%)	None	17	12	11	13.0 abc	53.6 abcde
	CP 400 lb/a	R. strip (38%)	None	30	19	14	14.0 ab	56.1 abc
	CP 400 lb/a	R. strip (38%)	VIF	28	17	12	15.5 a	56.8 abc
1-B[b]	Control	None	None	0	0	0	9.0 de	46.5 de
	MB, 1 lb / tree site	Tree site	None	0	0	-1	11.1 bcd	48.3 cde
	CP, 1 lb / tree site	Tree site	None	-13	0	5	9.7 cde	50.5 bcde
	Telone II, 1 lb / tree site	Tree site	None	-11	-7	-1	10.1 cde	47.8 cde
2[c]	Control	None	None	0	0	0	13.1 abc	60.7 abcd
	Control	None	VIF	-3	-2	1	11.7 bcd	57.8 abcd
	MB, 400 lb/a	Br. (100%)	None	-5	2	1	14.8 ab	62.7 ab
	MB, 400 lb/a	R. strip (38%)	None	-9	-5	-2	12.4 abcd	60.1 abcd
	MB, 400 lb/a	R. strip (23%)	None	-9	-3	0	12.7 abc	58.4 abcd
	MB, 400 lb/a	R. strip (38%)	VIF	-10	-4	-1	12.5 abcd	61.8 abc
	Telone II, 340 lb/a	Br. (100%)	None	-5	-3	1	9.4 cd	57.6 abcd
	Telone II, 340 lb/a	R. strip (38%)	None	-4	-2	0	11.2 bcd	57.7 abcd
	Telone II, 340 lb/a	R. strip (38%)	VIF	-8	-4	0	8.9 d	55.1 d
	Telone C35, 535 lb/a	R. strip (38%)	None	-12	-5	-3	12.3 bcd	57.1 bcd
	Telone C35, 535 lb/a	R. strip (38%)	VIF	-10	-4	0	11.4 bcd	56.2 cd
	IM:CP (50:50), 400 lb/a	Br. (100%)	None	-4	-2	-2	16.1 a	63.1 a
	IM:CP (50:50), 400 lb/a	R. strip (38%)	None	-7	-3	-1	13.5 ab	61.1 abc
	CP 400 lb/a	R. strip (38%)	None	-5	-3	-1	11.7 bcd	56.8 cd
	CP 400 lb/a	R. strip (23%)	None	-3	-1	0	12.1 bcd	56.7 cd
	CP 400 lb/a	R. strip (38%)	VIF	-13	-7	-5	13.1 abc	56.8 bcd

[a]Fumigants applied 27 October 2003
[b]Fumigants applied 10 November 2003
[c]Fumigants applied 11 November 2003
[d]Means within an experiment (i.e., 1-A, 1-B, or 2) without letters in common are significantly different according to Duncan's multiple range test.

Table 4. Preliminary data from 2006 area-wide trial testing and demonstrating effects of different fumigants, fumigant rates, and treatment zones on performance of replanted almond trees

Trt.	Fumigant, rate per treated area[a]	Treated area in tree row (and % of total area)	Fumigant per orch. acre (lbs)	Disease severity rating (0 to 5 scale)	
				6/20/07	8/27/07
1	Control	None	0	1.8	1.6
2	Methyl bromide, 400 lb/a	8-ft strip (38%)	152	0.8	1.0
3	Telone II, 350 lb/a	8-ft strip (38%)	133	1.0	0.8
4	Chloropicrin (CP), 400 lb/a	8-ft strip (38%)	152	0.1	0.1
5	CP, 300 lb/a	8-ft strip (38%)	114	0.4	0.2
6	CP, 200 lb/a	8-ft strip (38%)	76	0.1	0.1
7	CP, 400 lb/a	8x8-ft tree sites (17%)	68	0.5	0.3
8	Midas (IM:CP. 50:50), 300 lb/a	8-ft row strip (38%)	152	0.3	0.1
9	Telone C35, 550 lb/ac	8-ft row strip (38%)	209	0.1	0.1
10	Pic-clor 60, 550 lb/ac	8-ft row strip (38%)	209	0.0	0.1
11	Pic-clor 60, 400 lb/ac	8-ft row strip (38%)	152	0.3	0.2
12	Telone C35, 550 lb/ac	8x8-ft tree sites (17%)	93	0.3	0.2
13	Telone C35, 550 lb/ac	Broadcast (100%)	550	0.1	0.1
Minimum significant difference based on 95% confidence intervals:				*0.5*	*0.5*

[a]Applied by shank in late summer or early fall 2006 after <1 year of fallow. Previous crop was almond on Nemaguard rootstock. IM=Iodomethane. Disease severity rating scale extremes: 0= healthy, 5=dead.

Table 5. Overview of bacterial communities sampled from roots in orchard and microplot trials

Sampling Method[a]	Trial Location	Year	Pre-plant soil treatment	Number of sequenced 16S rDNA fragments	Number of OTUs[b]	Chao1 diversity estimate (95% confidence interval)	Shannon Index (95% confidence interval)
CD	Orchard	2003	Control	94	8	8.3 (8.0-14.0)	1.18 (0.96-1.40)
			Chloropicrin	115	4	5.0 (2.0-8.6)	0.62 (0.10-0.83)
		2004	Control	139	25	64.0 (35.1-175.8)	2.39 (2.20-2.59)
			Chloropicrin	136	19	28.0 (21.0-59.4)	1.93 (1.72-2.14)
	Microplots	2003	Control	75	15	20.2 (16.0-42.6)	1.90 (1.63-2.17)
			Chloropicrin	106	19	31.0 (21.7-73.2)	2.16 (1.93-2.38)
			Methyl Bromide	85	15	43.0 (22.4-120.3)	1.73 (1.45-2.01)
		2004	Control	147	24	59.0 (33.7-150.5)	1.99 (1.46-2.22)
			Chloropicrin	146	20	75.0 (37.6-192.0)	1.88 (1.66-2.10)
			Methyl Bromide	127	20	29.0 (22.0-60.4)	2.10 (1.90-2.30)
CI	Orchard	2003	Control	170	45	95.0 (62.7-186.6)	3.05 (2.85-3.24)
			Chloropicrin	162	42	56.0 (47.0-84.8)	2.94 (2.74-3.15)
		2004	Control	162	92	270.8 (179.2-458.4)	4.17 (4.00-4.34)
			Chloropicrin	127	67	149.1 (103.2-252.9)	3.92 (3.76-4.08)
	Microplots	2003	Control	135	52	66.2 (56.8-94.7)	3.63 (3.50-3.76)
			Chloropicrin	160	65	108.2 (88.2-127.2)	3.78 (3.61-3.95)
			Methyl Bromide	158	62	91.1 (73.9-132.4)	3.69 (3.53-3.84)

[a] Culture-dependant sampling consisted of bacterial dilution plating on 5% tryptic soy broth agar, followed by PCR amplification and sequencing of rDNA (800-900 bp). Culture-independent sampling consisted of direct extraction of DNA followed by PCR amplification and sequencing of 16S rDNA (800-100bp).
[b] Based on sequencing of 16s rDNA, followed by sequence alignment using MUSCLE, trimming sequences to a common end point, using PHYLIP to obtain a DNA distance matrix, and clustering of OTUs using DOTUR with a 92% genetic similarity threshold for OTU resolution.

Table 6. Results of analysis of molecular variance in 16s rDNA representing bacteria cultured from roots of healthy almond trees in fumigated soil and RD-affected almond trees in non-fumigated soil[b]

Location	Year of sampling	Root extraction treatment[c]	Source of variation	Observed Partition		F_{ct}	P_e
				Variance	% of Total		
Orchard	2003	Rinse	Among trts.	4.88	10.44	0.104	0.023
			Among pops. within trts.	3.00	6.41		
			Within all pops.	38.90	83.15		
		Bleach	Among trts.	0.37	0.28	0.003	0.511
			Among pops. within trts.	13.77	25.62		
			Within all pops.	38.81	74.10		
	2004	Rinse	Among trts.	0.16	0.16	0.002	0.324
			Among pops. within trts.	2.70	2.71		
			Within all pops.	102.20	97.13		
		Bleach	Among trts.	0.22	0.30	0.003	0.184
			Among pops. within trts.	0.97	1.28		
			Within all pops.	76.22	98.42		
Microplots	2003	Rinse	Among trts.	0.07	0.08	0.001	0.356
			Among pops. within trts.	4.90	5.20		
			Within all pops.	89.24	94.72		
		Bleach	Among trts.	18.62	23.17	0.232	0.005
			Among pops. within trts.	4.47	5.74		
			Within all pops.	55.31	71.09		
	2004	Rinse	Among trts.	1.97	2.23	0.022	0.112
			Among pops. within trts.	5.77	6.52		
			Within all pops.	80.77	91.25		
		Bleach	Among trts.	5.47	5.86	0.059	0.026
			Among pops. within trts.	8.09	8.67		
			Within all pops.	79.80	85.48		

[a]Bacterial isolates were randomly chosen after dilution plating on 5% tryptic soy broth agar. After subculturing, isolates were subjected to PCR amplification and sequencing on 16s rDNA fragments (800-900 bp). The sequences were aligned using MUSCLE, trimmed to common end points before analysis of molecular variance using Arlequin V3.01. Depending on the location and year of sampling, there were 25 to 65 16s rDNA sequences per pre-plant soil treatment within a root surface sterilization treatment.

[b] Pre-plant soil treatments, which were the non-fumigated control, fumigation with chloropicrin, and, only at the micrplot location, fumigation with methyl bromide.

[c] Rinse root segments were vortexed in sterile distilled water and the liquid was used for dilution plating. Bleach root segments were surface sterilized in sodium hypochlorite, rinsed and ground in sterile distilled water, and the resulting suspension was used in dilution plating.

Table 7. Results of analysis of molecular variance of 16s rDNA obtained from culture-independent sampling[a] of roots affected by Prunus replant disease in non-fumigated soil and roots of healthy trees in pre-plant fumigated soil [b]

Location	Year of Sampling	Source of Variation	Observed Partition		F_{ct}	P_e
			Variance	% of Total		
Orchard	2003	Among trts.	0.06	0.03	0.0003	0.281
		Among pops. within trts.	0.12	0.06		
		Within all pops.	203.24	99.91		
	2004	Among trts.	6.75	2.92	0.029	0.035
		Among pops. within trts.	1.44	0.62		
		Within all pops.	222.84	96.45		
Microplots	2003	Among trts.	0.17	0.08	0.001	0.297
		Among pops. within trts.	3.34	1.53		
		Within all pops.	214.39	98.39		

[a]DNA was extracted directly from roots and rhizosphere soil and used for PCR amplification and sequencing of 16S rDNA (800-100bp). The sequences were aligned using MUSCLE, trimmed to common end points before analysis of molecular variance using Arlequin V3.01. Depending on the location and year of sampling, there were 127 to 170 16S rDNA sequences per pre-plant soil treatment.

[b] Pre-plant soil treatments, which were the non-fumigated control, fumigation with chloropicrin, and, only at the micrplot location, fumigation with methyl bromide.

Table 8. Results of redundancy analysis ordinations for operational taxonomic units and environmental variables in orchard and microplot replant trials[a]

Sampling Method[b]	Trial	Year	Canonical Axis	Eigenvalue	Cumulative percentage of variance in species data explained	P_e
Culture-dependent	Orchard	2003	1	0.049	7.4	0.538
			2	0.014	9.5	0.526
			3	0.002	9.9	
		2004	1	0.185	25.1	0.006
			2	0.18	27.5	0.008
			3	0.007	28.5	
	Microplots	2003	1	0.125	14.4	0.008
			2	0.049	20.0	0.010
			3	0.033	23.7	
			4	0.013	25.3	
		2004	1	0.178	22.7	0.002
			2	0.048	28.9	0.002
			3	0.012	30.5	
			4	0.007	31.4	
Culture-independent	Orchard	2003	1	0.249	40.4	0.118
		2004	1	0.235	48.9	0.068
	Microplots	2003	1	0.159	21.5	0.246
			2	0.099	34.8	0.094

[a] Redundancy analysis was performed using CANOCO software v. 4.5. For culture-dependent sampling, pre-plant soil fumigation and root extraction treatments were independent (environmental) variables and operational taxonomic units (OTUs) were independent variables. For culture-independent sampling, pre-plant fumigation treatment was the independent variable and OTUs were dependent variables. For both sampling methods, blocks were covariables.

Table 9. Operational taxonomic units (OTUs) occurring at significantly different incidences in subpopulations from roots of trees affected by Prunus replant disease in non-fumigated soil and roots of healthy trees in pre-plant fumigated soil [a]

Sampling method	Trial	Year	Root treatment[b]	OTU[c]	% of population from RD-affected trees	% of population from healthy trees	P value
Culture-dependent	Orchard Plots	2003	Bleach	Pseud-2	4.1	24.1	0.002
			Rinse	Pseud-2	7	25.4	0.008
		2004	Bleach	Vario-1	15.8	34.8	0.004
	Microplots	2003	Bleach	Pseud-1	11.4	29.9	0.008
				Pseud-2	9.1	23.7	0.021
				Rhiz-1	13.6	0	0.001
				RhizA-1	22.7	5.1	0.001
		2004	Bleach	Pseud-2	15.7	34.8	0.004
				Rhiz-1	10.5	0	0.001
				RhizA-1	15.8	3.4	0.001
				RhizA-2	42.1	16.4	0.001
				Vario-1	18.4	32.2	0.015
			Rinse	Pseud-3	12.3	1.5	0.001
				Vario-1	16.4	45.8	0.001
Culture-independent	Orchard Plots	2003	None	Strept-2	16.5	4.9	0.001
			None	Vario-1	8.2	3.1	0.022
		2004	None	Strept-1	9.9	3.1	0.012
			None	Strept-2	14.8	7.8	0.031

[a]Culture-dependent or culture-independent OTUs were determined by PCR amplification of the 16s rDNA, alignment of the sequences MUSCLE, trimming to a common end point, calculating a DNA distance matrix using PHYLIP, and clustering of OTUs by using DOTUR with an 8% genetic dissimiliarity threshold for OTU resolution.

[b] Rinsed root segments were vortexed in sterile distilled water and the liquid was used for dilution plating. Bleached root segments were surface sterilized in sodium hypochlorite, rinsed and ground in sterile distilled water, and the resulting suspension was used in dilution plating.

[c] Prefixes of "Pseud," "Rhiz," "RhizA," "Strept," and "Vario" indicate OTUs of the taxons *Pseudomonas*, *Rhizobium*, Rhizobiaceae, *Streptomyces*, and *Variovorax*. Suffixes differentiate OTUs within taxons.

Table 10. Fungal taxons detected by culture-based isolations and represented at an incidence of at least two percent among root isolates from the orchard replant trials near Durham, 2003-04

Fungal taxon	Abbreviation in ordination diagrams	Total no. of isolates	% of population	% from control plots (RD-affected)	% from fumigated plots (Healthy)
Alternaria	*Alt*	104	2.5	48.1	51.9
Cylindrocarpon sp.	*Cyl*	423	10.2	72.6	27.4
Fusarium sp. "Ukn"	*FusUnk*	210	5.1	59.5	40.5
Fusarium sp. "A"	*FusA*	197	4.7	83.2	16.8
Fusarium sp. "C"	*FusC*	251	6.0	84.5	15.5
Fusarium sp. "D"	*FusD*	163	3.9	49.7	50.3
Fusarium sp. "E"	*FusE*	106	2.6	68.9	31.1
Fusarium sp. "H"	*FusH*	301	7.2	64.5	35.5
Fusarium sp. "I"	*FusI*	129	3.1	36.4	63.6
Gliocladium sp.	*Glio*	233	5.6	27.5	72.5
Mortierella sp.	*Mort*	962	23.2	62.8	37.2
Pythium sp.	*Pyth*	348	8.4	71.0	29.0
Rhizoctonia sp.	*Rhizoc*	108	2.6	73.1	26.9
Unknown fungus "gen"	*Unkgen*	434	10.4	51.6	48.4
Unknown fungus "A"	*UnkA*	186	4.5	36.0	64.0
Total:		4155	100.0	61.1	38.9

Table 11. Fungal taxons detected by culture-based isolations and represented at an incidence of at least two percent among root isolates from the microplot replant trials near Parlier, 2003-04

Fungal taxon	Abbrev.	Tot. no. of isol-ates	% of popu-lation	% in non-fumigated plots (RD-affected)	% in MB-fumigated plots (400 lb/a) (RD-affected)[a]	% in MB-fumigated plots (2500 lb/a) (Healthy)	% in CP-fumigated plots (400 lb/a) (Healthy)	% in CP-fumigated plots (2500 lb/a) (Healthy)
Cylindrocarpon sp.	*Cyl*	217	9.1	2.0	2.5	0.5	1.8	2.2
Fusarium sp. "A"	*FusA*	221	9.2	4.0	1.2	0.3	1.9	2.0
Fusarium sp. "C"	*FusC*	90	3.8	1.5	0.4	0.1	0.8	0.9
Fusarium sp. "C"	*FusD*	89	3.7	0.8	0.5	0.9	0.8	0.8
Fusarium sp. "Ukn"	*FusUnk*	115	4.8	2.1	0.9	0.6	1.0	0.3
Gliocladium sp.	*Glio*	166	6.9	0.4	1.1	0.6	1.5	3.3
Mortierella sp.	*Mor*	359	15.0	4.1	3.3	3.7	1.8	2.2
Penicillium sp.	*Pen*	73	3.0	0.5	0.8	1.2	0.5	0.2
Pythium sp.	*Pyt*	183	7.6	3.5	0.7	1.4	1.6	0.5
Rhizoctonia sp.	*Rhiz*	154	6.4	4.4	0.4	0.4	0.8	0.5
Trichoderma sp.	*Trich*	290	12.1	0.7	1.4	0.5	3.1	6.3
Unk. fungal sp."gen"	*Unkgen*	247	10.3	2.6	2.3	1.6	2.0	1.8
Unk. fungal sp."G"	*UknG*	131	5.5	1.0	1.3	0.9	0.8	1.5
Unk. fungal sp."M"	*UknM*	61	2.5	0.5	0.4	0.9	0.5	0.3
Total:		2396	100.0	28.0	17.0	13.6	18.9	22.6

Table 12. Fungal taxons detected by culture-based isolations and represented at an incidence of at least two percent among root isolates from the orchard replant trial near Firebaugh, 2006

ngal taxon	Abbrev.	Tot. no. of isolates	% of population	% in non-migated plots RD-affected)	% in CP-migated plots (400 lb/a) (Healthy)
remonium sp.	*remon*	5	3.3	2.0	1.3
pergillus sp.	*per*	6	3.9	3.9	0.0
Cladosporium sp.	*Clad*	32	21.1	21.1	0.0
Fusarium sp. "1"	*Fs1*	8	5.3	5.3	0.0
Fusarium sp. "3"	*Fs3*	8	5.3	5.3	0.0
Fusarium sp. "4"	*Fs4*	7	4.6	3.3	1.3
Fusarium oxysporum	*Foxy1*	22	14.5	14.5	0.0
Gliocladium sp.	*Glio-2*	57	37.5	1.3	36.2
Unknown fungal sp. "C"	*Unk-C*	5	3.3	3.3	0.0
Unknown fungal sp. "GM"	*Unk-GM*	2	1.3	1.3	0.0
Total:		152	100.0	61.2	38.8

Table 13. Fungal (and nematode) taxons detected by culture-independent PCR at an incidence of at least 0.6 percent among root isolates from the orchard replant trials near Durham, 2003-04

TU	AST identification results with ≥99% rDNA sequenc homology	Tot. no. of isolates	centage of bacterial population		
			All plots	Non-fumig. plots (RD-affected)	P-fumig. plots (Healthy)
9	*Ectria-Gibberella-Fusarium* spp. complex	586	23.6	65.5	34.5
8	*Ectria-Gibberella-Fusarium* spp. complex	429	17.3	65.5	34.5
1	*Lithyrella candolleana*	111	4.5	28.8	71.2
3	*Pseudogymnoascus*	106	4.3	5.7	94.3
9	*Chaetomium globosum, Madurella mycetomas, Acrocladium asperum, Humicola grisea*	89	3.6	56.2	43.8
7	*Leptoshpaeria* spp., *Ophiosphaerella herpotricha, Microcurbitaria elongata, Herpotrichiia parasitica*	85	3.4	43.5	56.5
1	*Colletosphaerella cucumerina, Verticillium dahliae*	72	2.9	37.5	62.5
4	*Chaetomyces pannorum,Pseudogymnoascus roseus, Acrocladium patulum, Tetrachaetum elegans, Geniculosporia tetracladia*	68	2.7	11.8	88.2
5	*Lithyrella* spp. *Lacrymaria velutina*	47	1.9	34.0	66.0
5	*Pencholaimus* sp. nematode	38	1.5	23.7	76.3
03	*Geniculospora curvula*	37	1.5	83.8	16.2
6	*Leptoshpaeria* spp., *Ophiosphaerella herpotricha, Microcurbitaria elongata, Herpotrichiia parasitica, Pleomassariothyrium palmarum*	36	1.5	30.6	69.4
1	Cultured ascomycete AB074660.1	32	1.3	46.9	53.1
4	*Chaetomium globosum, Madurella mycetomas, Acrocladium asperum, Humicola grisea*	27	1.1	48.1	51.9
4	*Ceratobasidium* spp.	23	0.9	47.8	52.2
2	*Chaetomium globosum, Madurella mycetomas, Acrocladium asperum, Humicola grisea, uncultured Eukaryote, Neurospora crassa*	16	0.6	50.0	50.0
6	*Pencholaimus* sp. nematode	16	0.6	31.3	68.8

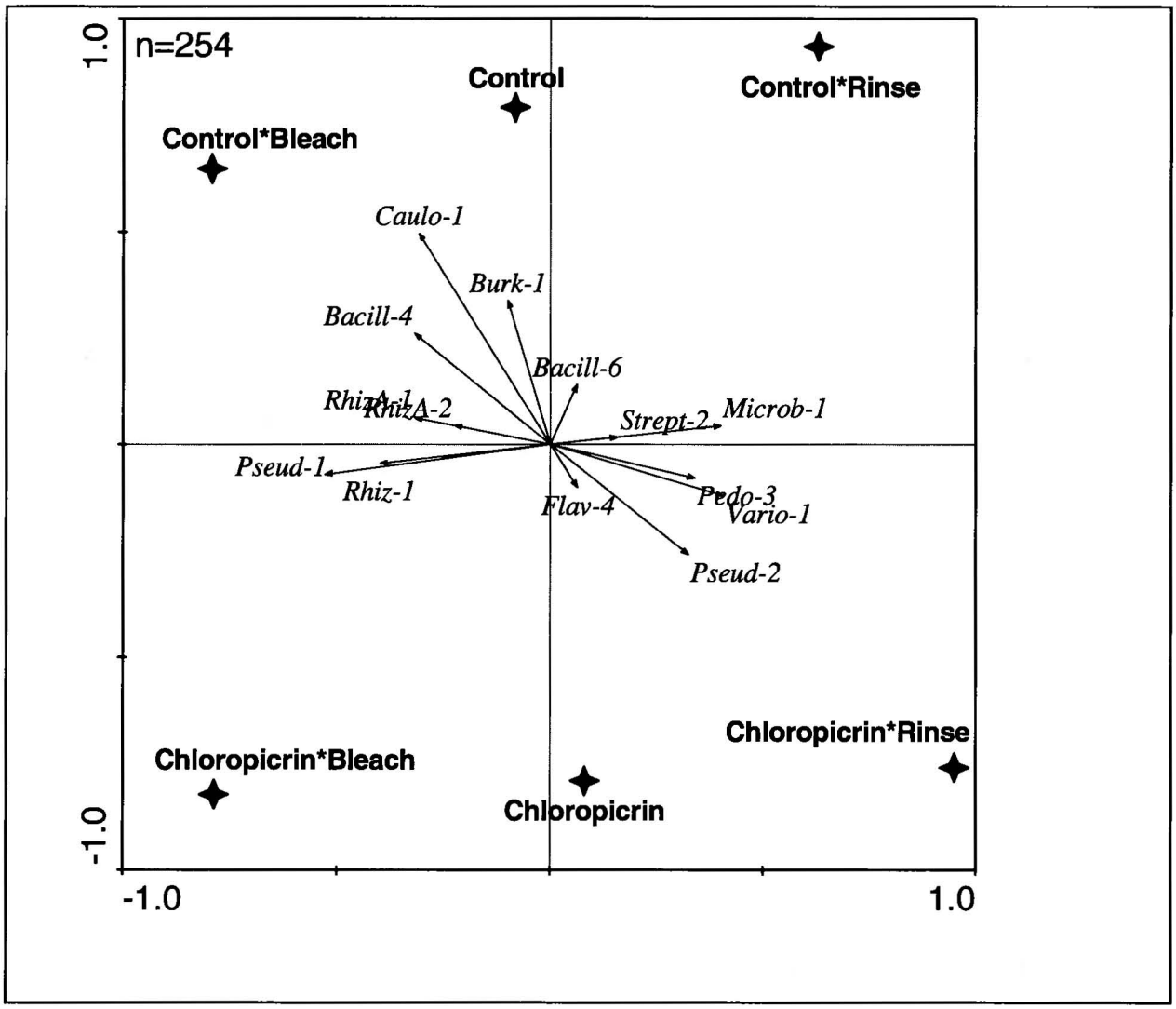

Fig. 1. Redundancy analysis ordination biplot of bacterial operational taxonomic units (OTUs, in italics) detected by culture-dependent sampling and their relationship to environmental variables (i.e., pre-plant soil fumigation treatments and their combinations with treatments that root samples were given before culture isolations) for culture-dependent sampling in the 2004 orchard trial near Durham. All plants in control plots were affected by replant disease, while those in chloropicrin-fumigated plots were healthy. Genera discriminated (and unique intra-genus OTUs) included *Bacillus* (Bacill-4, and Bacill-6), *Burkholderia* (Burk-1), *Caulobacter* (Caulo-1), *Flavobacterium* (Flav-4), *Microbacterium* (Microb-1), *Pedobacter* (Pedo-3), *Pseudomonas* (Pseud-1, and Pseud-2), *Rhizobium* (Rhiz-1), Unclassified Rhizobiaceae members (RhizA-1, and RhizA-2), *Streptomyces* (Strept-2), and *Variovorax* (Vario-1). Axis 1 P=0.006, Axis 2 P=0.006.

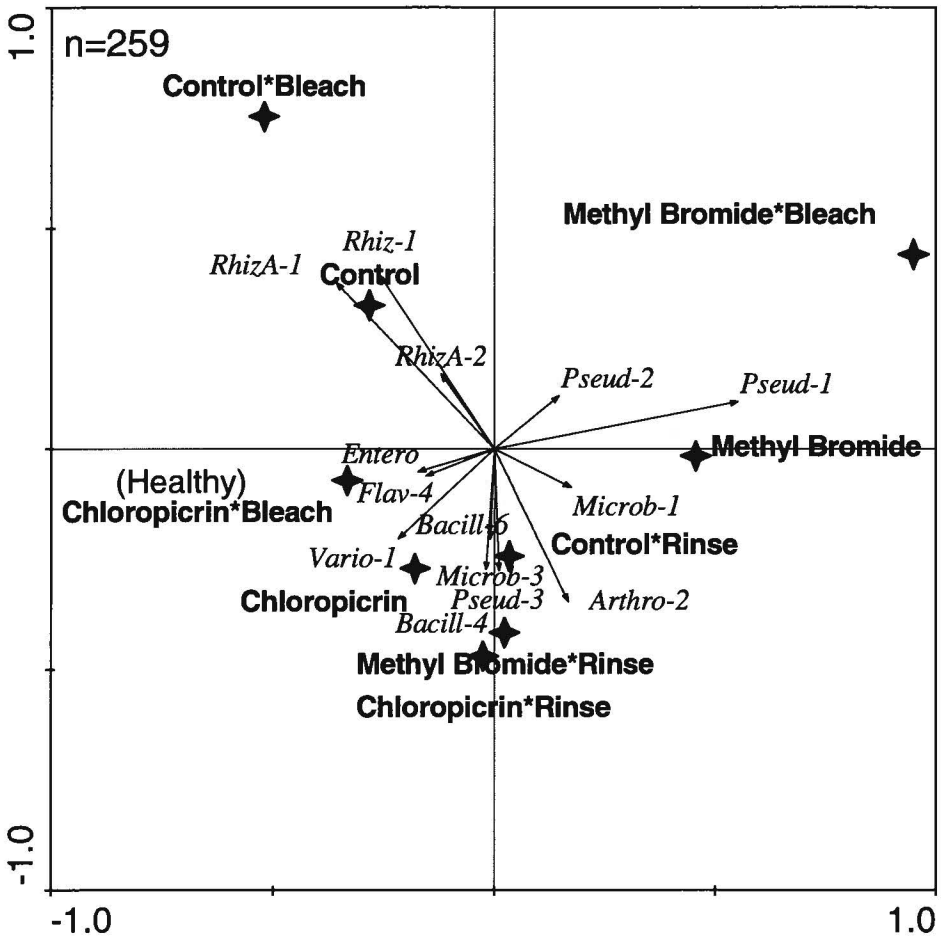

Fig. 2. Redundancy analysis ordination biplot of bacterial operational taxonomic units (OTUs) detected by culture based sampling and their relationship with environmental variables (pre-plant soil fumigation treatments and their combinations with treatments that roots were given before culture isolations) for the 2003 microplot trial near Parlier. All plants in control plots were affected by replant disease, while those in chloropicrin- or MB-fumigated plots were healthy. Genera discriminated (and unique intra-genus OTUs) included *Arthrobacter* (Arthro-2), *Bacillus* (Bacill-4, and Bacill-6), *Enterobacter* (Entero), *Flavobacterium* (Flav-4), *Microbacterium* (Microb-1 and Microb-3), *Pseudomonas* (Pseud-1, Pseud-2, and Pseud-3), *Rhizobium* (Rhiz-1), unclassified members of Rhizobiaceae (RhizA-1, and RhizA-2), and *Variovorax* (Vario-1). Axis 1 P=0.008, Axis 2: P=0.002.

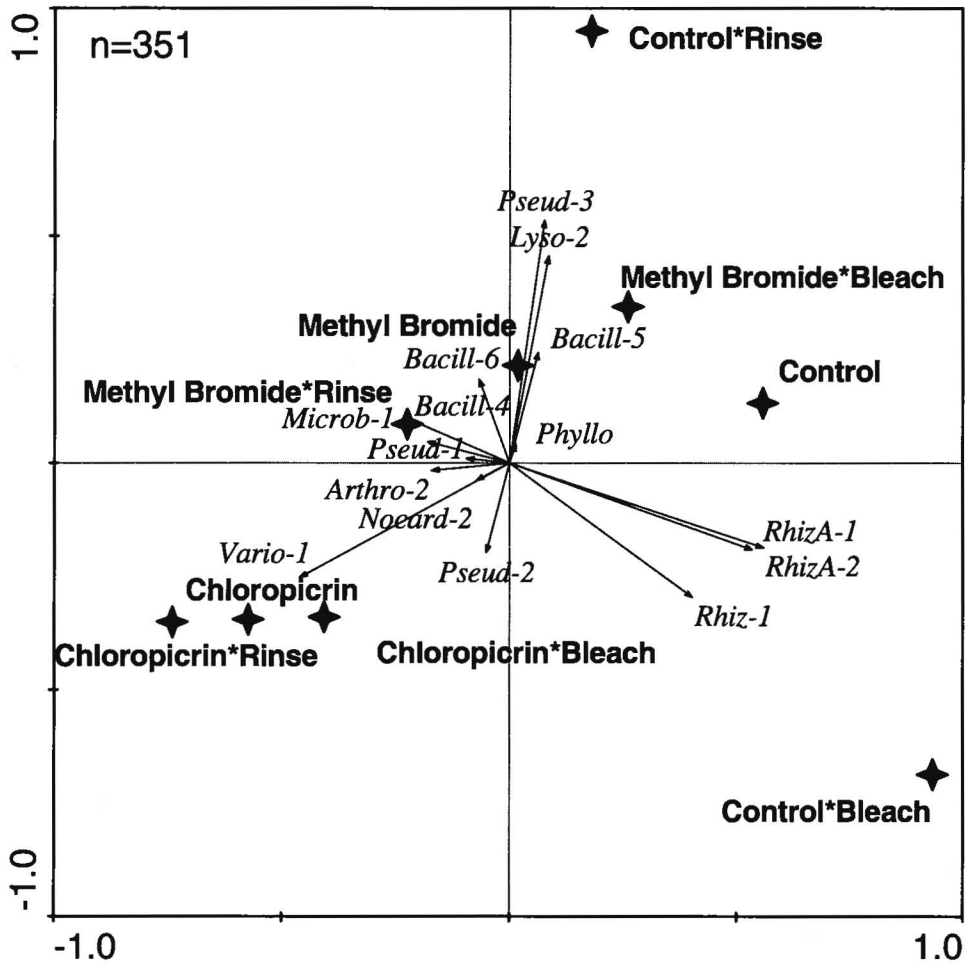

Fig. 3. Redundancy analysis ordination biplot of bacterial operational taxonomic units (OTUs) detected by culture-dependent sampling and their relationship to environmental variables (pre-plant soil fumigation treatments and their combinations with treatments that roots were given before culture isolations) for the 2004 microplot trial near Parlier. All plants in control plots were affected by replant disease, while those in chloropicrin-fumigated plots were healthy. OTUs were discriminated by PCR amplification and sequencing of 16S rDNA, followed by genetic-distance-based clustering of the sequences using DOTUR software. Genera discriminated (and unique intra-genus OTUs) included *Arthrobacter* (Arthro-2), *Bacillus* (Bacill-4, Bacill-5, and Bacill-6), *Lysobacter* (Lyso-2), *Microbacterium* (Microb-1), *Pseudomonas* (Pseud-1, Pseud-2, and Pseud-3), *Rhizobium* (Rhiz-1), unclassified members of Rhizobiaceae (RhizA-1, and RhizA-2), and *Variovorax* (Vario-1). Axis 1: P=0.002, Axis 2: P=0.002.

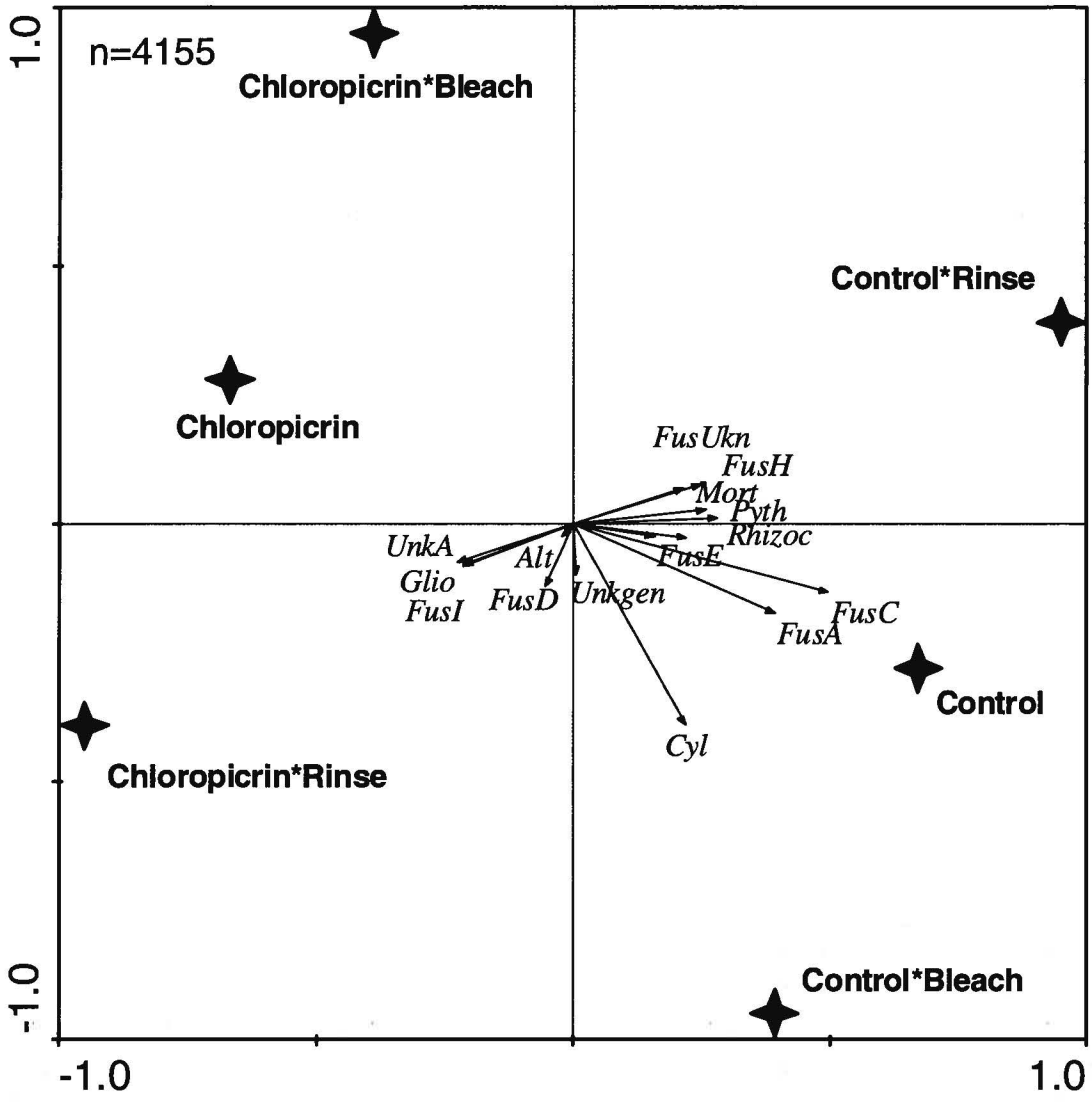

Fig. 4. Redundancy ordination analysis of fungal taxons detected by culture-based sampling of roots collected from healthy trees in chloropicrin-treated plots and diseased trees in non-fumigated plots near Durham, 2003-04. All plants in control plots were affected by replant disease, while those in chloropicrin-fumigated plots were healthy. For simplicity, the ordination was restricted to taxons representing at least 2% of the isolates in the population sample. A key to taxon abbreviations is given in Table 10. The ordination was significant at P=0.002 for each axis.

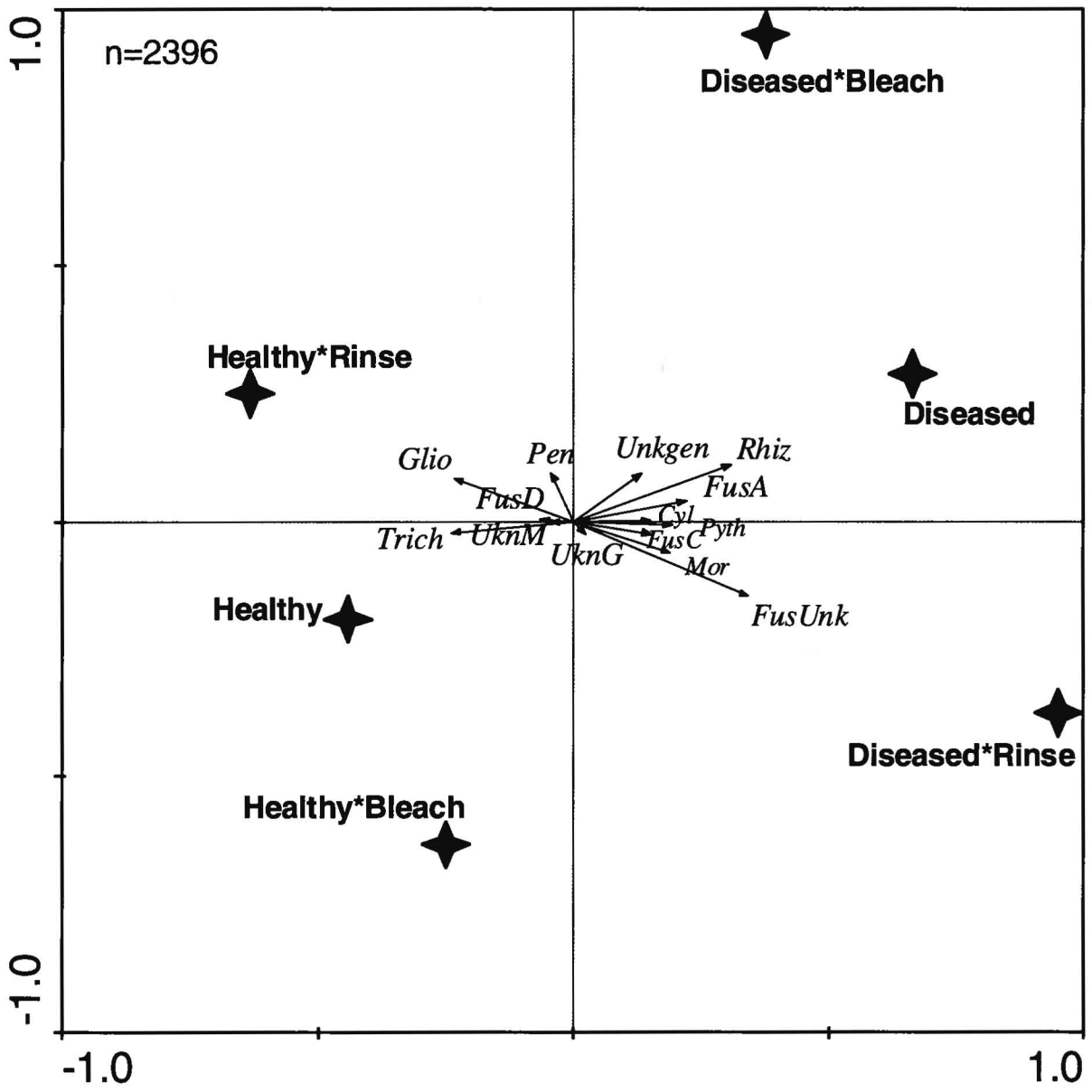

Fig. 5. Redundancy ordination analysis of fungal taxons detected by culture-based sampling of roots collected from healthy trees in chloropicrin-treated microplots (400 to 2500 lb/a) and microplots treated with a high rate of MB (2500 lb/a) and diseased trees in non-fumigated microplots and microplots treated with a standard rate of MB (400 lb/a) near Parlier, 2003-04. (The low rate of MB did not prevent RD). For simplicity, the ordination was restricted to taxons representing at least 2% of the isolates in the population sample. A key to taxon abbreviations is given in Table 11. The ordination was significant at P=0.002 for each axis.

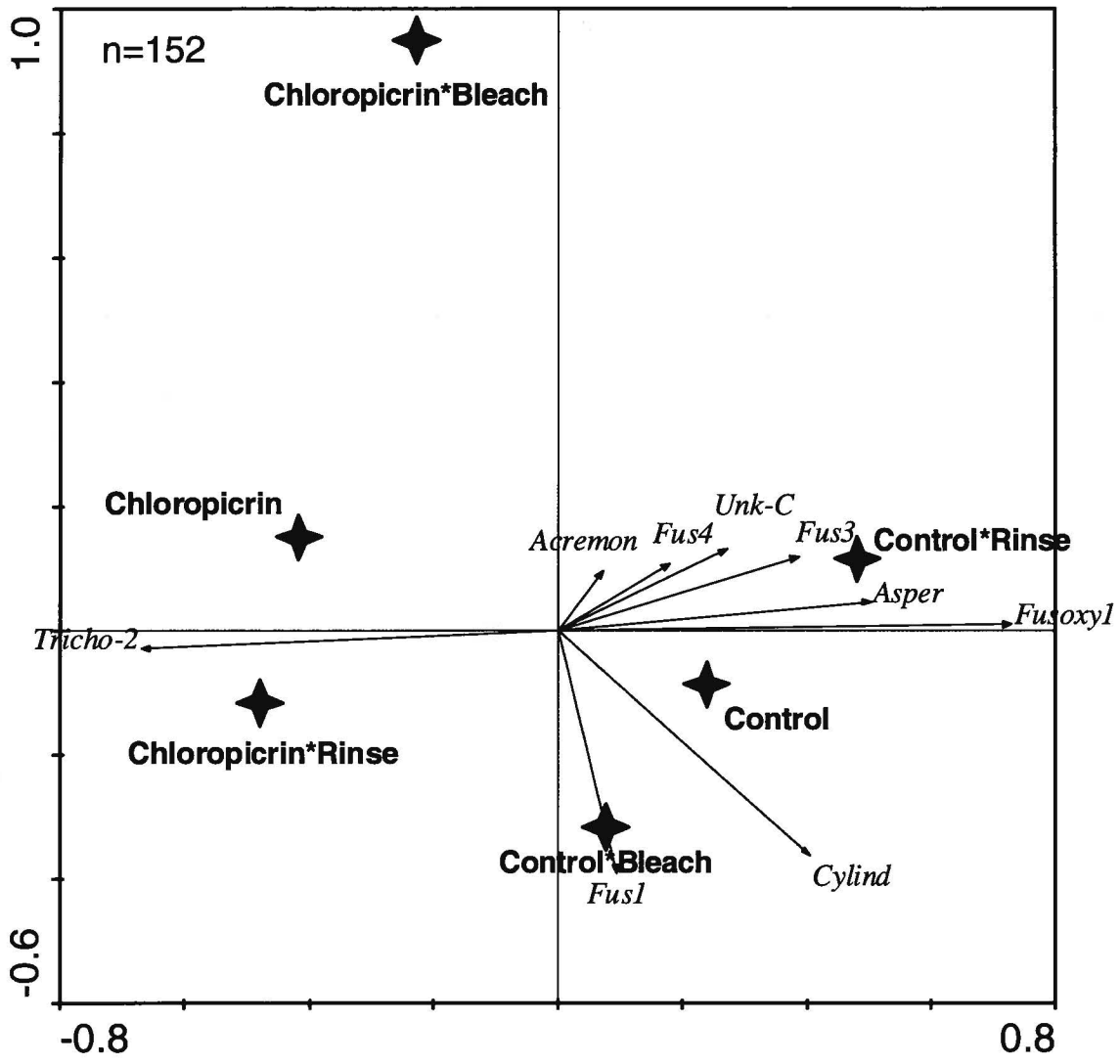

Fig. 6. Redundancy ordination analysis of fungal taxons detected by culture-based sampling of roots collected from healthy trees in chloropicrin-treated plots (400 lb/a) and diseased trees in non-fumigated plots near Firebaugh, 2006. All plants in control plots were affected by replant disease, while those in chloropicrin-fumigated plots were healthy. For simplicity, the ordination was restricted to taxons representing at least 2% of the isolates in the population sample. A key to taxon abbreviations is given in Table 12. The ordination was significant at P=0.004 for each axis.

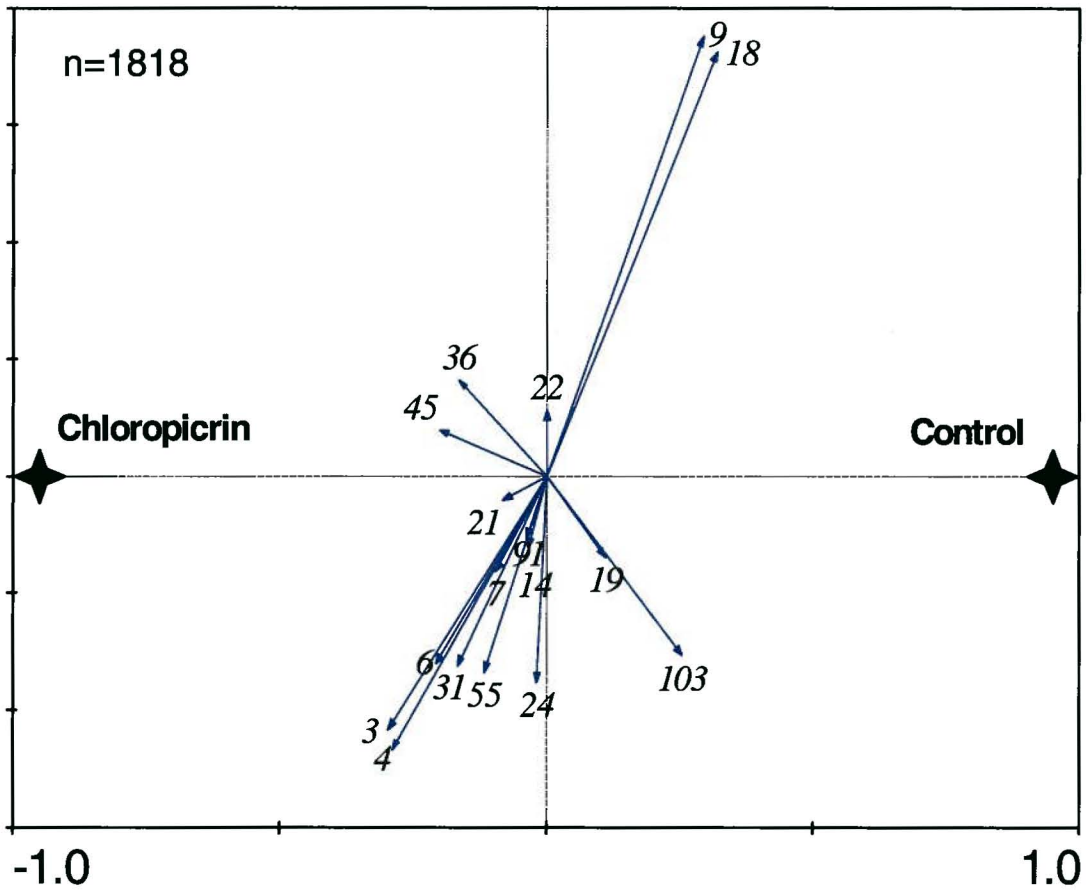

Fig. 7. Redundancy ordination analysis of fungal taxons detected by culture-independent PCR sampling of roots collected from healthy trees in chloropicrin-treated plots and diseased trees in non-fumigated plots near Firebaugh, 2006. All plants in control plots were affected by replant disease, while those in chloropicrin-fumigated plots were healthy. For simplicity, the ordination was restricted to taxons representing at least 0.6% of the isolates in the population sample. A key to taxon numbers is given in Table 13. The ordination was significant only at a level of P=0.10.

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