MANAGEMENT OF LETHAL PHYTOPHTHORA CANKER AND BIOLOGY AND CONTROL OF REPLANT DISORDER

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Abstract

Research was conducted to determine seasonal effects on development of lethal Phytophthora canker (LPC) on almond, develop improved practical management strategies for the disease, and investigate management approaches and unknown causes for replant disorder (RD). Following monthly almond orchard inoculations with *P. cactorum* and *P. citricola* in Kern County, resulting canker lengths produced in 3-week incubation periods were roughly proportional to the average prevailing air temperatures, except that some of the hottest summer temperatures (i.e., average maximums >33 C) slowed or prevented *P. citricola* cankers. Average air temperatures less than 10 C in winter resulted in negligible canker lengths within the initial 3-week incubation period, but the cankers expanded after temperatures warmed in the spring. During moderate temperatures, frequent inspection of an orchard at risk for LPC is needed to detect cankers at an early stage and prevent further spread. A foliar spray and micro sprinkler chemigation test was completed in Kern County with Phostrol (a new phosphonate product for which almond fungicide registration is being pursued by the manufacturer, Nufarm Americas, Inc.). One foliar spray with Phostrol (3.3 pts./A) in mid October provided significant canker suppression through mid May (57 to 43 % smaller for *P. cactorum*, 86 to 59% for *P. citricola*, depending on inoculation and incubation dates), but chemigation with Phostrol at the same rate and time had no significant effect on disease development. The experiment is being repeated following a spring foliar spray and chemigation. At a cleared old almond orchard site that had exhibited a severe replant disorder near Chico, preplant shank treatments of methyl bromide, Telone, and chloropicrin were tested (360 to 374 lb/A, non-tarped). Only the chloropicrin treatment resulted in commercially acceptable growth of replanted almond on Marianna 2624 rootstock. Tree-site treatments with methyl bromide or Telone (1 lb per site) or use of Lovell peach rootstock provided benefit, but not enough to be commercially acceptable. The replant disorder appeared to result from decay of fine roots as they were produced on the young trees. Isolations from diseased and healthy roots provided preliminary evidence for an association between the disorder and infection by certain fungi (i.e., *Cylindrocarpon*, *Fusarium*, and others), but much more research is needed to confirm and expand these results and determine unknown roles of other biological agents in this and other replant disorders.

Background

This research is concerned with two important soilborne problems affecting almond production—"lethal Phytophthora canker" (LPC) disease and "replant disorder" (RD) (also known as "replant problem"). Lethal Phytophthora canker can be caused by either *Phytophthora*

cactorum or *P. citricola* and often kills mature almond trees within 1 to 3 years of infection. It is recognized by profusely gumming cankers that expand rapidly, especially in vertical directions. Cankers caused by *P. cactorum* or *P. citricola* usually grow more rapidly than those caused by *Ceratocystis*. The LPC pathogens can persist from year to year until the scion is girdled, unlike pruning wound cankers caused by *P. syringae*, which "die out" in hot weather. Several Kern County almond orchards have lost more than 5% of their trees due to LPC. Replant disorder can occur when stone fruit or nut trees are planted without precautions at sites previously devoted to a closely related crop. The symptoms include poor growth, delayed crop production, and, in severe cases, tree death. Some known causes for the poor establishment include parasitic nematodes, oak root fungus, and *Phytophthora*. Additional, but presently unknown, biological causes are likely, however, because RD can occur in absence of the known pests, and pre-plant fumigation can eliminate or reduce severity of the unknown cause(s).

We report below on three main research objectives for the year, including further determinations of seasonal effects on development of LPC, further improvement of LPC management practices through experimentation with chemigation and foliar applications of phosphonate, and a new emphasis on etiology and management of RD. Seasonal effects on development of LPC were followed for another year, and the findings will provide a basis for timing inspections and treatments for orchards affected by the disease. Efficacy of Phostrol (a "new" phosphonate) following a fall foliar spray vs. a fall chemigation was compared to help facilitate registration of the product for control of LPC and determine the most effective and lowest-cost application methods. Our previous work with Nutriphite (a fertilizer containing phosphonate, which has systemic activity against many *Phytophthora* diseases) demonstrated that a single fall or spring foliar spray with the material protected against LPC for several months, but it is unknown whether chemigation through micro sprinklers is similarly effective. Work was begun to determine unknown causes and develop practical control measures for a severe RD problem that has affected replanted almond orchards in Butte County. Due to the phase out of methyl bromide, new knowledge and management strategies are needed for this and other types of RD.

Objectives

1. Determine seasonal effects on LPC development.
2. Develop improved management strategies for LPC.
3. Determine unknown causes and improved management strategies for replant disorder.

Determining seasonal effects on LPC development. Different sets of almond trees, cultivar Nonpareil, were inoculated over time, one set per month. The first 18 successive inoculations (October 1999 through April 2001) were conducted in a block of 10-yr-old trees near Shafter, CA, and the most recent 12 inoculations (May 2001 to April 2002) occurred in a nearby 12-yr-old orchard (Fig. 1A). Each month, eight randomly selected trees in the test block were wound inoculated-- four with *P. cactorum* and four with *P. citricola*. Each of the selected trees received one of the pathogens on two primary or secondary scaffold branches and sterile agar as a control on a third scaffold branch. The inoculants were introduced under a 12 x 12 mm patch of bark and held in place with a branch wrap of duct tape. Three weeks after inoculation, length and width of resulting cankers were measured. Air temperature data (max., min., avg.), collected by the California Irrigation Management System at Shafter, CA were tabulated for each 3-week period of incubation (Fig. 1B).

Shoots segments were collected from the field trees used for the seasonal study and inoculated in the lab under standardized temperature and moisture conditions. The segments, approximately 5 to 15 mm diameter and 20 cm in length, were cut from randomly selected trees in the blocks used for monthly field inoculations, placed in polyethylene bags, and kept cool until inoculation. For each date of testing, 10 shoot sections were inoculated with *P. cactorum,* 10 with *P. citricola*, and four with sterile agar as a control. The inoculants consisted of 5-mm-diameter V8 juice agar disks colonized by one of the pathogens or sterile V8 juice agar disks (the control). Near the center of each shoot, one of the disks was placed on exposed cambium and wrapped with electrical tape. After inoculation, all of the shoots were placed in a humid chamber (100% r.h.) and incubated at 22 to 24 C for 5 days. Susceptibility of the shoots to the pathogen was assessed according to canker length at the end of the incubation period.

Following the inoculations in the orchard, average air temperatures less than 10 C in winter resulted in negligible canker lengths within the initial 3-week incubation period (12/99, 11/00, and 12/00, 1/ and 11/01; Fig. 1A, B). However, remeasurement on 4/9/01 of cankers that had initially failed to expand during cool temperatures in the 3 weeks following 11/16/00, 12/15/00, and 1/15/01 inoculations revealed that all of the cankers grew rapidly as temperatures warmed in the spring (Fig. 2). As temperatures warmed in spring, successive inoculation and incubation periods tended to result in progressively longer cankers. Spring and summer temperatures generally were conducive to canker development, except that some of the hottest temperatures (i.e., averages >25 C and maximums >33 C) slowed or prevented *P. citricola* cankers (6 and 8/01, Fig. 1 A, B).

Following the excised shoot inoculations in the lab, canker lengths fluctuated across the different monthly testing intervals, but the shoots generally exhibited susceptibility, regardless of whether the trees were dormant or actively growing when the shoots were collected (Fig. 3).

The fact that LPC cankers expand rapidly for much of the year indicates that orchards at risk for the disease should be surveyed frequently to insure timely treatment. Infections occurring during cool fall or winter temperatures may not be apparent until later in the spring when warmer temperatures allow canker expansion. The results from excised shoot inoculations provided no evidence for large seasonal fluctuations in susceptibility of the host to the pathogens, suggesting that temperature is a major factor governing canker expansion in the field.

Develop improved management strategies for LPC: relative effectiveness phosphonate applied by chemigation vs. by foliar spraying. In October 2001 a trial was established to compare effectiveness of phosphonate treatment by foliar spraying vs. by chemigation for management of LPC. On 10/19/01, the experimental treatments were applied and included 1) a foliar spray with Phostrol (Nufarm Americas, Inc.) (3.3 pints of formulation per acre in 150 gallons of water per acre), 2) chemigation with Phostrol through micro sprinklers (3.3 pints of formulation per acre injected 3 to 4 hr before completion of an 18-hr irrigation set that applied 1.2" water), and 3) a water control (150 gallons per acre of foliar water spray and 1.2" of irrigation water, both without Phostrol). To avoid confounding Phostrol treatments with water application, the trees that received the foliar Phostrol spray also received 1.2" of irrigation water, and trees given the Phostrol by chemigation received a foliar water spray (150 gal/A). The experiment used a split-plot design; there were four replicate 15-tree mainplots per Phostrol and control treatment, arranged in randomized complete blocks. Single trees within each mainplot

were randomly allocated to each of nine subplot treatments, which included the possible combinations of three inoculants (*P. cactorum*, *P. citricola*, or a control) and three inoculation dates (11/16/01, 2/15/02, or 4/1/02). Phostrol treatment efficacy was assessed according to the length and width of bark cankers resulting after inoculation with the pathogens; incubation periods of 90, 45, and 45 days were allowed after the inoculations on 11/16/01, 2/15/02, and 4/1/02, respectively, before the cankers were measured.

A bioassay method also was used to assess effectiveness of the Phostrol treatments. Basal segments of shoots (vigorous "water sprouts", about 10 to 15 mm diameter and 20 cm long) were collected from each of the Phostrol and control plots described above on each of the field inoculation dates. In the lab, the middle of each shoot piece was wound inoculated with a V8 juice agar disk covered with mycelium of *P. cactorum* or *P. citricola* or a sterile V8 juice agar disk (the inoculation control) using a No. 1 cork borer; a bark disk was removed and replaced with one of the agar disks, then the inoculated area was wrapped with electrical tape. The inoculated shoots were incubated for 1 week in a humid chamber (100% r.h.), and resulting canker length was used to assess effects of the orchard treatments.

In the orchard test, the pre-inoculation foliar Phostrol spray treatment significantly suppressed cankers caused by *P. cactorum* and *P. citricola*, but the Phostrol chemigation treatment had no significant effect (Fig 4). Depending on the inoculation/ incubation interval, cankers on trees inoculated with *P. cactorum* averaged 57 to 43% smaller (based on canker lengths) in Phostrol-sprayed plots than in the water control plots. Similarly, cankers on trees inoculated with *P. citricola* averaged 86 to 59% smaller in Phostrol plots than in control plots.

In the lab test with excised shoots, following the first inoculation test with *P. cactorum* (11/24/01), cankers were 36% smaller on shoots from the Phostrol-sprayed trees than on the control trees, but there was no effect of the treatment on the second and last dates of inoculation (2/19/02, 4/3/02) (Fig. 5). Following inoculation with *P. citricola*, cankers were 61 and 38% smaller on shoots from Phostrol-sprayed trees on the first and second dates of inoculation, respectively, but there was no significant effect of the treatment on the last date of inoculation. There was no measurable effect of the Phostrol chemigation in the orchard on subsequent development of cankers on the excised shoots.

Our field results indicate that a foliar spray with Phostrol in mid October provides many months of strong suppression of cankers caused by *P. cactorum* or *P. citricola*. The benefit offered by this treatment was similar in magnitude to that observed in our earlier experiments with Nutriphite formulations (2000 and 2001 Almond Board Reports). If registered as a fungicide, Phostrol could provide a valuable treatment for management of LPC. Our data to date indicate no benefit for LPC management from chemigation with Phostrol in the fall. The fact that excised shoot test results did not fully reflect results from the field experiments highlights the importance of orchard tests for this type of evaluation. A spring repeat of the Phostrol orchard experiment has been established and includes Phostrol applied by foliar spray and chemigation on 4/24/02. Compared to the fall experiment, the spring repeat is occurring when more water will be taken up by the trees, and it is hoped that this will result in more uptake of the phosphonate applied by chemigation.

Determine unknown causes and improved management strategies for replant disorder.

Field tests. Replicate fumigated and non-fumigated plots were established at an almond replant site near Chico, CA in cooperation with Paiva Farms, Chico; Tri-Cal, Inc., Hollister; and Cardinal Professional Products, Woodland. The tests were designed to determine effective management approaches and cause(s) for the type of replant problem occurring at the site. A grower attempt to replant the block with almond on Marianna 2624 rootstock had failed in spring/summer 2000 (approx. 70% of trees died or failed to grow), and the circumstances were similar to those observed at several other almond replant sites in the county in previous years. Sampling in the affected block to date has not detected significant parasitic nematode populations (S. Schneider, USDA-ARS, Fresno). Experiment 1 at the Chico site included pre-plant treatments of methyl bromide (MBr) (360 lb/A), chloropicrin (374 lb/A), Telone (360 lb/A), and a non-fumigated control, each imposed on four replicate 19- x 22-m replicate (18-tree-site) plots in randomized complete blocks. The fumigants were injected by shank over entire plots on 10/21/00 without plastic mulch. The plots were replanted in January 2001 with almond on Marianna 2624. Experiment 2 at the Chico orchard involved pre-plant tree-site fumigation on 10/21/00 with methyl bromide or Telone (1.0 lb injected in one probe per tree site, non-tarped) and a non-fumigated control; the plots were replanted in January 2001 with almond on Marianna 2624, or in the case of the non-fumigated control sites, also with almond on Lovell peach rootstock. Experiment 2 had four replicate three-tree-site plots per treatment in randomized complete blocks.

Only the pre-plant chloropicrin treatment resulted in satisfactory growth and grower retention of replanted trees (Table 1, Experiments 1 and 2). By some measures, pre-plant methyl bromide fumigation slightly improved tree performance in Experiment 1, but it was less effective than chloropicrin. Telone provided no benefit in Experiment 1. In Experiment 2, pre-plant use of methyl bromide or Telone or use of Lovell rootstock rather than Lovell rootstock improved tree performance compared to that of almond on Marianna 2624 in non-fumigated plots, but the improvement was insufficient to be commercially acceptable level (Table 1, Experiment 2). Repeat and expanded experiments have been established at the Chico test orchard and are needed to confirm the first-year results. The favorable tree response following pre-plant chloropicrin treatment appears promising, especially with the looming loss of methyl bromide.

Sampling and culturing roots from replant disorder-affected and healthy trees. On four occasions (4/26, 5/14, 5/31, and 8/13/01) in Experiment 1 at Chico, roots from three to four RD-symptomatic and three to four healthy trees in non-fumigated and fumigated plots, respectively, were inspected and sampled for lab isolations. When possible, the root samples were subdivided into healthy (relatively white cortex and no obvious decay) and diseased (dark, with damaged cortex) classes. Twenty-four to 32 root pieces from each class were either rinsed in sterile water or surface sterilized in 10% commercial bleach adjusted to pH 7.2 before culturing on water agar amended with ampicillin (100 ppm) (a non-selective medium for isolating many fungi) or PARP medium (semi-selective for oomycetes such as *Pythium* and *Phytophthora*). All fungi growing from cultured roots were subcultured on 0.2-x ampicillin-amended potato dextrose agar plates and identified to genus level according to morphology. Isolations also were made for bacteria. For each sampled tree and available root health class, 0.2 g of roots was subjected to dilution plating on 0.1x tryptic soy agar. Separate dilution plates were prepared for the rhizosphere (i.e., the soil and other particles adhering to the root surface, suspended by vigorous vortexing) and

the surface and internal portions of roots (prepared by rinsing, surface sterilizing, and grinding the roots before plating). Bacteria growing on the isolation plates were sampled randomly and preserved for future characterization.

The root inspection and sampling at Chico revealed decay of the fine roots (≤1mm dia.) on diseased trees in non-fumigated plots. Compared to the root systems in non-fumigated plots, those in chloropicrin-fumigated plots had more and healthier new roots. Results of the isolations from sampled roots suggested an association of root disease with species of *Cylindrocarpon* and *Fusarium* (data from 5/14 and 8/13 isolations shown; Tables 2 and 3, respectively). Many other fungal species were isolated from the roots at relatively low frequency. Representative isolates of fungi from the Chico trial root systems are being tested for pathogenicity in a greenhouse.

Greenhouse bioassays with soil from RD sites. To provide more insight on unknown causes of RD, a greenhouse bioassay test was conducted with soil from plots in Chico Experiment 1 (only soil from methyl bromide and control plots was collected). The soil samples were subjected to semi-selective chemical treatments or autoclaving, distributed to 0.6-liter pots, and planted with Nemaguard peach seedlings and Marianna 2624 plants in the greenhouse. Resulting health and vigor of the plants was used as an indicator of soil treatment effects. The soil treatments included the possible combinations of two heat treatments (no heating and autoclaving) and six chemical treatments: a non-treated water control, difenoconazole (Dividend 3MG, 20 mg a.i./kg soil), fludioxonil (Maxim 4 FS, 20 mg ai/kg), chloramphenicol + streptomycin (each antibiotic at 10 mg/kg), mefenoxam (Ridomil Gold EC, 10 mg a.i./kg), and fenamiphos (Nemacur 3, 30 mg ai/ kg). Difenconazole and fludioxonil are general fungicides, chloramphenicol and streptomycin are bacteriocides, mefenoxam suppresses *Phytophthora* and *Pythium* spp., and fenamiphos kills nematodes. All treatments initially were applied at 3 days before planting by atomization into mixing soil samples. The first three chemical treatments were repeated at 1 and 2 mos after planting by soil drenching, whereas the other treatments were applied only once. Three months after transplanting, the root systems of the test plants were washed free from the soil, and effects of the soil treatments were judged according to shoot weights and root mass and health. In addition, isolations were conducted from roots on plants grown in autoclaved and non-autoclaved soil samples that had not received a chemical treatment; for each treatment healthy and diseased root segments (0.5 to 1 cm length, ≤1 mm dia) were sampled from four replicate plants of Marianna 2624 plum and four of Nemaguard peach. The root segments were rinsed in sterile water or bleached (10% commercial bleach, pH 7.0 to 7.2) and cultured on water agar amended with tetracycline (100 mg/liter) or PARP. All fungal isolates were subcultured on one-fifth strength PDA + tetracycline (100 ppm) and identified to genus according to morphology.

Among the treatment factors of field fumigation with methyl bromide, autoclaving, and chemical amendment, only heating and chemical amendment had significant effects on health of the roots on Marianna 2624 and Nemaguard assay plants (Table 4). Fludioxonil treatments significantly reduced the amount of root discoloration that occurred in non-autoclaved soil, compared to that in non-autoclaved soil in the other treatments (Table 4). None of the other chemical amendments significantly affected root health. Autoclaving the soil prevented the root discoloration symptom. Chemical treatment had a negligible effect on top fresh weight of the plants (P=0.06), which was primarily due to slightly lower top fresh weights in the fludioxonil treated plants (Table 4). Root weights were not affected significantly by chemical or heat treatments. Incidence of *Cylindrocarpon*, *Fusarium*, *Pythium*, and *Rhizoctonia* exhibited some association with symptoms of root disease (Tables 5, 6).

Table 1. Effects of pre-plant treatments on first-year performance of almond trees planted at the site of an old cleared almond orchard

Experiment	Pre-plant fumigation treatment*	Rootstock	Increase in trunk diameter (mm)**	Final tree height**	Disease rating (0 to 5 scale)**	Final tree retention (%)**
1, Large plot	None (control)	Marianna 2624	1 a	1.0 a	3.4 a	2 a
	Methyl bromide (360 lb/A)	Marianna 2624	4 b	1.2 a	2.1 b	21 a
	Chloropicrin (374 lb/A)	Marianna 2624	10 c	1.7 b	0.3 c	96 b
	Telone (360 lb/A)	Marianna 2624	2 a	1.1 a	2.9 a	1 a
2, Tree sites	None (control)	Marianna 2624	1 a	0.9 a	3.5 a	0
	None (control)	Lovell peach	6 b	1.3 b	1.2 b	33
	Methyl bromide (1 lb/tree site)	Marianna 2624	7 b	1.4 b	0.9 b	58
	Telone (1 lb/ tree site)	Marianna 2624	8 b	1.3 b	1.4 b	67

*Large-plot treatments applied with shanks spaced at 2 ft; tree-site treatments applied with one probe per tree site.
**There were four replicate groups of 18 trees per treatment in the large-plot experiment and four replicate groups of three trees in the small-plot experiment. All trees planted January 2001. All tree measurements made 8/13/01. Tree disease rating scale: 0=healthy tree with good growth; 1=slight shoot stunting apparent; moderate shoot stunting; 3=no recent shoot growth; no recent growth, and leaf burning, discoloration or wilting; 4=tree nearly dead, 5=tree dead with dry leaves. Tree retention determined week of 10/10/01; only trees ≥1.5 m height retained; mean separations not attempted for this variable for Experiment 2.

Table 2. Incidence of selected fungi on roots sampled on 5-14-01 from replanted almond trees on Marianna 2624 rootstock in Chico Experiment 1*

			Incidence of isolation from roots (%)						
Pre-plant fumigation treatment	Status of root sample	Root trt.	*Aspergillus*	*Cylindroarpon*	*Fusarium*	*Mortierella*	*Penicillium*	*Rhizoctonia*	*Trichoderma*
None	Healthy	None	0	0 b	0 c	0 b	0	0	0
		Bleach	0	0 b	0 c	0 b	0	0	0
	Diseased	None	4	4 b	75 a	4 b	17	0	4
		Bleach	4	33 a	67 a	0 b	0	4	0
Chloropicrin	Healthy	None	0	8 b	25 bc	25 a	17	0	8
		Bleach	0	0 b	0 c	0 b	8	0	0
	Diseased	None	8	21 ab	8 bc	25 a	13	8	29
		Bleach	0	13 ab	33 b	0 b	0	8	0

*Roots were sampled from trees planted in plots given the indicated pre-plant treatments, categorized as being healthy (relatively white cortex and no obvious decay) or diseased (dark, damaged cortex), and either rinsed in sterile water or surface sterilized in 10% commercial bleach adjusted to pH 7.2 before culturing on water agar amended with ampicillin (100 ppm). Twenty-four root pieces were cultured for each combination of field treatment, root health, and root surface sterilization treatment. All fungi growing from cultured roots were identified according to morphology. Mean separation letters were assigned only in columns where there were significant differences among the means; those within a column and without letters in common differ significantly according to the Waller-Duncan k-ratio test.

Table 3. Incidence of selected fungi on roots sampled on 8-13-01 from replanted almond trees on Marianna 2624 rootstock in Chico Experiment 1*

			Incidence of isolation (%) from roots												
Pre-plant soil fumigation treatment	Status of root sample	Root sample trt.	*Alternaria*	*Basidomycetes*	*Cylindrocarpon*	*Fusarium*	*Rhizoctonia*	*Mortierella*	*Penicillium*	*Pythium*	*Peyroniella*	*Trichoderma*	*Gliocladium*	*Wardomyces*	Other
Chloropic.	Healthy	None	9	3	0 b	22 b	13	13 ab	3	0	0	3	0	6	3
Chloropic.	Diseased	None	16	0	3 b	16 b	13	22 a	6	0	0	3	6	3	0
None	Diseased	None	6	0	14 b	80 a	2	2 bc	2	2	2	0	5	0	6
Chloropic.	Healthy	Bleach	0	0	0 b	22 b	3	0 c	0	0	0	3	0	0	3
Chloropic.	Diseased	Bleach	0	0	6 b	16 b	6	0 c	0	0	0	0	0	0	13
None	Diseased	Bleach	2	8	34 a	48 ab	3	0 c	6	0	0	0	0	0	8

*Roots were sampled from trees planted in plots given the indicated pre-plant treatments, categorized as being healthy (relatively white cortex and no obvious decay) or diseased (dark, damaged cortex), and either rinsed in sterile water or surface sterilized in 10% commercial bleach adjusted to pH 7.2 before culturing on water agar amended with ampicillin (100 ppm). Thirty-two to 64 root pieces were cultured for each combination of field treatment, root health, and root surface sterilization (root sample) treatment. All fungi growing from cultured roots were identified according to morphology. Mean separation letters were assigned only in columns where there were significant differences among the means; those within a column and without letters in common differ significantly according to the Waller-Duncan k-ratio test.

Table 4. Effects of semi-selective soil chemical treatments and pre-plant soil autoclaving on growth and health of Marianna 2624 and Nemaguard cuttings planted in the greenhouse in potted samples of soil from the replant disorder study near Chico[a]

Chemical treatment[b]	Soil heating	Top weight (g)	Root weight (g)	Percentage of discolored roots
Control	None	4.8	5.7	49
	Autoclaved	4.7	7.1	18
Chloramphenicol + streptomycin	None	4.6	6.1	48
	Autoclaved	3.9	5.6	21
Difenoconozole	None	4.7	6.5	47
	Autoclaved	4.2	5.8	13
Fludioxonil	None	3.8	6.6	17
	Autoclaved	3.7	6.5	7
Mefenoxam	None	4.0	6.5	49
	Autoclaved	5.1	7.2	18
Fenamiphos	None	4.7	5.4	43
	Autoclaved	4.5	6.2	20
L.S.D.[c]		NS	NS	12

[a]Soil was collected from methyl bromide-fumigated and non-fumigated plots in Chico Experiment 1. Effects of fumigation and rootstock did not interact significantly with chemical or soil heating treatments above, so the data were combined across the former two factors for presentation above. For the percentage of discolored roots, here was significant statistical interaction between soil heating treatment and rootstock (i.e., Marianna 2624 had 51 and 13% diseased roots in non-heated and autoclaved soil treatments, respectively whereas Nemaguard had 34 and 19% diseased roots in the same treatments)

[b]Treatments of difenoconazole (Dividend 3MG, 20 mg a.i./kg soil), fludioxonil (Maxim 4 FS, 20 mg ai/ kg), and chloramphenicol + streptomycin (each antibiotic at 10 mg/kg) were applied 3 days before planting by atomization into mixing soil samples, and the treatments were repeated at 1 and 2 mo after planting by soil drenching. Ridomil Gold EC (mefenoxam, 10 mg a.i./kg) and Nemacur 3 (fenamiphos, 30 mg ai/kg) were applied only pre-plant by the atomization method. Controls received water alone.

[c]Least significant difference according to 95%confidence interval for means, "NS" indicates there was no significant difference. Note that only the autoclaving or treatment with fludioxonil reduced severity of root disease symptoms.

Table 5. Incidences of selected fungi on roots of Marianna 2624 and Nemaguard rootstock plants grown in the greenhouse in potted samples of soil from the Chico replant disorder study[a]

Pre-plant soil heat treatment	Apparent status of root sample	Root sample surface sterilization treatment	Incidence of isolation per root piece (%)				
			Cylindrocarpon	*Fusarium*	*Mortierella*	*Rhizoctonia*	*Trichoderma*
None	Healthy	None	17.2	77	8.5	3.1	9.7
		Bleach	37.0	46	0.2	0.0	2.9
	Diseased	None	5.2	87	4.3	7.8	11.3
		Bleach	22.4	82	0.2	10.9	2.9
Autoclaving[b]	Healthy	None	0.0	77	20.3	0.0	9.4
		Bleach	0.0	41	1.6	0.0	1.6
	Diseased	None	0.0	80	15.6	0.0	18.8
		Bleach	0.0	95	0.0	1.6	4.7

[a]For each treatment combination, four healthy and four discolored root segments (0.5 to 1 cm length, ≤1 mm dia) were sampled from eight replicate plants (four Marianna 2624 plum and four Nemaguard peach plants, data combined across rootstock) that had grown for 3 months in potted 0.6-liter samples of soil from the Chico replant trial. The root segments were rinsed in sterile water or bleached (10% commercial bleach, pH 7.0 to 7.2) and cultured on water agar amended with tetracycline 100 mg/liter. Three days after culturing the roots, all fungal isolates were transferred individually to one-fifth strength PDA + tetracycline (100 ppm) and identified to genus according to morphology. Additional fungi, not listed above, were isolated at low incidence (generally less than 5%).
[b]Autoclaved on 3 successive days at 110° C

Table 6. Incidence of *Pythium* spp. on roots of Marianna 2624 and Nemaguard rootstocks grown in the greenhouse in potted samples of soil from the Chico replant study[a]

Plant selection	Soil fumigation treatment	Soil heat treatment[c]	Apparent status of root sample	Incidence of *Pythium* (%)
Marianna 2624	Non-fumigated	None	Healthy	6
			Diseased	19
		Autoclaved	Healthy	0
			Diseased	0
	MBr-fumigated[b]	None	Healthy	0
			Diseased	0
		Autoclaved	Healthy	0
			Diseased	0
Nemaguard	Non-fumigated	None	Healthy	38
			Diseased	69
		Autoclaved	Healthy	0
			Diseased	0
	MBr-fumigated	None	Healthy	0
			Diseased	13
		Autoclaved	Healthy	0
			Diseased	0
Significant statistical effects[d]				Rootstock x fumig. x heat (P=0.01); heat x roothealth (P=0.04)

[a]For each treatment combination, four healthy and four discolored root segments (0.5 to 1 cm length, ≤1 mm dia) were sampled from eight replicate plants of Marianna 2624 plum and Nemaguard peach that had grown for 3 months in potted 0.6-liter samples of soil from the Chico replant trial. The root segments were rinsed in sterile water or bleached (10% commercial bleach, pH 7.0 to 7.2), and cultured on water agar amended with tetracycline 100 mg/liter. Three days after culturing the roots, all fungal isolates were transferred individually to one-fifth strength PDA + tetracycline (100 ppm) and identified to genus according to morphology.
[b]360 lb/A, pre-plant shank injected on 10/21/01, no tarp.
[c]Applied before planting in the greenhouse, after soil collection from Chico plots. Autoclaved on 3 successive days at 110 C.
[d]According to analysis of variance, SAS Version 8.

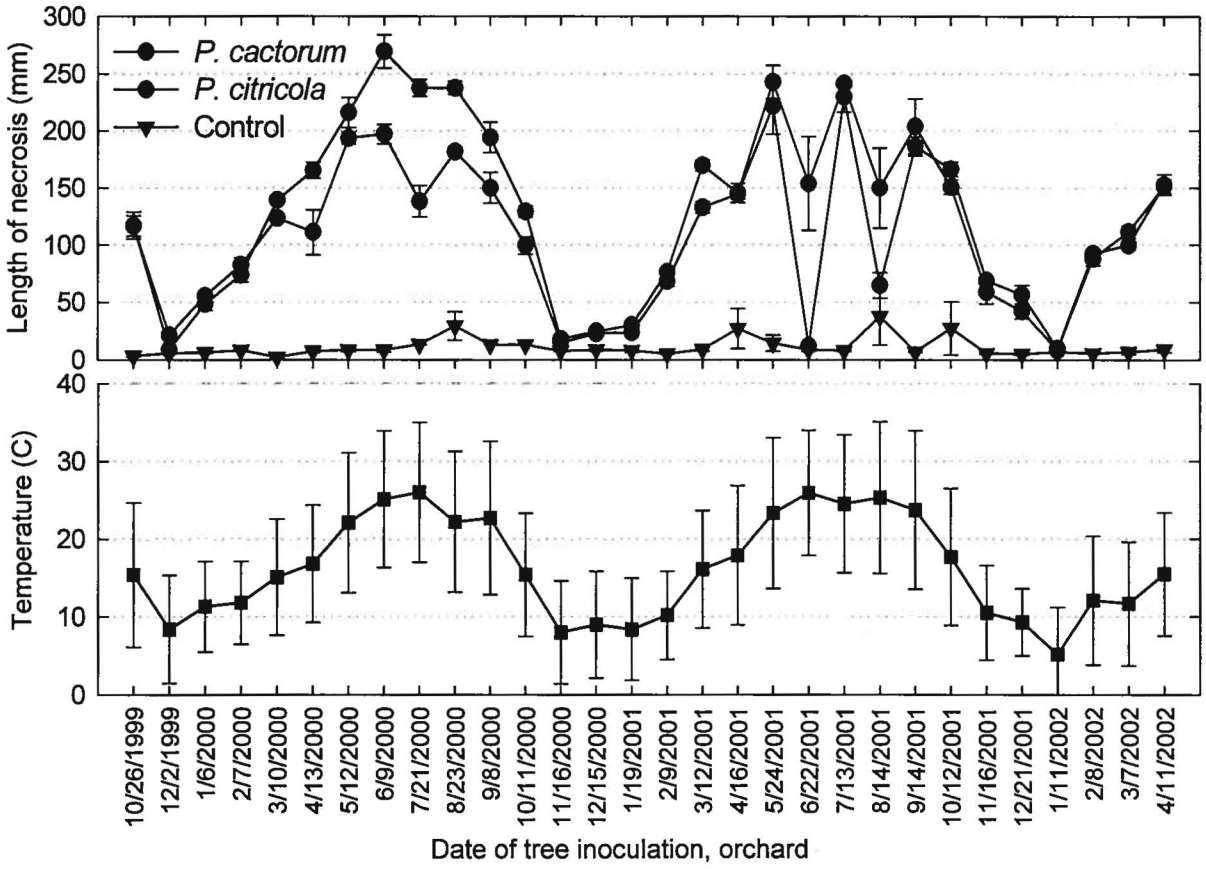

Fig. 1. A, Effect of date of inoculation on amount of canker development during 3-week incubation periods in a commercial almond orchard near Shafter, CA. Each data point represents eight separate inoculations. Vertical bars indicate standard errors for the means. **B**, Air temperatures (Shafter, CA) during the 3-week incubation periods; vertical bars indicate the average maximum and minimum temperatures for the periods, and points indicate averages of the average extreme temperatures.

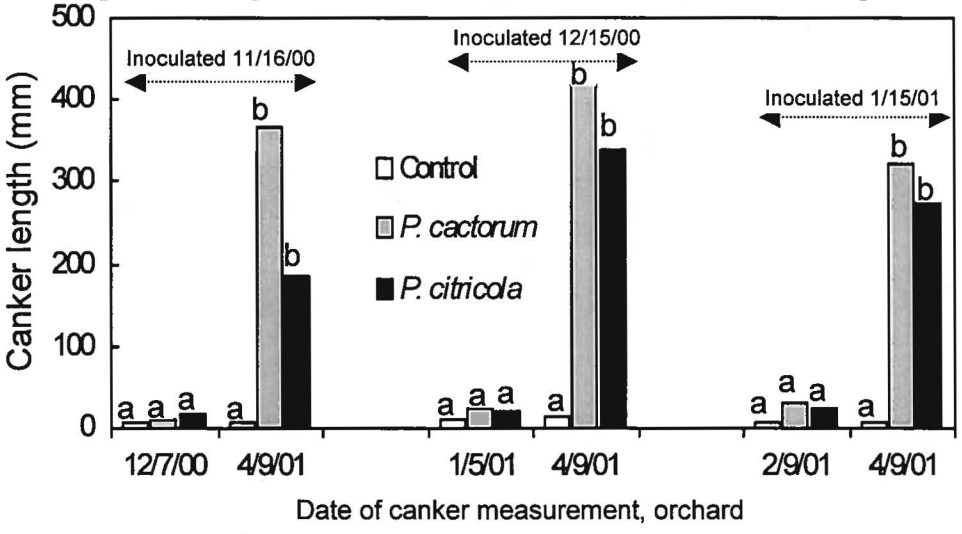

Fig. 2. Expansion in spring of the experimental orchard cankers that had grown little during initial 3-week winter incubation periods. Small cankers resulting from inoculations in December, January, and February were remeasured on 4/9/01.

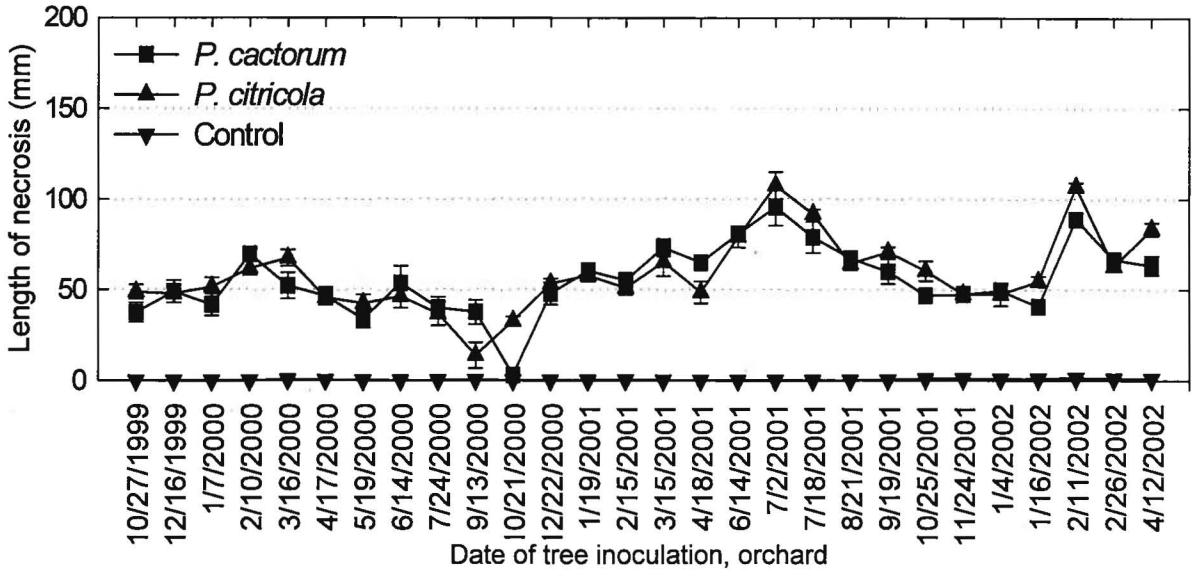

Fig. 3. Temporal variation in susceptibility of excised almond shoots to *Phytophthora cactorum* and *P. citricola.* Shoot segments (approx. 20 cm long) were excised from the trees used for monthly inoculations near Shafter (Figs. 1,2), wound inoculated on dates indicated, and incubated for 5 days at 20 to 24 C in humid chambers before disease assessment. Vertical bars indicate mean standard errors.

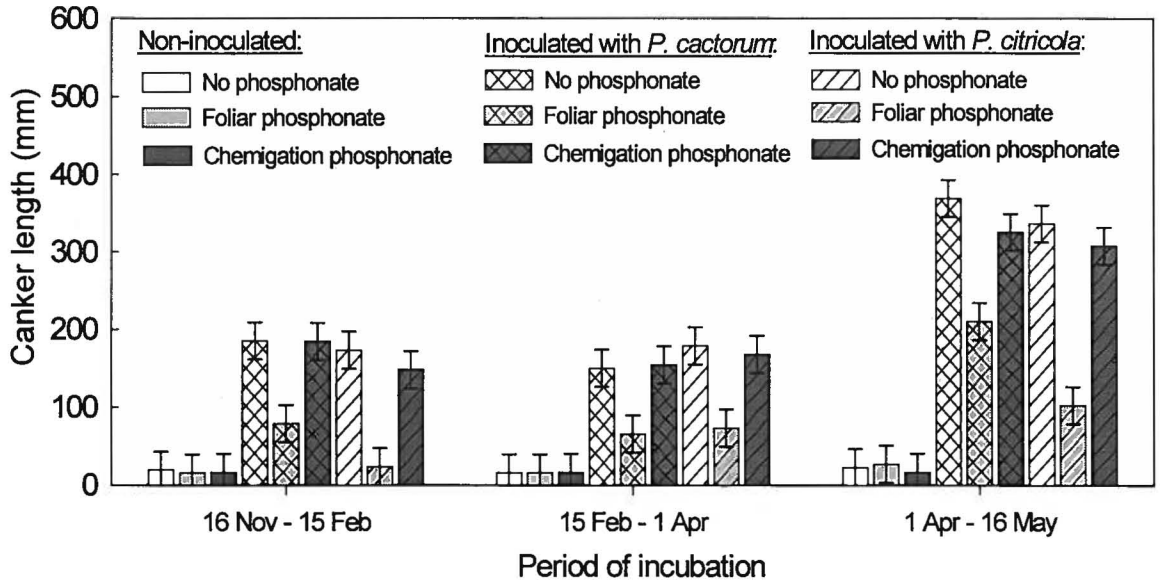

Fig. 4. Relative efficacy of foliar and micro sprinker applications of phosphonate for control of lethal Phytophthora cankers on commercial almond trees in a fall 2001-spring 2002 experiment. Phostrol (an experimental phosphonate, Nufarm Americas Inc.) was applied at 3.3 pints/acre on 10/19/01, either by a complete foliar spray (in 150 gal water per acre, conventional air blast) or by chemigation through micro sprinklers (the formulation was injected 3 to 4 hr before completion of an 18-hr irrigation set that applied 1.2" water). Treatment effectiveness was determined by inoculating groups of trees with *Phytophthora cactorum* or *P. citricola* at intervals of time after the phosphonate treatments. Control trees received the same amounts of water by spray and irrigation, but without Phostrol. Date ranges on the X-axis start with the date of inoculation and end with the date of canker measurement for the test intervals. Note that the Phostrol foliar spray suppressed canker development whereas the Phostrol chemigation did not. The experiment is being repeated in spring/summer 2002.

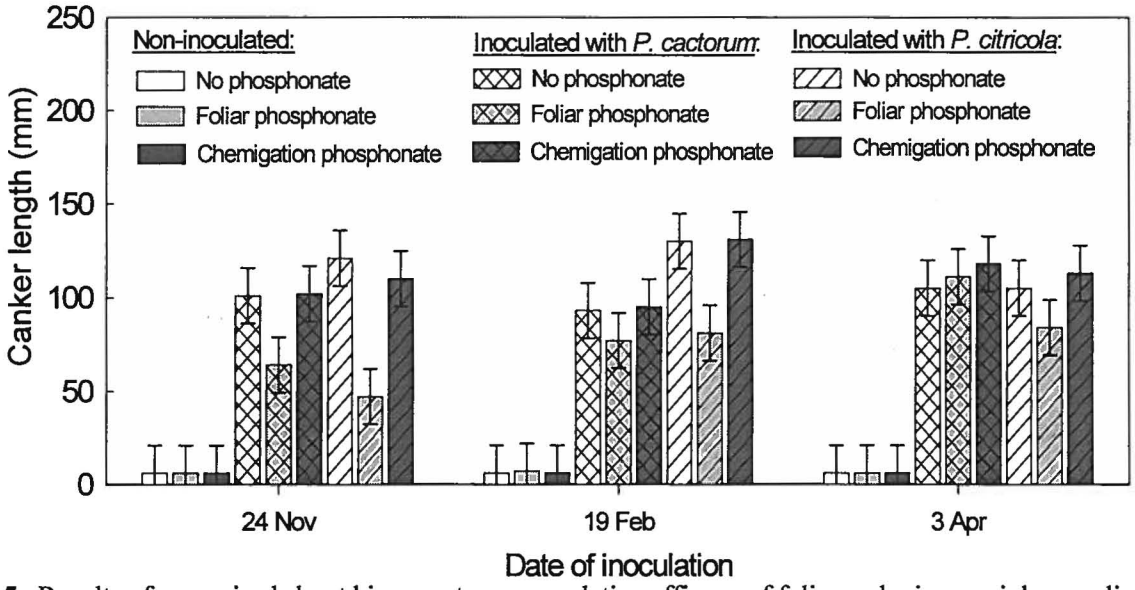

Fig. 5. Results of an excised shoot bioassay to assess relative efficacy of foliar and micro sprinker applications of phosphonate for control of lethal Phytophthora cankers. Basal segments of shoots (vigorous "water sprouts", about 10 to 15 mm diameter and 20 cm long) were collected from each plot described above on the field inoculation dates. The shoot segments were wound inoculated with *P. cactorum*, *P. citricola*, or sterile agar (the inoculation control). The inoculations with *P. cactorum* or *P. citricola* used 12 shoots per combination of inoculation date and Phostrol treatment, while those for the control used eight. After inoculation, the shoots were incubated for 1 week in a humid chamber (100% r.h.), and resulting canker length was used to assess effects of the orchard phosphonate treatments. Note that foliar-spray-induced canker suppression detected by the bioassay (above) did not last as long as that detected in the orchard experiment (Fig. 4).