BIOLOGY AND MANAGEMENT OF REPLANT DISEASE AND PERENNIAL PHYTOPHTHORA CANKER

G. Browne[1], J. Connell[2], S. McLaughlin[1], R. Lee[1], S. Schneider[3], D. Kluepfel[1], and T. Trout[3]
[1]USDA-ARS, Dept. of Plant Pathology, Univ. of Calif., Davis; [2]UCCE, Oroville, CA; and
[3]USDA-ARS WMRL, Parlier, CA

Introduction.

This research addresses two major soilborne problems faced by the California almond industry, replant disease (RD) and perennial Phytophthora canker disease (PPC). RD is a specific replant problem that complicates orchard establishment on sites with a recent history of closely related crops. It can occur in absence of other general replant problems such as nutrient deficiencies and toxicities, improper soil pH, limiting soil physical conditions, poor plant-soil water relations, and root or vascular system dysfunction caused by plant parasitic nematodes or fungi. RD has been severe in several almond plantings in Butte County, where is has resulted in more than 50% loss of trees in large areas of the orchards in their first year of growth, but more typically it causes reduced vigor in orchards without killing trees. Although RD is most evident in the first few years after planting, yield may be impacted for most of the orchard's life. In contrast to RD, PPC has only been observed in mature, bearing almond orchards. It results from infection by either *Phytophthora cactorum* or *P. citricola*, and it typically kills trees within a few years of canker initiation. It is recognized by perennial branch and trunk cankers that extend vertically several feet within a year. In contrast to pruning wound cankers, which cease development in the summer and usually are not lethal, PPC cankers can persist through the summer and girdle the scion.

Objectives, 2003/04 research cycle:

1. Develop improved management strategies for replant disease (RD).
2. Determine unknown causes of RD.
3. Improve control strategies for perennial Phytophthora canker (PPC).

Objective 1. Develop improved management strategies for RD.

Fumigant-based approaches. Multiple fumigation trials were established from 2000 to 2004 in commercial almond orchards in near Durham, CA and micro plots near Parlier, CA. The trials involved soils that were conducive to RD and lacked significant populations of plant parasitic nematodes. The experiments were designed to test efficacy of fumigant alternatives to methyl bromide (MB) and support determinations of PRD etiology.

In two Durham orchards, tree-site treatments with MB or alternative fumigants including chloropicrin (Pic), Telone II (1,3-D), Telone C35 (1,3-D:Pic), Midas (iodomethane [IM], or IM:Pic [50:50]) were applied at 0.22 or 0.45 kg per tree site in early Nov 2002 after the soil at each tree site had been loosened with a tractor-mounted auger (each hole 60 cm deep, 60 cm diameter, soil replaced in hole after auger operation). The tree sites were planted with bare-root almond trees on Marianna 2624 rootstock in Feb 2003. Efficacy of the treatments was determined according to subjective visual disease ratings, growth in trunk diameter, and growth

in tree height during 2003 and 2004. Without pre-plant fumigation, only 0 to 17% of replanted trees (depending on orchard) became commercially acceptable trees (attained height of at least 1.2 m in 2003 and maintained healthy shoots in 2004) (**Table 1**). In one of the Durham orchards, where pre-plant Pic treatments of 0, 0.11, 0.22, 45, and 0.91 kg per tree site were compared in a replicated trial; Pic was highly effective at all of the rates (**Fig. 1**).

In Parlier micro plot trials, pre-plant treatments with MB and Pic (448 and 3024 kg per hectare, each fumigant) were compared for effects on incidence and severity of RD in Nemaguard peach seedlings in 46 cm dia x 120 cm deep micro plots. Three experiments were established: one each year in 2001/02, 2002/03, 2003/04. In each test, replicate micro plots filled with soil from a RD-affected peach orchard nearby received the fumigation treatments (or a non-fumigated control) in November. The following spring, Nemaguard peach seedlings were transplanted into the soil, and the seedling growth was monitored for 6 months. In all three experiments, growth of peach seedlings was improved dramatically with either rate of Pic (**Fig. 2**, 2004 results). MB treatment at the low rate improved seedling growth, but only the high MB rate was as effective as Pic (**Fig. 2**).

Effects of rootstock susceptibility. In the two Durham orchards described above, we evaluated the relative susceptibility to RD in the almond rootstocks Marianna 2624, Nemaguard peach, and Lovell peach. Growth and survival data indicated that although all of the rootstocks are subject to RD, almond on Marianna 2624 is especially susceptible (**Table 2**).

Effects of peach vs. grape cropping history. Because a significant number of vineyards are being shifted to almond production, we are investigating cross-specificity between RD of almond (peach rootstock) and grape. Parlier micro plots (46 x 120 cm) were filled with non-fumigated soil sampled from a peach orchard or, alternatively, with non-fumigated soil from an adjacent vineyard. In Nov 2003, the soils in micro plots were either left non-fumigated or pre-plant fumigated with Telone, MB, Telone C35, or Pic (448 to 600 kg per hectare). In Apr 2004, the micro plots were planted with Nemaguard peach seedlings and rooted Thompson Seedless grape cuttings; each crop was replanted in 6 replicate plots per combination of soil and fumigation treatment. Effects of the pre-plant crop history fumigation treatments are being assessed by monitoring growth and health of the peach plants.

The results to date (**Fig. 3**) indicate that there is a high degree of specificity between peach and grape RD. In the soil from the peach orchard, peach plants were severely stunted unless pre-plant fumigation was applied, and the fumigation treatments increased maximum length of peach shoots by 30 Aug by 56 to 136% (**Fig. 3**). In contrast, peach seedlings grew well in grape soil, regardless of fumigation treatment, and pre-plant fumigation improved growth by 12 to 13%. There was a lesser, converse effect of the soil treatments on growth of grape; by 30 Aug fumigation improved grape shoot growth by 0 to 12% in peach soil and 17 to 21% in grape soil (data not shown).

Effects of pre-plant fallowing and crop rotation. There is potential for using short-term crop rotation or fallowing to manage RD. We evaluated short-term fallow and cover crop rotations for effects on RD in micro plots near Parlier. Micro plots (60 x 120 cm) were filled with soil from a RD-affected orchard in Apr 2002. Treatments in 2002 included: 1) almond on Nemaguard peach rootstock (A/NG) Jun-Nov; 2) A/NG Jun-Nov + MB:Pic (50:50, 448 kg per hectare, Nov); 3) bare fallow Apr-Nov; 4) fallow Apr-Nov + MB:Pic Nov; 5) field corn Jun-Nov; 6) Piper sudan grass Jun-Nov; 7) Penewawa wheat Nov-Mar; and 8) Piper Sudan Jun-Nov + Penewawa wheat Nov-Mar (**Table 3**). After each crop's growth period, the roots (sudan, A/NG) or roots and shoots (corn, wheat) were chopped and incorporated into the soil. Treatment

efficacy was assessed according to growth of Nemaguard peach seedlings, planted four per plot Apr 2003. A repeat experiment was initiated in 2003 using the same treatment schedule as described for the 2002 experiment (**Table 3**).

Without fumigation, only sudan and wheat rotations (Trts. 6 and 7, **Fig. 4**) significantly and consistently improved growth of replanted peach seedlings relative to that following the non-fumigated, non-fallowed control (Trt. 1). Corn rotation (Trt. 5) and sudan plus wheat rotation (Trt. 8) were less effective in 2003 than in 2004, whereas fallowing alone (Trt. 3) was less effective in 2004 than in 2003 (Fig. 4). The effective rotations approached, but did not consistently match, the benefit of pre-plant fumigation with MB:Pic to peach seedling growth (Treatments 2 and 4, **Fig. 4**).

Conclusions. Our results indicate that tree site treatments with chloropicrin, IM, Telone each can prevent RD at sites not infested with significant populations of plant parasitic nematodes. Chloropicrin and fumigant mixtures containing it are particularly effective for prevention of the disease. Additional research is needed to optimize application methods for tree site and row-strip treatments.

Our results suggest that peach is not subject to RD in old grape vineyard soils, but additional experiments with peach and grape soils are needed to confirm this. Our rotation and fallowing experiments suggest that short-term crop rotations involving cultivars of sudan, corn, or wheat can be used to manage RD and may provide more benefit than fallowing alone. Further research is needed to test the rotations on a field scale.

Objective 2. Determine unknown causes of replant disease.

Plots used for sampling healthy and RD-affected trees. Trials were established to identify microorganisms associated with RD in field experiments near Durham and Parlier, CA. The Durham trials were established in two orchards where severe RD had occurred in the previous year; the experimental treatments were pre-plant fumigation with chloropicrin (0.5 kg per tree site) and no fumigation. Replicate interplant sites between the growers' permanent tree sites were assigned randomly to the treatments. The Parlier trials occurred in micro plots (concrete cylinders,1.2m deep, 46 cm dia.) that were filled with soil from an adjacent peach orchard with a history of RD. The micro plot treatments were chloropicrin or methyl bromide, each at 448 or 3024 kg per hectare, and a non-fumigated control. Fumigation treatments were imposed in October or November 2003, and the plots were planted in January to March 2004, depending on the experiment. Standard commercial bare-root almond trees (cv. Carmel on M2624 rootstock) were planted in the Durham plots, and greenhouse-grown peach seedlings (Nemaguard) were planted in the Parlier plots.

Culture-based isolations from healthy and RD-affected trees. At multiple intervals in late spring and summer, four replicate trees were destructively sampled from the fumigated and non-fumigated plots near Chico by carefully digging up the root systems and lifting roots from the surrounding soil. Samples from the Parlier micro plots were collected and processed in the same manner as for Chico plots, except that a 13-cm diameter soil coring device was installed around the upper 30 cm of each micro plot root system and used to lift the enclosed roots and soil from the micro plots.

Isolations for fungi were completed using 1-cm segments of the fine roots ($\leq$1 mm dia.) on water agar medium amended with ampicillin and a richer potato dextrose agar medium amended with semi-selective fungicides and antibiotics. Isolations from the roots for oomycetes (i.e.,

Pythium and *Phytophthora spp.*) were completed using PARP medium. For each sampled replicate plot and for each isolation medium, 16 root segments were plated directly after rinsing in sterile distilled water (sdw), and an additional 16 root segments were surface sterilized in bleach solution (0.6% sodium hypochlorite, pH 7.2) for 1 to 2 min, rinsed and plated. All fungi isolated from the roots were subcultured on 20% Potato Dextrose Agar (PDA) for subsequent characterization. *Phytophthora* and *Pythium spp.* isolated on PARP culture plates were identified to genus directly on the plates.

Bacteria were cultured from the same type of roots as used for the fungal isolations described above. From each replicate field plot, 0.4 g of the roots and tightly adhering rhizosphere soil was vortexed for 10-15 sec in 10 ml sdw. The resulting soil suspension was serially diluted and plated onto Tryptic Soy Agar (TSA) and incubated at 28°C. Two days later the colonies were tallied and colony forming units (CFU) per gram root tissue was calculated. The remaining washed roots were surfaced sterilized in 0.6% sodium hypochlorite for 1 to 2 min, rinsed in sdw, and then macerated using a mortar and pestle. The plant tissue homogenate was filtered through Miracloth, serially diluted and plated on 5% TSA. After two days incubation at 28°C, the colonies were tallied and CFU/g root tissue determined. After quantification, randomly selected bacterial isolates from the rhizosphere and root isolations (30 per root system; 15 from the rhizosphere soil and 15 from the root tissue) were single-colony purified and preserved at -80°C for identification and pathogenicity/growth stimulation tests with peach seedlings (described below).

Several thousand cultured fungal and bacterial and bacterial isolates have been preserved, and subsets of both the fungal and bacterial collections are being identified and tested for pathogenicity. Starting in summer 2004, a graduate student has been focused on characterizing the bacterial populations associated with healthy and RD-affected trees in the Durham and Parlier plots; the intent is to determine whether certain members of bacterial populations are associated with incidence or RD in non-fumigated soil or growth stimulation in fumigated soil. The bacteria are being identified by sequencing rRNA gene regions. Representatives of the collection will be tested for effects on growth and health of Nemaguard peach seedlings in the greenhouse.

DNA-based characterizations. In addition to the culture-based methods described above, DNA-based culture-independent methods are being used to characterize microbial roles in RD. The main reason for using culture-independent approaches to study RD etiology is that most organisms in soil can not be isolated and grown in culture media, and molecular approaches potentially provide a wider "view" into microbial communities. Our goal is to associate particular rRNA gene sequences with RD incidence or suppression. The sequences can be traced, via DNA sequence data bases, to particular species of bacteria or fungi. Briefly, steps in our approach include: 1) collection and preservation of samples from healthy and RD-affected trees, 2) extraction of all DNA from the samples, 3) use of the polymerase-chain-reaction (PCR) with appropriate primers to amplify diagnostic rDNA fragments from bacterial and fungal fractions of the DNA, 4) separation, discrimination, and identification of the amplified bacterial and fungal rDNA fragments.

Step 1 was completed on several dates for Durham and Parlier trials in 2003 and 2004. Samples were collected on the same dates and from the same trees as described above for the culture-based isolations. Approximately 20 g of fine roots ($\leq$ 2 mm diameter) and 100 g of the surrounding soil were sampled from soil depths of 5 to 40 cm for each single-tree plot (Durham) or micro plot (Parlier); the samples were put in polyethylene bags, placed on dry ice for transport

to the lab, and stored at -80°C. Steps 2 and 3 have been completed for one set of samples from the Durham trials. We are using denaturing gradient gel electrophoresis (DGGE) as a first approach in Step 4. As proposed for 2004/05, we also will adapt a more sensitive technique, oligonucleotide fingerprinting of ribosomal genes (OFRG), for completion of Step 4. DGGE and OFRG analyses offer powerful approaches to examining RD biology, but the work will likely require multiple years for completion. Dr. Dan Kluepfel's lab (USDA-ARS, Davis) is collaborating with us on molecular aspects of this research.

Objective 3. Improve control strategies for Perennial Phytophthora canker (PPC).

In 2003/04, we completed the last of a series of three experiments that compared efficacy of preventive foliar spraying vs. chemigation with phosphonate. Although some of the data from the first two experiments were presented in previous reports, we provide here a complete summary of all of the data. The experiments and corresponding phosphonate sources, amounts, and treatment dates were: Experiment 1, Phostrol® (Nufarm Americas, Inc.; Burr Ridge, IL), 3.8 liters/ha (2.1 kg phosphonic acid/ha), applied 19 October 2001 by a foliar spray or a chemigation; Experiment 2, Phostrol, 4.4 liters/ha (2.3 kg phosphonic acid/ha) applied 24 April 2002 by a foliar spray or a chemigation; and Experiment 3, NutriPhite P foliar (4-30-8) 4.3 liters/ha (2.2 kg phosphonic acid/ha) applied by a foliar spray or a chemigation, and NutriPhite P Soil® (0-60-0) 4.3 liters/ha (4.4 kg phosphonic acid/ha), applied only by chemigation. All Experiment 3 treatments were applied 15 July 2003. Additional treatment details are available on request (gtbrowne@ucdavis.edu). To prevent confounding foliar spraying or irrigation with phosphonate treatment, all phosphonate chemigation plots received a water-control foliar spray, all phosphonate sprayed plots received a water-control "chemigation", and all non-phosphonate (control) plots received both of the water controls. The phosphonate and control treatments each were applied to four replicate 15-tree mainplots in a randomized complete block design. Within the mainplots, nine single-tree subplots were assigned randomly to factorial combinations of inoculants (control, *P. cactorum*, and *P. citricola*) and inoculation dates (three successive dates per experiment).

The efficacy of the phosphonate treatments was tested by inoculating the treated trees and/or shoot or trunk bark disks removed from them at intervals of time after phosphonate application. In Experiments 1 and 2, we inoculated tree branches and shoots collected from them with *P. cactorum* and *P. citricola*; the branches were inoculated in the orchard on intact trees, but the shoots were cut off and inoculated in the lab. For both methods, the amount of disease (i.e., the length of cankers) was used to assess treatment efficacy. In Experiment 3, only excised shoots and excised disks of bark from the treated trees were inoculated; all of the inoculations occurred in the lab. Cankers on the shoots in Experiment 3 were measured as described for Experiments 1 and 2, but for the trunk bark inoculations, necrotic areas were traced, scanned into grayscale images, and measured using Assess software (APS Press, St. Paul).

In Experiment 1, the preventive foliar phosphonate spray on 19 October significantly suppressed canker development in orchard trees during the next 6 months (incubation periods beginning 16 November, 15 February, 1 April), but the chemigation was ineffective (**Table 4**).

In Experiment 2, effects of preventive foliar and chemigation treatments with phosphonate on 24 April varied with assay method, incubation period, and *Phytophthora* sp. (**Table 4**). Branch cankers caused by *P. cactorum* or *P. citricola* were 19 to 32% smaller on trees treated with phosphonate by foliar spraying or chemigation than on trees treated with water alone.

Excised shoot cankers produced by *P. cactorum*, however, only were suppressed by the preventive foliar spray and only during the first two incubation intervals (starting 17 May, 24 June). Excised shoot cankers produced by *P. citricola* were suppressed by the foliar spray during all three incubation intervals (starting 17 May, 24 June, 24 July) and by the chemigation during the last incubation interval.

In Experiment 3, the preventive foliar spray with phosphonate on 15 July inhibited growth of cankers produced by *P. cactorum* and *P. citricola* on excised shoots during all three incubation intervals, up to 5 months after treatment (**Table 5**). On the other hand, chemigation with phosphonate (either NP 4-30-8 or NP 0-60-0) only inhibited shoot cankers caused by *P. citricola*, and only during the first month after treatment (Table 2, incubation starting 15 August). On the excised bark disks, contaminating fungi prevented meaningful treatment assessment after the 15 August incubation. For the 13 October and 16 December incubation periods, benomyl (7 g a.i. per liter, sprayed on bark disks immediately after inoculation) was used to prevent the contamination. Necrotic areas induced by *P. cactorum* during the 13 October incubation were smaller on the disks from NP 0-60-0 chemigation plots than on those from the other treatments (**Table 6**). Necrotic areas produced by *P. citricola* during the incubation periods starting on 13 October were significantly smaller on disks from all phosphonate-treated trees (i.e., those given NP 4-30-8 foliar spray, NP 4-30-8 chemigation, or NP 0-60-0 chemigation) than on disks from trees treated with water alone (**Table 6**). During the last incubation (starting 16 December), only the foliar phosphonate treatment significantly inhibited development of cankers, and only for *P. cactorum*.

Conclusions. These results confirm that fall and spring foliar sprays give afford valuable protection from scion cankers caused by *P. cactorum* and *P. citricola*, but they also indicate that growers should limit phosphonate chemigation treatments for PPC control to periods of relatively high crop evapotranspiration (ET_C). The fall chemigation on 19 October was ineffective, possibly because of insufficient water (and phosphonate) uptake by the tree roots. Normal crop evapotranspiration (ET_C) for southern San Joaquin Valley almonds in October and November totals about 30 mm, which is only about 10% of that occurring during April and May or July and August, the sets of months that included and followed dates of effective chemigation treatments in Experiments 2 and 3. The fact that canker suppression sometimes occurred on shoots when it did not occur on trees, and visa versa, suggests that multiple test methods are advisable when assessing disease suppression by phosphonate treatments in a perennial plant. The excised assays are desirable because they permit evaluation of systemic treatments without irreversible tree damage.

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Table 1. Effect of pre-plant fumigation treatments on performance of almond trees in two commercial orchards near Durham, CA and affected by severe replant disease[a]

Fumigant	Rate of fumigant per tree site (lb)	Increase in trunk diameter (mm) by 9 Dec 2003		Increase in trunk diameter (mm) by 31 Aug 2004		Acceptable trees (%) 31 Aug 2004	
		Orchard 1	Orchard 2	Orchard 1	Orchard 2	Orchard 1	Orchard 2
None	0	7	3	15	17	0	17
MB	1.0	18	11	42	32	92	75
Pic	0.5	25	17	53	40	100	100
Pic	1.0	23	17	50	37	100	92
IM	0.5	--	12	--	34	--	100
IM	1.0		14	48	35	92	100
IM:Pic	0.5	22	16	43	39	92	100
IM:Pic	1.0	21	16	42	39	92	100
Telone II	0.5	17	13	46	30	92	100
Telone II	1.0	20	15	46	35	83	100
Telone C35	0.5	20	14	50	35	92	100
Telone C35	1.0	24	15	48	36	92	100
Min. sig. dif.:		*6*	*4*	*13*	*7*	*29*	*25*

[a]Orchards 1 and 2 were fumigated on 1 Nov and 30 Oct, 2002, respectively. A "-" indicates treatment not administered in Orchard 1

Table 2. Effect of rootstocks and pre-plant fumigation treatments on performance of almond trees in two commercial orchards near Durham, CA and affected by severe replant disease[a]

Rootstock	Fumigant	Increase in trunk diameter (mm) by 9 Dec 2003		Increase in trunk diameter (mm) by 31 Aug 2004		Acceptable trees (%) 31 Aug 2004	
		Orchard 1	Orchard 2	Orchard 1	Orchard 2	Orchard 1	Orchard 2
Mar.2624	None	8	2	19	19	50	0
	MB:Pic	23	16	48	39	100	90
	Pic	26	18	50	43	100	100
Lovell	None	11	8	36	27	100	60
	MB:Pic	25	18	48	41	100	100
	Pic	27	18	54	45	100	100
Nemaguard	None	10	4	33	25	100	30
	MB:Pic	22	13	46	39	100	100
	Pic	24	16	49	42	100	100
Min. sig. dif.:		*6*	*5*	*9*	*8*	*21*	*33*

[a]Orchards 1 and 2 were fumigated on 1 Nov and 30 Oct, 2002, respectively.

Table 3. Pre-plant treatments applied to Parlier micro plots filled with soil from a peach orchard affected by Prunus replant disease

Trt. No.	Pre-plant cropping status in summer (Jun-Nov)	Fumigation treatment (Nov)	Pre-plant cropping status in winter/spring (Nov-Mar)
1	Almond on Nemaguard	None	Bare fallow
2	Almond on Nemaguard	MB:Pic, 448 kg/ha	Bare fallow
3	Bare fallow	None	Bare fallow
4	Bare fallow	MB:Pic, 448 kg/ha[b]	Bare fallow
5	Corn hybrid N8214[a]	None	Bare fallow
6	Piper sudan grass	None	Bare fallow
7	Bare fallow	None	Penewawa wheat[c]
8	Piper sudan grass	None	Penewawa wheat

[a]Syngenta Seeds, NK Brand, Western Ag Services, Clovis, CA.
[b]methyl bromide/chloropicrin mixture (50:50, w:w).
[c]Lake Seed, Inc., Ronan MT.

Table 4. Effects of preventive foliar and chemigation treatments with phosphonate on development of cankers caused by two species of *Phytophthora* in Experiments 1 and 2, Kern County

Experiment no.[a]	Date of chemical treatment	Inoculation method[b]	Incubation period(s)	Phosphonate treatment	Inoculant and mean canker length (mm)[e]		
					Sterile control	*P. cactorum*	*P. citricola*
1	19 Oct 2001	Orchard tree	16 Nov 2000-15 Feb 2001	Water control	19	185	173
				Foliar spray	16	79*	24*
				Chemigation	16	184	148
			15 Feb-1 Apr 2002	Water control	16	150	179
				Foliar spray	16	66*	73*
				Chemigation	16	155	168
			1 Apr-16 May 2002	Water control	23	369	336
				Foliar spray	27	210*	103*
				Chemigation	17	325	308
		Excised shoot	Three combined[c]	Water control	0	94	113
				Foliar spray	0	78	65*
				Chemigation	0	99	112
2	24 Apr 2002	Orchard tree	Three combined[d]	Water control	19	452	368
				Foliar spray	17	358*	251*
				Chemigation	20	365*	262*
		Excised shoot	17 May-24 May 2002	Water control	1	120	102
				Foliar spray	2	55*	28*
				Chemigation	3	126	82
			24 Jun-1 Jul 2002	Water control	0	121	124
				Foliar spray	0	84*	47*
				Chemigation	0	115	97
			24 Jul-31 Jul 2002	Water control	0	109	121
				Foliar spray	0	85	24*
				Chemigation	0	93	47*

[a]In Experiments 5 and 6, Phostrol® was applied at 3.8 and 4.4 liters/ha (2.1 and 2.3 kg of phosphonic acid/ha), respectively; within an experiment, the spray and chemigation treatments received the same amount of phosphonate and all treatments received the same amounts of water by foliar spray and irrigation.

[b]In the orchard tree method, wounds on two intact branches on each of four replicate trees were inoculated per factorial treatment combination of incubation period, phosphonate treatment, and inoculum treatment. In the excised shoot method, three shoot segments from each of four replicate plots were wound inoculated per treatment combination of incubation period, phosphonate treatment, and inoculation treatment involving *P. cactorum* or *P. citricola*; two shoot segments from each of four replicate plots were inoculated per treatment combination involving the sterile control.

[c]Data from three incubation periods (24 Nov 2000 to 3 Dec 2001, 19 Feb to 28 Feb 2002, and 3 Apr to 10 Apr 2002) were combined due to a lack of interaction between incubation period and the other treatment factors (P=0.33).

[d]Data from three incubation periods in 2002 (15 May to 18 Jun, 18 Jun to 23 Jul, 22 Jul to 28 Aug) were combined due to lack of interaction between incubation period and the other treatment factors (P=0.17).

[e]Within an incubation period and inoculant, asterisks indicate means from foliar or chemigation treatments with phosphonate that differ significantly from the corresponding mean for the water control, based on 95% confidence intervals.

Table 5. Effects of preventive phosphonate treatments applied by foliar spraying or chemigation on development of cankers caused by two species of *Phytophthora* on excised shoots in Experiment 3, Kern County, 2003[a]

7-day incubation period	Phosphonate treatment[a]	Phosphonic acid (kg/ha)	Inoculant and mean canker length (mm)[b]		
			Non-inoculated control	*P. cactorum*	*P. citricola*
15 Aug-22 Aug	Water control	0	1	115	123
	Foliar spray, NP 4-30-8	2.2	1	41*	30*
	Chemigation, NP 4-30-8	2.2	0	104	54*
	Chemigation, NP 0-60-0	4.4	1	86	35*
13 Oct-20 Oct	Water control	0	1	95	54
	Foliar spray, NP 4-30-8	2.2	0	34*	16*
	Chemigation, NP 4-30-8	2.2	2	89	45
	Chemigation, NP 0-60-0	4.4	1	72	29
16 Dec-23 Dec	Water control	0	0	60	56
	Foliar spray, NP 4-30-8	2.2	0	24*	13*
	Chemigation, NP 4-30-8	2.2	0	46	38
	Chemigation, NP 0-60-0	4.4	0	38	34

[a]The phosphonate sources were NutriPhite P foliar 4-30-8, applied by foliar spraying or chemigation at 4.3 liters/ha or NutriPhite P soil Hi-Grade 0-60-0 applied by chemigation at 4.3 liters/ha. The control received equivalent amounts of water applied by spraying and irrigation.

[b]For each combination of incubation period, phosphonate treatment, and species of *Phytophthora*, three excised shoot segments from each of four replicate field plots were wounded and inoculated on one side with mycelium of the pathogen on V8 juice agar disks. On the side opposite from the wound inoculated with a *Phytophthora* sp., each shoot segment was wounded and inoculated with sterile V8 juice agar as a control. Within incubation periods and inoculation treatments, asterisks indicate phosphonate treatment means that differ significantly from the corresponding water-control means, based on 95% confidence intervals.

Table 6. Effects of preventive phosphonate treatments applied by foliar spraying or chemigation on development of necrosis in bark disks excised from tree trunks in Experiment 3, Kern County, 2003[a]

Incubation period	Phosphonate treatment[a]	Phosphonic acid (kg/ha)	Inoculant and mean area of necrosis (mm^2)[b]		
			Non-inoculated	*P. cactorum*	*P. citricola*
13 -20 Oct	Water control	0	25	662	601
	Fol. spray, NP 4-30-8	2.2	27	707	288*
	Chemigation, NP 4-30-8	2.2	25	551	182*
	Chemigation, NP 0-60-0	4.4	21	350*	130*
16 -23 Dec	Water control	0	36	571	483
	Fol. spray, NP 4-30-8	2.2	36	310*	343
	Chemigation, NP 4-30-8	2.2	38	511	298
	Chemigation, NP 0-60-0	4.4	36	380	319

[a]The phosphonate sources, all applied 15 July 2003, were NutriPhite P foliar 4-30-8, applied by foliar spraying or chemigation at 4.3 liters/ha or NutriPhite P soil Hi-Grade 0-60-0 applied by chemigation at 4.3 liters/ha. The control received equivalent amounts of water applied by spraying and irrigation.

[b]Four replicate 5-cm-diameter excised bark disks (one per replicate field plot) were wound inoculated per treatment combination. Within incubation periods and inoculation treatments, asterisks indicate phosphonate treatment means that differ significantly from the corresponding water-control means, based on 95% confidence intervals.

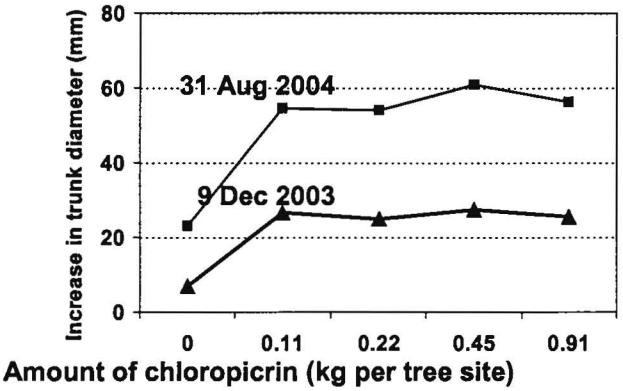

Fig. 1. Effect of pre-plant soil fumigation with chloropicrin on growth of almond trees on Marianna 2624 rootstock in an orchard affected by PRD near Durham, CA. The trees were planted in Feb 2003 after application of the treatments in Nov 2002.

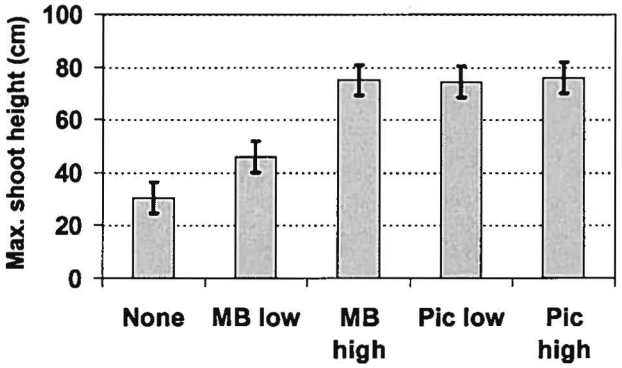

Fig. 2. Effect of pre-plant treatments with chloropicrin and methyl bromide on incidence and severity of PRD in micro plots near Parlier, CA. For each fumigant, low and high rates were 448 and 3024 kg per hectare, respectively. Data collected 30 Aug 2004. Vertical bars are 95% confidence intervals.

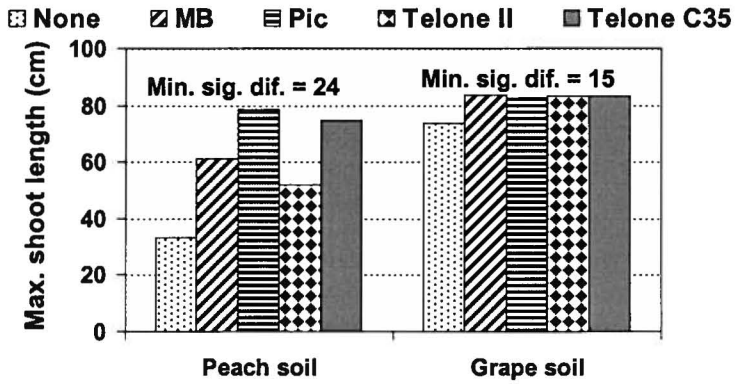

Fig. 3. Effect of long-term pre-plant soil history and pre-plant fumigation treatments on growth of Nemaguard peach seedlings in Parlier micro plots. The "peach" and "grape" soils had been cropped peach and grape, respectively for more than 15 years before they were used to fill the micro plots. The peach seedlings were planted in Apr 2004 and shoot length was measured on 30 Aug 2004.

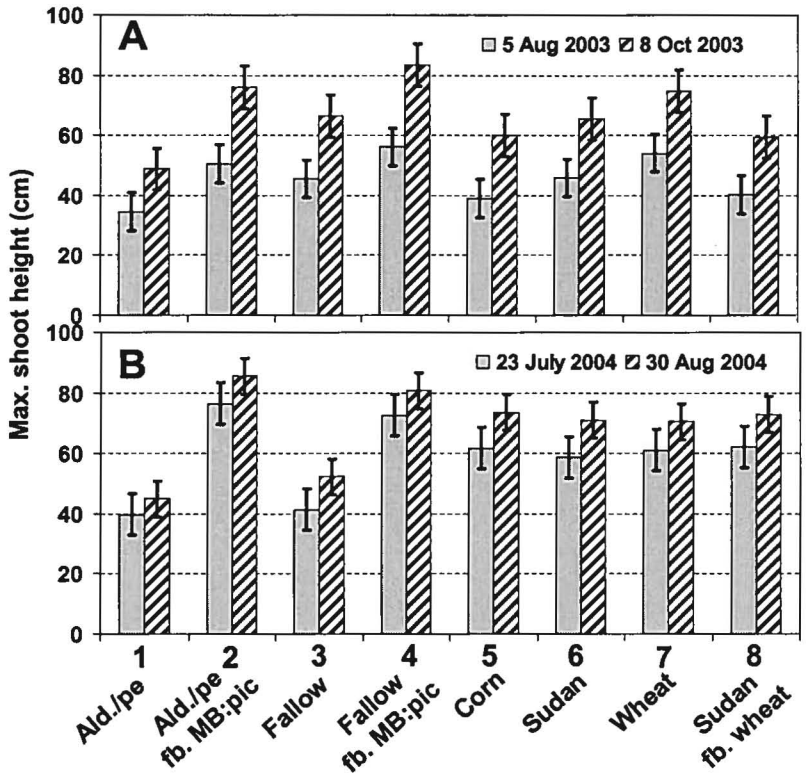

Fig. 4. Effect of short-term fallowing, short term crop rotation, and pre-plant fumigation on growth of Nemaguard peach seedlings planted in micro plots near Parlier, CA. **A**, Experiment 1 (2002/03) and **B**, Experiment 2 (2003/04). See Table 3.