Replant Problems: Causes and Improved Strategies

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Project Leader: Greg T. Browne
USDA-ARS, Department of Plant Pathology
University of California - Davis
One Shields Ave.
Davis, CA 95616
(530) 754-9351
Gregory.Browne@ARS.USDA.GOV

Project Cooperators: L. Schmidt, D. Doll, R. Bhat, D. Kluepfel, B. Holtz, B. Lampinen, S. Upadhyaya, B. Hanson, D. Wang, S. Gao, J. Gerik, M. Shafii, S. Metcalf, V. Udompetaikul, J. Gartung, N. Goodell, C. Rose, R. Catania,D. Elam, J. Edstrom, S. Cutter, P. Domecq

Interpretive Summary:

The objectives of this research are to: 1) determine biological causes of and environmental contributions to replant disease (RD) of almond and 2) support development of new management strategies for RD and other replant problems. The approach to these objectives includes conducting replant trials in commercial and research orchards to evaluate the new management strategies and extensive use of root and soil samples from selected treatments in the field trials to determine the fundamental microbial causes of RD and environmental contributions to it. The approach used for Objective 1 is organized around completion of Koch's postulates for microbes associated with RD. Koch's postulates are the logical scientific steps required to establish a causal role for pathogen(s) in a disease. We have secured funding assistance for the field trials from the USDA-ARS Pacific Area-Wide Pest Management Program for Methyl Bromide Alternatives (PAW-MBA).

Under Objective 1 for 2007/08, we tested pathogenicity of bacteria that were previously found to be positively or negatively associated with RD in microplots near Parlier, CA and commercial orchards in Butte County (see Browne et al. report to Almond Board, 2007). The pathogenicity trial was conducted in a greenhouse in fall 2007. In the trial, none of the bacteria caused measurable disease or growth stimulation on Nemaguard peach seedlings. The negative results were obtained following root dip and soil drench treatments with the bacteria, and no significant effect of the inoculants was observed, whether they were administered to plants growing in non-autoclaved RD soil: sand mixture (i.e., 2 parts soil collected around Nemaguard rootstock affected by RD, 1 part sterile sand, v:v), or autoclaved RD soil:sand mixture (i.e., autoclaved 2:1 RD soil:sand).

Symptoms of RD (growth stunting, root cortex necrosis), developed on the Nemaguard seedlings grown in non-autoclaved soil, regardless of whether the soil received a bacterial inoculant or not. Conversely, no symptoms or RD developed on the seedlings grown in the autoclaved soil mixture, regardless of bacterial inoculant. Therefore, the pathogenicity test provided no evidence for a causal role of bacteria in almond RD, yet it confirmed that heat-sensitive elements of the soil system can induce the disease. A repeat of this trial is underway. In previous years, greenhouse pathogenicity tests provided preliminary evidence that several fungi and oomycetes associated with RD in replant trials near Parlier and Chico (i.e., *Cylindrocarpon* sp., *Fusarium* sp., *Humicola* sp., and *Pythium* spp.) can induce root cortex necrosis, a symptom of RD (Browne et al. reports to the Almond Board, 2004-06). In the 2007/08 period, we worked towards confirming fungal associations with the disease and fungal pathogenicity on Nemagurad rootstock. This began with root isolations for and identifications of fungi and oomycetes from the fine roots ($\leq$ 1 mm dia) of replicate healthy and RD-affected trees (in chloropicrin-fumigated and non-fumigated plots, respectively) in a replant trial started in 2006 near Firebaugh. Additional isolations were conducted from roots of replicate healthy and RD-affected Nemaguard peach seedlings that had been grown in a greenhouse trial in pots of autoclaved and non-autoclaved mixtures of RD soil, respectively. The RD soil had been collected from a Parlier replant site for use in the greenhouse trial. For all of the isolations, half of the root sample pieces from each tree were subjected to rinsing alone, while the other root pieces were subjected to an ethanol dip (70% solution, 1 sec) and bleaching (0.6% sodium hypochlorite, 1 min) before rinsing in sterile water. The former treatment favored isolation of organisms from the rhizosphere and external surfaces of the roots, while the latter treatment favored isolation of organisms from interior portions of the root. The fungi and oomycetes detected in the samples from the Firebaugh and greenhouse trials were grouped according to their morphology, hyphal tipped to ensure purity, and subjected to PCR and sequencing of their rDNA to determine their identity. Data matrices summarizing incidence of each of the organisms on each health class of roots were prepared and subjected to ordination analysis (i.e., a statistical method for plotting and analyzing community shifts associated with environmental or biological conditions). The ordinations were statistically significant ($P=0.002$) and associated RD with incidence of *Cylindrocarpon* sp., *Pythium* sp., several *Fusarium* spp., and other fungi, whereas healthy root status was associated with incidence of *Trichoderma* and *Chaetomium* spp. Inocula of the fungi positively and negatively associated with RD have been prepared and will be used shortly for a pathogenicity trial.

Previously, culture-independent PCR analysis of bacterial and fungal communities from healthy and RD-affected trees in Chico and Parlier area replant trials did not reveal additional bacteria or fungi associated with RD, compared to those detected with culture-based isolations (see report to the Almond Board, Browne et al 2007). Nevertheless, it is important to repeat the culture-independent work at additional RD sites just as we have done for the culture-dependent isolations. We have initiated the culture-independent examination of root samples from the Firebaugh replant trial described above.

Under Objective 2, we established three field trials including treatments with an improved version of Shrini Upadhyaya's GPS-controlled spot fumigation system and one trial including spot drip fumigation treatments. The spot treatments, which applied chloropicrin, Telone C35, or Inline, were compared with shank-applied strip and broadcast treatments with methyl bromide, Telone II, Telone C35 and Midas (50% iodomethane: 50% chloropicrin). The preliminary data from these four trials confirm that, at least in early years after planting when RD is most severe, shank-applied spot treatments are as effective as conventional shank-applied strip and broadcast treatments for preventing the disease. The drip-applied spot treatments, although helpful, were less effective than shank-applied treatments. The zone of treated soil apparently was too small in spot drip plots. It is likely that the spot drip treatment can be improved; in a 2005 trial use of larger drip treatment zones was effective. Improving spot treatments and determining their limitations are critical due to the current regulatory mandate to reduce fumigant emissions. Two of the replant trials also are examining use of short-term crop rotations as a means to reduce reliance on soil fumigation and improve replanted orchard performance. Preliminary data from these trials indicate significant tree growth benefit resulting from sudan rotation, both in combination with pre-plant soil fumigation and without soil fumigation.

Objectives:

1. Determine biological causes of and environmental contributions to replant disease (RD).
2. Support development of new management strategies for RD and other replant problems.

Materials and Methods:

Objective 1

Testing pathogenicity of bacteria positively or negatively associated with RD. Bacterial inocula for greenhouse pathogenicty tests consisted of 18-day-old 10% Tryptic Soy broth (TSB) cultures of individual bacterial taxons determined by ordination analyses to be positively associated with RD (i.e., isolated more frequently from trees affected by RD), or negatively associated with the disease (i.e., isolated more frequently from healthy trees). The concentration of bacteria in the broth cultures was adjusted to approximately10^8 cells/ml for plant inoculations. Three-week-old Nemaguard peach seedlings growing in 2 x 4-cm cells in trays were uprooted gently and the roots were dipped in cups of the bacterial inoculants for approximately 1 min. The seedlings then were transplanted into 1-liter pots containing either 1) a mixture of 2 parts autoclaved Hanford Sandy Loam collected from an RD-affected orchard near Parlier and 1 part autoclaved sand (v:v), or 2) a mixture consisting of the same ingredients and proportions, except that the field soil was left non-autoclaved. There were five replicate seedlings per treatment combination, arranged in randomized complete blocks. After the transplanting, each seedling received a root drench; a 10-ml aliquot of the broth culture that had been used for root dipping was applied to the soil around the base of the plant's stem. Control seedlings received root dips and drenches with sterile 10%

TSB. The peach seedlings were watered as needed. Two months after the inoculations, the roots were rinsed free from the soil, and effects of the bacterial inoculants and soil treatments were assessed according to severity of root cortex necrosis and shoot and root fresh weights.

Examining fungi and oomycetes associated with RD. We previously associated several species of fungi and *Pythium* (although *Pythium* is fungal-like, it is classified as an oomycete), with RD in Chico and Parlier trials, but, because an essential step in Koch's postulates is establishing a consistent association between suspect pathogens and the disease of interest, we worked towards confirming the disease association with specific fungi in a new replant trial located near Firebaugh. The trial received pre-plant fumigation treatments in October 2006 and was replanted in Jan. 2007 (2007 report to the Almond Board, Browne et. al). In June 2007 and again in August 2007 we sampled feeder roots (roots $\leq$ 1 mm diameter) from RD-affected and healthy trees in non-fumigated and fumigated plots of the trial, respectively. Roots were collected from six replicate trees in each health class on each sampling date. (Results of the sampling in June 2007 were partially reported on last year but are presented in this report due to refinements in the fungal identifications). The root samples were used for isolations of fungi on water agar amended with ampicillin and isolations of oomycetes on PARP (corn meal agar amended with pimaricin, ampicillin, rifampicin and pentachloronitrobenzene) as described previously (2005 Comprehensive Report to the Almond Board, Browne et al.). As in previous isolations, before culturing, the root pieces were either 1) rinsed only in distilled water or 2) dipped in 70% ethanol for 1 sec and soaked for 1 min 0.6% in sodium hypochlorite before rinsing in water; the former treatment favors isolation of organisms plentiful near the root surface whereas the latter treatment favors isolation of organisms from the interior portions of the root. All true fungi isolated were subcultured and identified from the June 2007 samples (oomycetes were not quantified for this sampling date), and all oomycetes as well as fungi were subcultured from the August 2007 samples. The isolates were grouped and identified according to morphological characteristics (i.e., colony and spore types) and sequences in their rDNA. The rDNA was amplified using PCR primers ITS 1 and 4 based on our experience that the NCBI sequence database is best represented for fungi for DNA sequences between these priming sites. After the fungi were identified, the data matrices tabulating incidence of each organism for roots from each replicate healthy and diseased tree were prepared and subjected to redundancy analysis (RDA, which is a type of ordination method useful for visualizing community shifts associated with environmental variables).

In addition to the fungal isolations and identifications from the replant plots near Firebaugh, we also conducted similar isolations from selected plants in the bacterial pathogenicty test (described above) at the trial's conclusion. For the latter, RD-affected Nemaguard roots were sampled and used for isolations from five replicate plants exposed to the mixture of non-autoclaved RD soil and sand (only the control plants that did not receive a bacterial inoculant were sampled), and five replicate plants exposed to the autoclaved soil:sand mixture that did not receive a bacterial inoculant.

Testing for fungal and oomycete pathogenicity. In an approach similar to that used for the bacterial pathogenicity tests, isolates of fungi and *Pythium* sp. determined by RDA to be positively or negatively associated with RD will be tested for pathogenicity in a greenhouse. Pathogenicity of the isolates will be assessed according to growth of the Nemaguard peach seedlings and health of their roots two to 3 months after inoculation. Two mixtures of corn meal: sand mixture has been used to grow the required inocula.

Objective 2

Four new replant trials were planted in the 2007/08 project cycle. The trials and treatments they emphasize are 1) the Nickels Soil Lab trial near Arbuckle, which is focused on GPS-controlled spot fumigation treatments in comparison with conventional strip treatments; 2) the Madera trial, which includes GPS-controlled spot, conventional strip, and broadcast treatments with varied fumigants and rates; 3) an almond replant trial at USDA-ARS Parlier, which tests factorial combinations of pre-plant cropping treatments with shank fumigation; and 4) a peach replant trial at USDA-ARS Parlier, which is evaluating factorial combinations of spot and strip fumigation treatments with a rotation of sudan grass.

All but one of the fumigation treatments were applied in October 2007 by TriCal, Inc. with shanks at 18" depth beneath the soil surface under conditions (e.g., soil moisture, soil sealing methods, etc.) required for commercial applicators. The exception was a drip-applied spot treatment with Inline (a 61:35 mixture of 1,3-dichloropropene and chloropicrin). This treatment was applied along with the shank treatments in October 2007, but it was administered through a resident microirrigation system connected with "spaghetti-tube spikes" at each site to be planted with a tree the following January (Fig. 1). Trees for the Nickels trial were planted in April 2008, while those for the other three trials were planted in Jan. 2008. Initial trunk diameters were determined shortly after planting to facilitate growth assessments at the end of the 2008 growing season. Depending on the trial, there are five to six replicate plots (each containing four to 30 trees per treatment combination).

Results and Discussion:

Objective 1

Testing pathogenicity of bacteria positively or negatively associated with RD. None of the tested bacteria caused measurable disease or growth stimulation on Nemaguard peach seedlings (Table 1). No significant effect of the inoculants was observed whether they were administered to plants growing in the non-autoclaved RD soil: sand mixture or the autoclaved RD soil: sand mixture. Symptoms of RD (growth stunting, root cortex necrosis) occurred on the Nemaguard seedlings in non-autoclaved soil, regardless of whether or not the seedling and soil received a bacterial inoculant (Tables 1,2). Conversely, no symptoms or RD occurred on seedlings in the autoclaved soil mixture, regardless of bacterial inoculant. Therefore, results of the pathogenicity test provided no evidence for a causal role of bacteria in almond RD, but they confirmed that heat-sensitive elements of the soil system can induce RD. A repeat of this trial is underway.

Examining fungi and oomycetes associated with RD. Redundancy analysis of fungal and oomycete community composition changes related to RD in the root samples collected from the Firebaugh replant trial in June (Fig. 2) and August (Fig. 3) yielded a significant ordinations (P=0.002). As indicated in Fig. 2 and 3 by the graphical proximity of the taxon abbreviations to the treatment centroids (triangle symbols) for the Control*Bleach and Control*Rinse treatments, fungi isolated preferentially in one or both sampling periods from RD-affected trees included *Cylindrocarpon* sp. (Cyl), *Fusarium solani* (three types, Fus.sol1, Fus.sol2, and Fus.sol3), *Fusarium* sp. (Fus.sp), *F.incarnatium* (Fus.icar), *Psathyrella* (Psa), *Nectria* sp. (Nec.sp), *Aspergillus* (Asp), *Pythium* (Pyth) and an unknown fungus (Unk.z). Conversely, fungi isolated preferentially from the healthy root samples included *Chaetomium globosum* and *Chaetomium* sp. (Chae.glo and Chae.sp, respectively) and *Trichoderma* sp. (Trichod).

Redundancy analysis of the fungal and oomycete community composition changes related to RD in the root samples collected from the greenhouse pathogenicity trial also revealed a significant ordination (*P=0.0002*, Fig. 4). *Cylindrocarpon* spp. (Cyl1, Cyl2), *Fusarium oxysporum* (Fus.oxy2, Fus.oxy3), *Rhizoctonia* sp. (Rhizoc), *Fusarium* sp. (Fus1) *Acremonium* sp. (Acrem, Acrem2), *Pythium* sp. (Pyth, Pyth2), and *Humicola* sp. (Hum) were isolated preferentially from the diseased roots whereas *Penicillium* (Pen), *Trichoderma* sp. (Trich1), *Mucor* sp. (Mucor), *Cladosporium* sp. (Cladoac) and *Ulocladium* sp. (Ulo) were isolated preferentially from healthy roots (Fig. 4).

Preparing for tests of fungal and oomycete pathogenicity. Inocula of representative isolates that were positively and negatively associated with RD based on the analyses described above have grown satisfactorily in corn meal-sand mixture and will be used to establish a pathogenicity trial in the first week of September (see inoculants and treatments planned (Table 3). Results from this trial should be available in late fall 2008.

Objective 2

Among the four replant trials given pre-plant treatments in October 2007, only the one at the Nickels location is not responding significantly to pre-plant soil fumigation treatments (Table 4). Recent disease ratings have not been taken for the Madera trial (Table 5), but observations in June and July indicated incidence of RD in non-treated control plots and positive responses to some of the fumigation treatments. At each of the Parlier replant trials there were significant main effects of pre-plant fumigation treatments and to pre-plant crop rotation (Tables 6,7). All of the preliminary observations and disease ratings for these trials will be followed by disease ratings in late summer and trunk circumference measurements during dormancy, which will provide a more solid basis than the preliminary disease ratings for treatment evaluation. Ultimately, yield assessments and economic analyses conducted under the PAW-MBA program will afford further practical assessments of these treatments.

Table 1. Lack of pathogenicity of bacterial isolates on Nemaguard peach, fall 2007 greenhouse trial*

Bacterial inoculant	Soil autoclaving	Shoot fresh ht. (cm)	Root fresh wt. (g)	Estimated % of root length with cortex necrosis
Control	-	3.4	3.8	82
	+	6.3	8.1	16
Flavobacterium sp.	-	2.9	3.2	68
	+	7.1	8.2	19
Pseudomonas sp.1	-	4.0	4.0	86
	+	7.2	8.4	23
Pseudomonas sp.c	-	3.8	4.8	88
	+	7.3	9.5	14
Rhizobium sp.1	-	2.8	3.8	72
	+	6.1	8.2	22
Rhizobium sp.c	-	3.0	2.9	83
	+	6.0	6.8	42
Rhizobium sp.A-1	-	3.2	3.4	86
	+	7.3	8.4	17
Rhizobium sp.A-c	-	3.1	3.8	83
	+	5.8	8.2	18
Variovorax sp.1	-	3.7	4.0	75
	+	5.6	8.1	17
Variovorax sp.c	-	3.7	3.7	85
	+	5.4	7.3	24

*Effect of bacterial inoculant not significant (P=0.27, 0.31, and 0.32 for shoot wt., root wt., and % root cortex necrosis, respectively); effect of soil autoclaving highly significant (P<0.001 for all variables); and effect of bacterial inoculant × soil treatment not significant (P=0.40, 0.99, and 0.4 for shoot wt., root wt., and % root necrosis, respectively).

Table 2. Main effect of soil autoclaving on health and growth of Nemaguard peach seedlings, fall 2007 greenhouse trial*

Soil treatment	Shoot fresh wt. (g)	Root fresh wt. (g)	Estimated % of root length with cortex necrosis
Not Autoclaved	3.4 a	3.7 a	81 a
Autoclaved	6.4 b	8.1 b	21 b

*Effect of soil autoclaving highly significant (P<0.0001 for all variables).

Table 3. Design of pathogenicity trial for isolates negatively or positively associated with replant disease in the 2007 Firebaugh and greenhouse trials

Inoculant (isolate)	Source of inoculant	Pre-plant soil autoclaving	Planned rate of inoculant and substrate addition to soil
Cylindrocarpon sp.1	Firebaugh trial	+/-	1, 5, and 10%
Cylindrocarpon sp.1	Firebaugh trial	+/-	1, 5, and 10%
Cylindrocarpon sp.1	Firebaugh trial	+/-	1, 5, and 10%
Cylindrocarpon sp.1	Parlier soil / GH trial	+/-	1, 5, and 10%
Cylindrocarpon sp. 2	Parlier soil / GH trial	+/-	1, 5, and 10%
Fusarium solani	Firebaugh trial	+/-	1, 5, and 10%
F.solani	Firebaugh trial	+/-	1, 5, and 10%
F.solani	Firebaugh trial	+/-	1, 5, and 10%
F. oxysporum	Firebaugh trial	+/-	1, 5, and 10%
F. oxysporum	Parlier soil / GH trial	+/-	1, 5, and 10%
Fusarium sp. 1a	Parlier soil / GH trial	+/-	1, 5, and 10%
Fusarium sp. 2	Parlier soil / GH trial	+/-	1, 5, and 10%
Fusarium sp.3	Firebaugh trial	+/-	1, 5, and 10%
Fusarium sp. 5b	Parlier soil / GH trial	+/-	1, 5, and 10%
Fusarium sp. ("mac + chl")	Firebaugh trial	+/-	1, 5, and 10%
Fusarium sp. (“no mac.)	Firebaugh trial	+/-	1, 5, and 10%
Fusarium sp. ("web.")	Parlier soil / GH trial	+/-	1, 5, and 10%
Fusarium sp.(“web.”)	Firebaugh trial	+/-	1, 5, and 10%
Fusarium sp. ("wig.")	Firebaugh trial	+/-	1, 5, and 10%
Humicola sp.	Parlier soil / GH trial	+/-	1, 5, and 10%
Humicola sp.	Parlier soil / GH trial	+/-	1, 5, and 10%
Humicola sp.	Parlier soil / GH trial	+/-	1, 5, and 10%
Humicola sp.	Parlier soil / GH trial	+/-	1, 5, and 10%
Macrophomina sp.	Parlier soil / GH trial	+/-	1, 5, and 10%
Mortierella sp.	Firebaugh trial	+/-	1, 5, and 10%
Pythium sp.	Firebaugh trial	+/-	1, 5, and 10%
Pythium sp.	Firebaugh trial	+/-	1, 5, and 10%
Pythium sp.	Parlier soil / GH trial	+/-	1, 5, and 10%
Pythium sp.	Parlier soil / GH trial	+/-	1, 5, and 10%
Pythium sp.	Parlier soil / GH trial	+/-	1, 5, and 10%
Pythium sp.	Parlier soil / GH trial	+/-	1, 5, and 10%
Rhizoctonia sp.	Parlier soil / GH trial	+/-	1, 5, and 10%
Trichoderma sp.	Firebaugh trial	+/-	1, 5, and 10%
Trichoderma sp.	Firebaugh trial	+/-	1, 5, and 10%
Unkn. basidiomycete	Firebaugh trial	+/-	1, 5, and 10%

Table 4. Treatments and preliminary disease ratings in Nickels Estate trial, Arbuckle*

Fumigant, rate per treated acre	Treated area	Fumigant per orchard acre (lb)	Disease severity rating (0 to 5 scale)
Control		0	0.6
MB 98:2, 400 lb/a	Row strip (41%)	166	0.6
Telone II, 340 lb/a	Row strip (41%)	141	0.6
Pic 60, 400 lb/a	Row strip (41%)	166	0.6
Pic 60, 400 lb/a	Tree site (12%)	50	0.5

*No significant treatment effects

Table 5. Treatments included in 2007 replant trial, Madera

Trt.	Fumigant, rate per treated acre	Treated area	Fumigant / orchard acre (lb)
m1	Control	None	0
m2	Methyl bromide, 400 lb/ac	Row strip (38%)	152
m3	Telone II, 340 lb/ac	Row strip (38%)	129
m4	Iodomethane:chloropicrin (50:50), 400 lb/ac	Row strip (38%)	152
m5	Chloropicrin, 400 lb/ac	Row strip (38%)	152
m6	Chloropicrin, 300 lb/ac	Row strip (38%)	114
m7	Chloropicrin, 200 lb/A	Row strip (38%)	76
m8	Telone C35, 544 lb/ac	Row strip (38%)	207
m9	Pic-clor 60, 400 lb/ac	Row strip (38%)	152
m10	Chloropicrin, 400 lb/ac	Tree square (11%)	44
m11	Telone C35, 544 lb/ac	Tree square (11%)	60
m12	Telone C35, 544 lb/ac	Broadcast (100%)	544
r1	Control	Row strip (38%)	0
r2	Pic-clor 60, 100 lb/a	Row strip (38%)	38
r3	60, 200 lb/a	Row strip (38%)	76
r4	60, 300 lb/a	Row strip (38%)	114
r5	60, 400 lb/a	Row strip (38%)	152
r6	60, 400 lb/a	Tree square (11%)	44
r7	Control	Row strip (38%)	0
r8	Iodomethane:chloropicrin 50:50, 100 lb/a	Row strip (38%)	38
r9	Iodomethane:chloropicrin 50:50, 200 lb/a	Row strip (38%)	76
r10	Iodomethane:chloropicrin 50:50, 300 lb/a	Row strip (38%)	114
r11	Iodomethane:chloropicrin 50:50, 400 lb/a	Row strip (38%)	152
r12	Iodomethane:chloropicrin 50:50, 400 lb/a	Tree square (11%)	44

[a]Trial site was cleared almond orchard. Replanted to almond on Nemaguard rootstock Jan 2008.

Table 6. Preliminary disease ratings for fumigation × sudan rotation trial at USDA-ARS, Parlier*

Fumigation treatment (Oct 2007)	Fumigant per treated acre (lbs)	Fumigant per orchard acre (lbs)	Sudan grass rotation (Jul-Sep 2007)	Disease severity rating (0 to 5 scale) (7 Jul 2008)
Control	0	--	no	1.5
			yes	0.9
Telone C35, by GPS-controlled shanks to 5x 6' tree spots	540	81	no	0.7
			yes	0.5
Telone C35, by conventional shanks to 8'-wide row strips	540	227	no	0.3
			yes	0.3
Chloropicrin, by GPS-controlled shanks to 5x6' tree spots	400	60	no	0.6
			yes	0.2
Inline, by single drip emitters to 4'-dia. tree spots	540	43	no	0.8
			yes	0.6
MB, by conventional shanks to 8'-wide row strips	400	168	no	0.9
			yes	0.5
None, yeast extract root spray and drench at planting	0	--	no	1.2
			yes	1.0

* Disease rating scale: 0= no disease, vigorous growth; 1= moderate stunting; 2= severe stunting; 3=severe stunting and wilting or leaf discoloration; 4= tree near death; 5=dead tree. The main effect of fumigation treatment was significant at *P<0.0001*, the main effect of crop rotation treatment was significant at *P=0.001*, and the interaction of fumigation × crop rotation treatment was not significant (*P=0.7*).

Table 7. Preliminary disease ratings for fumigation × crop rotation trial at USDA-ARS, Parlier*

Pre-plant fumigation treatment	Pre-plant crop rotation	Disease severity rating 7 Jul* 2008
Control	Peach	0.9
	Fallow	0.7
	Mustard	0.7
	Wheat-Sudan	0.5
Chloropicrin 400 lb/A	Peach	0.4
	Fallow	0.3
	Mustard	0.2
	Wheat-Sudan	0.2

*Disease rating scale: 0= no disease, vigorous growth; 1= moderate stunting; 2= severe stunting; 3=severe stunting and wilting or leaf discoloration; 4= tree near death; 5=dead tree.
The main effect of fumigation treatment was significant at *P=0.06*, the main effect of crop rotation treatment was significant at *P=0.009*, and the interaction of fumigation × crop rotation treatment was not significant (*P=0.6*).

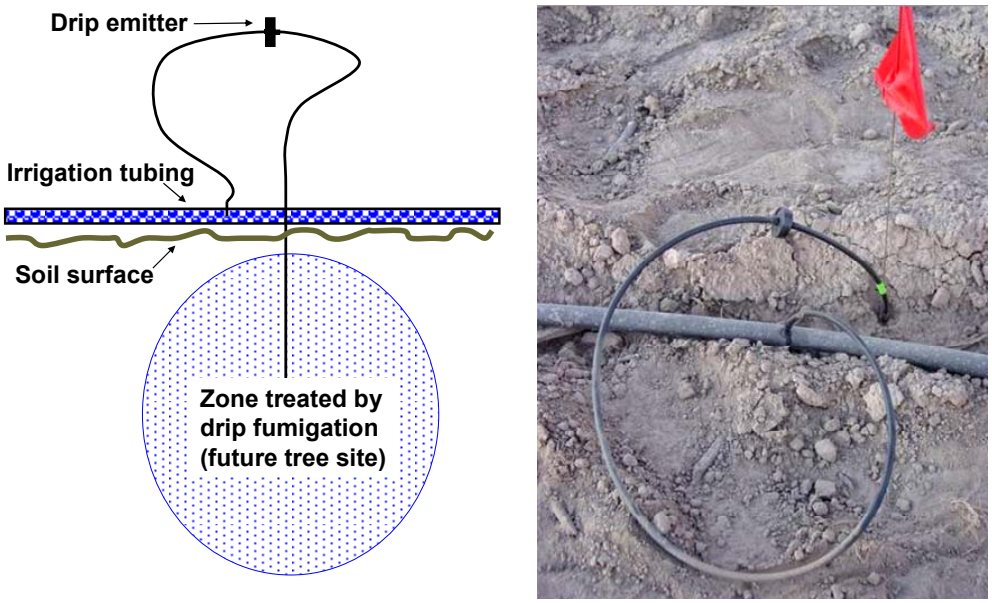

Fig. 1. Side-view diagram (left) and field photo (right) of spot drip fumigation set up used for treating tree sites (*experimental only*).

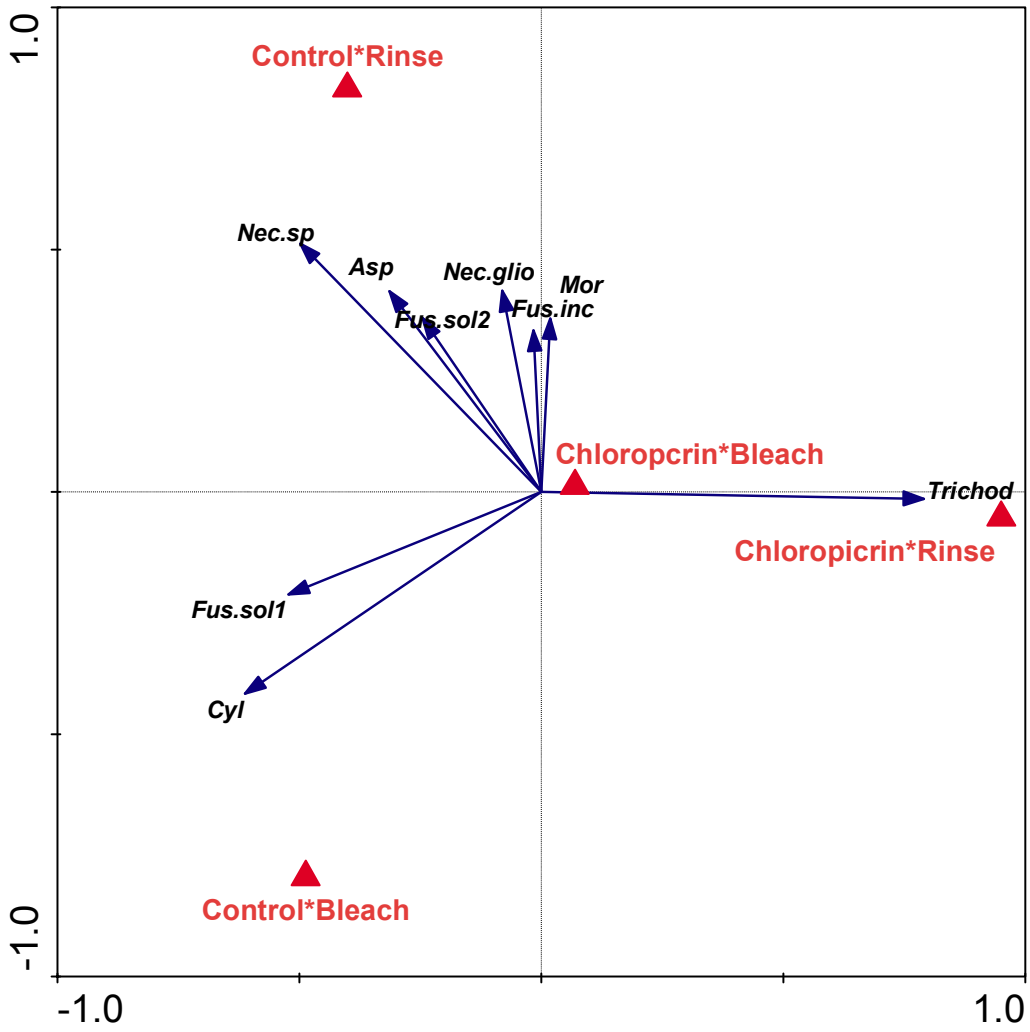

Fig. 2. Graphical representation of redundancy analysis ordination for fungi isolated from roots collected in June 2007 from RD-affected trees in non-fumigated control plots and healthy trees in chloropicrin-fumigated plots. Roots (≤ 1 mm dia) were sampled from six trees per treatment. The sampled roots were either rinsed in sterile water alone (represented by the triangular treatment symbols with "Rinse" suffix) or dipped in 70% ethanol for 1 sec and 0.6% sodium hypochlorite for 1 min prior to rinsing in sterile water (represented by the triangular treatment symbols with "Bleach" suffix). For details, please see Results section.

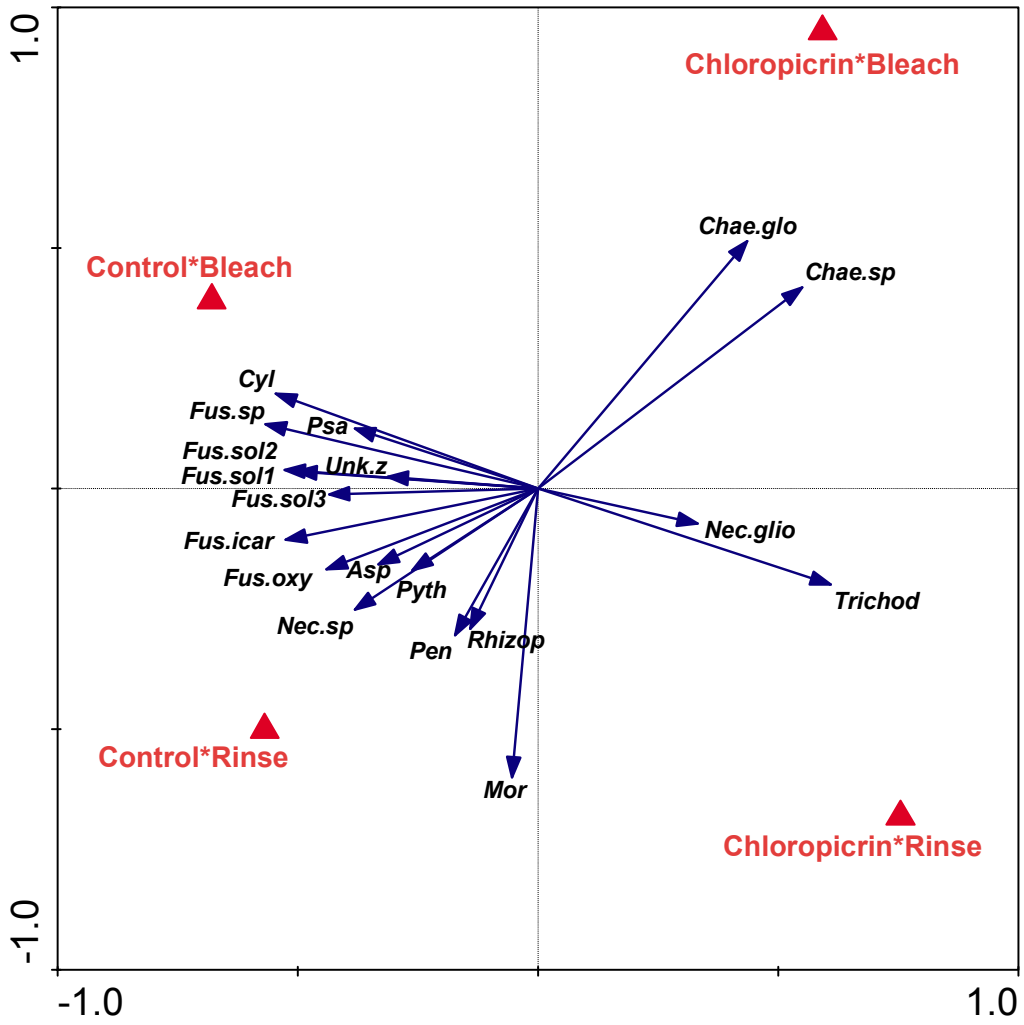

Fig. 3. Graphical representation of redundancy analysis ordination for fungi isolated from roots collected in August 2007 from RD-affected trees in non-fumigated control plots and healthy trees in chloropicrin-fumigated plots. Roots (≤ 1 mm dia) were sampled from six trees per treatment. The sampled roots were either rinsed in sterile water alone (represented by the triangular treatment symbols with "Rinse" suffix) or dipped in 70% ethanol for 1 sec and 0.6% sodium hypochlorite for 1 min prior to rinsing in sterile water (represented by the triangular treatment symbols with "Bleach" suffix). For details, please see Results section.

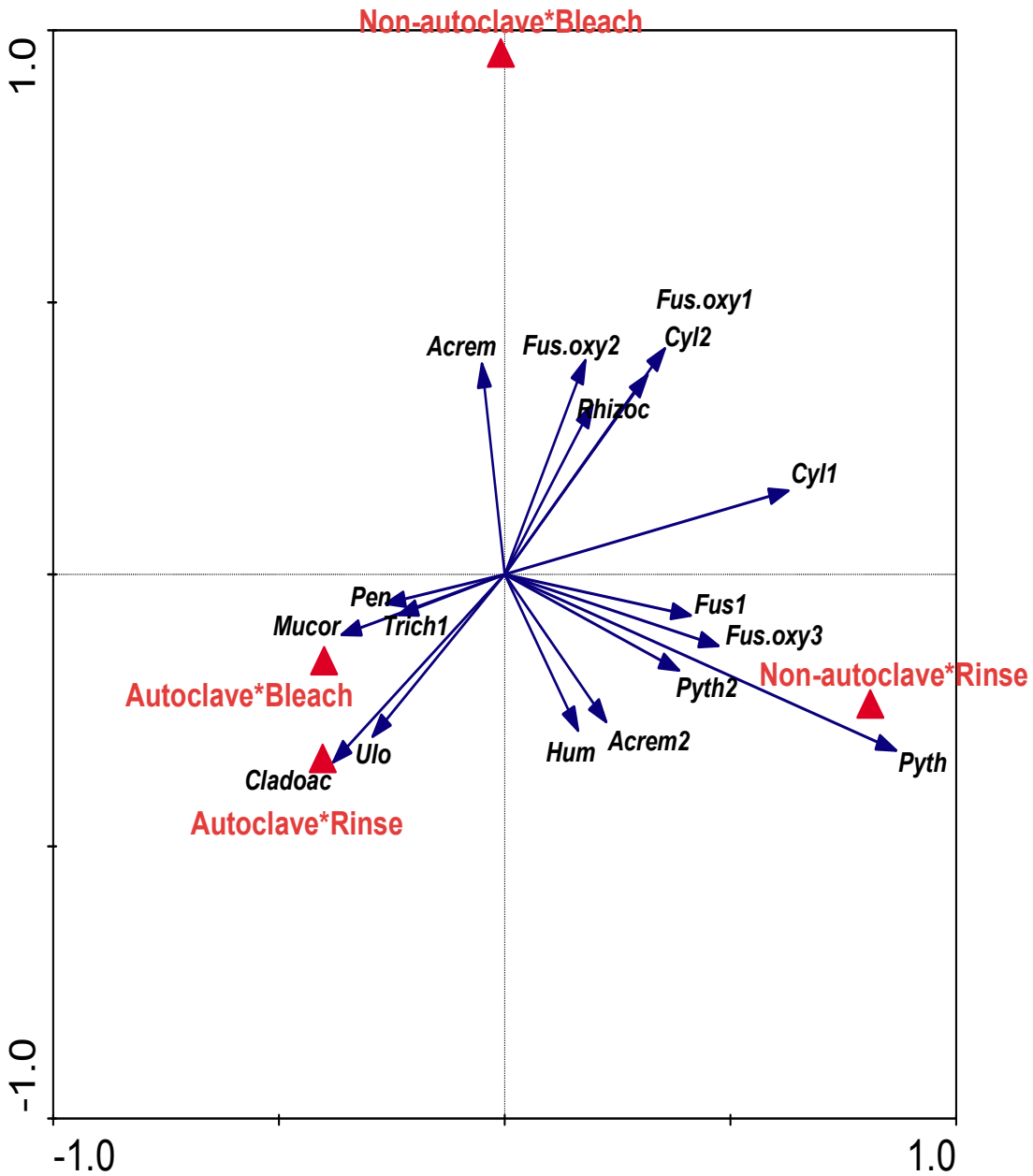

Fig. 4. Graphical representation of redundancy analysis ordination for fungi isolated from roots collected in November 2007 from RD-affected Nemaguard peach seedlings grown in non-autoclaved RD soil mixture and healthy Nemaguard seedlings grown in autoclaved RD soil mixture. Roots (≤ 1 mm dia) were sampled from five seedlings per treatment. The sampled roots were either rinsed in sterile water alone (represented by the triangular treatment symbols with "Rinse" suffix) or dipped in 70% ethanol for 1 sec and 0.6% sodium hypochlorite for 1 min prior to rinsing in sterile water (represented by the triangular treatment symbols with "Bleach" suffix). For details, please see Results section.

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