2005 COMPREHENSIVE REPORT TO THE ALMOND BOARD OF CALIFORNIA BIOLOGY AND MANAGEMENT OF ALMOND REPLANT DISEASE

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Introduction.
Replant disease (RD) is a specific replant problem that complicates orchard establishment on land areas with a recent history of closely related crops. The disease can occur alone or in combination with other 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 known as important pathogens. In Butte County, almond RD has resulted in more than 50% loss of trees in large areas of orchards in their first year of growth. More typically, RD causes reduced vigor in orchards without killing trees. Although RD is most evident in the first few years after planting, yield may be negatively impacted for most of the orchard's life. Our trials and those of others indicate that RD has broad economic significance on almonds and other *Prunus* species in California. As orchard districts age and regulatory restrictions on the use of soil fumigants increase, integrated control strategies and knowledge on RD etiology are becoming essential.

Objectives.
1) Develop improved management strategies for replant disease (RD) on California almonds.
   a) Evaluate efficacy alternative pre-plant soil fumigants for control of RD.
   b) Evaluate efficacy of short-term fallowing and crop rotations for control of RD.
   c) Examine specificity between RD on peach (rootstock for almond) and RD on grape.
2) Determine the unknown causes of RD on almond.
   a) Examine symptoms of RD on almond and peach.
   b) Examine possible roles of nematodes in RD.
   c) Examine possible roles of fungi in RD.
   d) Examine possible roles of bacteria in RD.

Organization of this report.

Section I, entitled "*Examination of symptoms and control measures for replant disease of almond and peach in California*" is a formal summarization of several years' completed work on Objectives 1a, 2a, and 2b. This section was submitted for peer review for publication.

Section II summarizes current status of ongoing research on Objectives 1a-c, 2c, and 2d.

SECTION I. (Formal summarization of completed work, Objectives 1a, 2a, and 2b).

Examination of symptoms and control measures for replant disease of almond and peach in California

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ABSTRACT

Browne, G.T., Connell, J.H., and Schneider, S.M. 2005. Examination of symptoms and control measures for almond and peach in California.

Trials were conducted in three orchards near Chico, CA and three sets of microplots near Parlier, CA to examine symptoms and control measures for replant disease (RD) on almond and peach. The orchards had sustained severe RD in almond, typified by cessation of shoot growth, chlorosis, wilting, defoliation, and ≥50% tree mortality the year of planting on land cleared of old almond trees. The affected areas were cleared again, given soil fumigation treatments in the fall, and replanted with almond trees the following winter. The replants in non-fumigated soil developed severe RD by the following summer, while those in most fumigated treatments remained healthy. Trees in non-fumigated soil developed smaller trunk diameters and fewer healthy roots ≤ 1mm diameter, compared to the healthy trees; all rootstocks (Marianna 2624, Lovell, Nemaguard) were affected. Pre-plant tree-site treatments with methyl bromide (MB), chloropicrin, 1,3-dichloropropene (1,3-D), 1,3-D + chloropicrin, iodomethane, and iodomethane + chloropicrin all prevented severe RD. Broadcast soil fumigation with chloropicrin also was effective, but broadcast MB and 1,3-D were ineffective. In microplots of RD-conducive soil, chloropicrin was more potent than MB for prevention of RD on Nemaguard peach. There was no association between nematodes and RD in orchards or microplots in these trials. On almond and peach, RD apparently is mediated by biological agent(s) other than nematodes and can be prevented by appropriate fumigation with chloropicrin or other MB alternatives.

Additional key words: *Prunus dulcis*, *Prunus persica*, stone fruit replant disorder

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INTRODUCTION

Young trees of stone fruits (i.e., species of *Prunus*, including almond and peach) often suffer from diverse replant problems that cause them to grow sub-optimally or, in severe cases, die when planted after other crops. In the broad sense, some replant problems are largely abiotic and non-specific in nature. For example, nutrient deficiencies and toxicities, improper soil pH, and soil compaction associated with previous crop production can impede development of replanted orchards (14). On the other hand, replant problems tend to have strong biotic components. For example, populations of plant parasitic nematodes, fungi, and *Phytophthora* spp. can increase in orchards and singly or collectively cause disease on replanted stone fruits (14). Several species of endo- and ecto- parasitic nematodes attack the roots of fruit and nut

crops in California (26,27), and pre-plant soil fumigation treatments are typically designed to control these pests. Even in absence of known pests and pathogens, however, young trees of *Prunus* spp. often lag in growth and productivity when planted after previous generation(s) of the same crop (14). Replant problems are usually most pronounced in the first year after planting, but economic consequences of the delayed tree growth tend to persist for the life of the orchard (14,18). We advocate using the terms "replant disorder" and "replant disease" in reference to replant problems resulting primarily from abiotic and biotic causes, respectively (28).

There is a long-standing history of replant disease (RD) on stone fruits in California (3,6,18,22-24), and the disease continues to cause serious economic loss. In the northern part of California's Central Valley (i.e., Butte County), we have repeatedly observed poor vegetative growth and high incidences of tree mortality (≥50%) in young almond orchards planted on land with a long-term history (i.e., more than 10 year) of almond production (Connell and Browne, *unpublished*). In the affected orchards, surveys and diagnostic sampling failed to link the young tree failures with poor horticultural practice or known pests and pathogens, yet the affected plants exhibited necrosis of the fine roots. Severe RD does not always occur when almond is planted after almond, and we have not observed the disease in almond planted after herbaceous crops. Similar, though less severe, replant problems have occurred in almond and peach plantings after removal of stone fruit orchards on peach rootstock in the San Joaquin Valley (3,18,30).

Previous research has illustrated the etiological complexity of replant disease (RD) in Rosaceous and other plants. In 1941, a "peach replant problem" not associated with plant parasitic nematodes or other known root pathogens was reported in California (24). The problem exhibited specificity, i.e. peach after peach was affected, but not peach after apple (23). Use of macro and micro nutrients failed to alleviate the problem. Evidence has been presented for a role of toxigenic peach root residues in peach replant problems (7,21,24), but the reports have not found consistent support (10,13). In recent preliminary experiments with soils from RD-affected peach and almond orchards, either heating the soil (50°C or autoclaving) or treating it with fludioxonil fungicide (Maxim®, Syngenta Crop Protection, Inc.; Greensboro, NC) reduced severity of root cortex necrosis on Nemaguard peach seedlings, and the soils did not contain populations of plant parasitic nematodes (4). Union mild etch, a disorder of young almond trees on the marginally compatible Marianna 2624 rootstock has interfered with the development of young orchards in Northern California (31). In the state of Washington, apple replant disease was shown to result primarily from root infection by *Cylindrocarpon destructans*, *Phytophthora cactorum*, *Pythium* spp. and *Rhizoctonia solani* (16). For many plant species, crop yield or growth depression has been associated with elevated populations of deleterious rhizosphere microorganisms that negatively influence plant growth without parasitizing plant tissue (1,25).

Improved management strategies are needed for RD. As orchard districts have aged in California, risk of replant problems has increased. Pre-plant soil fumigation with methyl bromide (MB) has been used to prevent replant problems in deciduous tree plantings, but the fumigant is being phased out due to its potential to deplete ozone in the stratosphere. Alternative fumigants are available, but research is needed to test their efficacy and optimize their application for management of replant problems. All fumigants may face increased regulatory constraints in the future, and research is needed to develop cultural and biological approaches for managing replant problems.

The objectives of the research reported here were 1) to examine effects of RD on growth and health of almond and peach trees, and 2) to develop effective control measures for the disease. A portion of this work was reported previously (3).

MATERIALS AND METHODS

Almond orchard sites. Trials involving RD on almond trees were conducted from 2000 to 2004 in three commercial orchards within 20 km of Chico, CA (Orchards 1,2, and 3; details in sections below). The soils were Nord loam or Farwell loam, depending on the orchard. The orchards had been in commercial almond production for at least 15 years before the experiments were initiated. In the year before the trials, the growers had cleared and replanted the orchards with almond trees. The young trees developed severe RD (i.e., failure of >50% of replanted trees in land areas covering >2 ha) in the first year after planting. The affected areas were cleared again, and replant trials were established where incidence and severity of RD had been greatest.

Peach microplot sites. Trials involving RD on Nemaguard peach rootstock were conducted from 2000 to 2004 in microplots at the USDA-ARS San Joaquin Valley Agricultural Science Center, near Parlier, CA. The microplots were constructed with open-ended sections of concrete pipe, each measuring 0.6 m diameter and 1.2 m long. The sections were inserted lengthwise into holes in the ground and filled with non-fumigated Hanford fine sandy loam collected from 0 to 0.2-m depth in an adjacent peach orchard. After filling and settling the microplots, the rims protruded about 10 cm above the surrounding soil surface and the elevation of the enclosed soil was approximately the same as that of soil outside the rim.

Pre-plant fumigation. In the almond trials, depending on the year and experiment, pre-plant fumigation occurred from 21 Oct. to 1 Nov., approximately 4 months before experimental trees were planted. Within an experiment, all fumigation treatments were applied within 1 day. The soil at 10 to 60 cm depth was 14 to 20 °C and had moisture contents from 0.14 to 0.31 kg/kg oven-dry soil.

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

Tree-site fumigation treatments were applied in several additional almond trials. The soil at the tree sites (i.e., the spots where trees were to be planted) was prepared for fumigation with a tractor-powered auger, which removed the soil from 50 to 60-cm-deep × 60-cm-diameter holes. The loose soil was pushed back into and mounded above the holes. Fumigation treatments were injected through a 1-cm-diameter hollow metal probe that was inserted to a depth of 45 to 50 cm in the center of the soil-filled holes. At its upper end the probe was connected to a pressurized supply of fumigant. Fumigant doses were measured volumetrically in a sealed chamber before injection. Fumigant dose weights were verified with an electronic scale placed under the supply cylinder. Pressurized nitrogen gas was used to force the fumigant through the probe and into the soil. After fumigation, soil around the injection hole was compressed to prevent premature escape of the fumigant.

In the peach replant trials conducted in microplots, pre-plant soil fumigation treatments were applied (depending on experiment) on 30 Apr. 2002, 20 Nov. 2002, and 19 Nov. 2003. Before fumigation, the soil was cultivated with a hand shovel to a depth of 0.4 m and tamped

moderately at the surface using the flat side of the shovel. The cultivation and tamping was intended to facilitate diffusion of fumigant through the subsoil while retarding diffusion through the soil surface. Oat seeds (20 to 30 cc, soaked in water for 1 to 2 hr before use) in cloth sample bags (5 x 8 cm, ASC Scientific, Carlsbad, CA) were placed in the soil (depths 0 to 6 cm and 30 to 35 cm) to provide a bioassay of fumigant efficacy based on subsequent seed germination. Fumigants were injected into the soil at a depth of 30 cm near the center of each microplot through an 8-mm-diameter hollow metal probe. The probe was connected by flexible tubing at its upper end to a frame-mounted gas-tight syringe (Hamilton Company, Reno, NV). The syringe was filled and emptied manually, using a closed, valve-controlled supply system, to deliver 4.5 or 30 ml (approximately 7 or 50 g, respectively) of liquid chloropicrin or methyl bromide to each plot. Pressurized nitrogen gas was used to force the fumigant into the soil before probe removal. After fumigation, the soil around the injection hole was compressed, and virtually impermeable film (VIF) mulch (Bromostop, Bruno Riminni, Ltd.; London, UK) was used to seal the top openings of the microplots. Control microplots were cultivated, tamped, and sealed with VIF, but they received no fumigant.

Planting and cultural practices. Unless specified otherwise, all almond orchard trials involved planting conventionally grown, dormant, bare-root almond trees into plots that had received a pre-plant fumigation or control treatment (details below). Depending on the year and experiment, trees were planted from the last week of Jan. to the first week of Mar., 3 to 4 months after soil fumigation. Immediately after planting, the tree stems were trimmed off at 0.6 m above the soil surface, lateral shoots were trimmed to stubs that retained 1 to 2 buds, and a wax-impregnated white paper tube (10 cm diameter, 0.4-m height) was slipped over each tree stem for protection from sun and herbicides. The trees were irrigated by high-impact sprinklers; up to one irrigation per week was applied to meet crop evapotranspiration needs.

All peach microplot trials involved planting 2- to 3-month-old Nemaguard peach seedlings into soil that had received pre-plant fumigation or control treatments (details below). The seeds were stratified for 2 months (8), planted and grown in a greenhouse for 2 to 3 months in trays of 2 × 2 × 4 cm cells filled with UC mix soil (15), trimmed to a main stem height of 10 cm, and transplanted into the microplots (three seedlings per plot). Depending on the experiment, transplanting occurred on 3 June 2002, 9 Apr. 2003, or 14 Apr. 2004. The microplots were irrigated daily with 0.4 to 1.5 liter water per microplot through a drip system and fertilized monthly with $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ applied to yield 28 to 56 kg/ha N per fertilization. Irrigation amounts were increased and decreased according to soil moisture level, which was kept near field capacity. Weed control in the plots was achieved by regular hand pulling.

Effects of alternative fumigation treatments (Experiments 1 to 8). Effects of pre-plant soil fumigation treatments on incidence and severity of RD were examined in Experiments 1 to 5 with almond trees in Orchards 1 to 3 near Chico, CA and Experiments 6 to 8 with peach seedlings in microplots near Parlier, CA. Experiment 1 compared broadcast applications of methyl bromide:choropicrin (MB) (98:2, wt:wt, TriCal, Inc.; Hollister, CA), chloropicrin (Pic) (Triclor®, TriCal, Inc.), and 1,3 dichloropropene (1,3-D) (Telone II®, Dow Agrosciences, Indianapolis, IN), all at 400 kg/ha, and a non-fumigated control at Orchard 1. Each treatment was applied to four replicate 19×22-m plots in randomized complete blocks. Each plot was planted with three rows of six almond trees on Marianna 2624 rootstock; trees were 6.4 and 3.6 m apart between and within rows, respectively.

Experiments 2 to 5 evaluated pre-plant fumigation of tree sites at Orchards 1, 2, and 3. Depending on the orchard, tree sites were 1.8 to 2.9 m apart within rows and 6.4 m apart between rows. Depending on the experiment, treatments included MB at 0.45 kg per tree site; two or more rates of iodomethane (IM), IM:Pic (50:50, wt:wt) (both referred to as formulations of Midas®, Arvesta Corporation, San Francisco, CA), Pic, 1,3-D, 1,3-D:Pic (61:35, Telone C35®, Dow Agrosciences); and a non-fumigated control. In Experiments 1 to 4, there were 12 or 18 trees per treatment arranged in 6 randomized complete blocks. In Experiment 5, there were five trees per treatment in a completely randomized design. The tree sites all were planted with Carmel almond on Marianna 2624 rootstock.

Experiments 6 to 8 in microplots examined effects of Pic and MB (each at 7 and 50 g per plot, which were equivalent to 426 and 3026 kg/ha, respectively) and a non-fumigated control on incidence and severity of replant disease on Nemaguard peach. The treatments were arranged in 12 randomized complete blocks; each block had one replicate microplot per treatment.

Effects of different scion-rootstock combinations (Experiments 9 to 11). Experiments 9, 10, and 11 were conducted in Orchards 1, 2, and 3 near Chico, respectively. In Experiment 9, Carmel almond trees on rootstocks of Marianna 2624 and Lovell peach were planted in tree sites that had been fumigated with MB:Pic (0.45 kg per site) or Pic (0.45 kg per site) or left non-fumigated; there were 18 trees per factorial treatment combination, allocated evenly among six randomized complete blocks. Experiments 10 and 11 were like Experiment 9, except that Nemaguard peach rootstock also was included, and there were 12 trees per treatment allocated among six randomized complete blocks.

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

To determine effects of RD on root length density, Carmel almond trees on Marianna 2624 and Lovell rootstocks were planted in Feb. 2004 in Pic-fumigated (0.45 kg/site, applied in fall 2003) and non-fumigated control tree sites in Orchards 2 and 3, adjacent to areas used for Experiments 3, 4, 10, and 11. The fumigation treatments were randomized in blocks containing one (Orchard 3) or two (Orchard 2) tree sites per fumigation treatment. The trees on Marianna 2624 and Lovell rootstocks were considered to be in separate experiments because separate sets of blocks were used for each rootstock. On 20 Oct. 2004, root systems were sampled from three randomly selected trees on each rootstock in each of the orchards. For each sampled tree, all roots within a 60-cm-diameter × 45-cm-deep cylinder of soil centered on its tree trunk were

excavated by digging with hand shovels. The roots and adhering soil were collected in plastic bags and stored at 4 °C for several days before they were separated from soil. The roots were gently washed of soil while being supported on a 2-mm mesh screen and blotted to remove free water. Roots that washed through the screen were collected and included in length analyses. An Epson 1640 XL scanner optimized for root system analyses by Regent Instruments, Inc. (Ste-Foy, QC, Canada) and WinRHIZO v.2004b software were used to capture and analyze images of the roots. The "Regents simple scanner interface" was used with 800 dpi grey scale images specified, and the roots were spread on the scanner glass so that there was minimal overlap between them. Exclusion regions were defined on the scanned images to eliminate contributions of debris before total root length was determined.

For experiments in peach microplots, effects on RD were assessed by measuring the height of plants and weighing their tops (i.e., the stems and shoots) on two occasions during the growing season. On each date, the heights and weights were obtained for all plants in four randomly selected blocks. Effects of RD on root length densities of Nemaguard peach plants were determined in the 2004 microplots (Experiment 8). The first week of Nov. 2004, 13-cm-diameter × 30-cm-deep cores of soil and the enclosed roots were collected from four randomly selected blocks of the microplots. Each soil-root core was centered around the stem of one peach plant, two plants were sampled per microplot. The roots were washed from the soil, scanned, and analyzed as described above for the almond tree root samples.

Examining plant-parasitic nematode populations. Samples of soil and roots were collected periodically from trees in the Chico and Parlier trials and assayed to determine whether certain bacteria, fungi, or plant-parasitic nematodes were associated with incidence of replant disease. Only examinations of nematode populations are reported on here. As the roots were collected, some of them were visually examined for symptoms of disease (i.e., necrosis, deformity, etc.). In Orchard 1, the samples were collected non-destructively on 11 Nov. 2002 from the trees in three randomly selected blocks of Experiment 9. A 500-cc sample of soil and roots was collected from depths of 5 to 45 cm below the soil surface within 30 cm of each tree's trunk. The soil samples were processed using the sieving/sugar flotation/centrifugation protocol with a 500-mesh sieve (25 μm opening) to extract nematodes (12). Nematodes were identified and counted under the microscope. In Orchards 2 and 3, samples were collected destructively in Oct. 2003 by excavating almond trees on Marianna 2624 rootstock; four trees were sampled per treatment in randomized complete blocks of non-fumigated and Pic-fumigated (0.45 kg per tree site) plots. These blocks of Pic and non-fumigated plots had been established adjacent to plots in Experiments 3,4, 10, and 11 solely for sampling purposes. At least 20 g of fine roots (diameter $\leq$3 mm) and 500 cc of adjacent soil (5 to 45 cm soil depth, $\leq$ 30 cm from the tree trunk) were collected from each of the trees. The soil samples were assayed for plant parasitic nematodes by the sieving/sugar flotation/centrifugation protocol described above and root samples were assayed using the mist chamber protocol (11). In Orchard 3, the destructive soil and root sampling procedures were repeated on 5 Oct. 2004 using an additional four blocks of single-tree Pic and control plots. These plots had been planted to almond trees on Marianna 2624 rootstock in Mar. 2004 after pre-plant fumigation in Nov. 2003, and they were sampled as described for the 2003 samples.

Sampling for nematodes in the Parlier microplots occurred on one or two occasions for each year's experiment (i.e., 13 Aug. and 25 Sep. for the 2002 trial, 14 Aug. for 2003, and 24 Oct. for 2004). On each sampling date, the microplots in four randomly selected replicate blocks were sampled. A soil sampling tube (2 × 45 cm) was used to collect multiple cores of soil and

roots, totaling 500 cc per microplot, from 0 to 45 cm soil depth within 20 cm of the experimental peach plants. Nematodes were extracted and counted from soil samples as described above. In addition, 20 g of the Nemaguard peach roots were collected from each microplot sampled on 24 Oct. 2004 and processed by the mist chamber protocol.

Data analyses. All plant growth and health data were subjected to analysis of variance (ANOVA) using PROC MIXED of SAS software (SAS, Release 9.1, Cary, NC). Data from sub sample trees (i.e., those given the same treatment within a microplot or block) were averaged before ANOVA. Block was specified as a random effect in experiments with randomized complete block designs. Confidence intervals (95%) were generated to facilitate mean separation.

RESULTS

Effects of alternative fumigation treatments (Experiements 1 to 8). For all tree response variables in experiments with almond trees on Marianna 2624 rootstock planted in orchards with a history of RD, effects of pre-plant fumigation treatments were significant or highly significant ($P<0.0001$ to 0.02, Tables 1,2). In Experiment 1, which involved broadcast shank applications of MB, Pic, and 1,3-D at 400 kg/ha at Orchard 1, only the Pic treatment was effective (Table 1). In the control plots, the almond trees grew little after planting (mean trunk diameter increase 1 mm, final tree height 1.0 m) and had high disease ratings (mean 3.4). Only 3% of the trees were commercially acceptable. The standard MB treatment increased trunk growth and decreased the disease rating significantly compared to the control, but the gains were small. Only 42% of the trees were commercially acceptable. The Pic treatment increased trunk diameter growth and decreased disease ratings by a factor of approximately 10, compared to the control, and 96% of the trees were commercially acceptable. The 1,3-D treatment did not significantly improve tree growth or health.

In Experiment 2, which involved fumigation of tree-sites in Orchard 1, all of the fumigation treatments (MB, 1,3-D, and Pic at 0.2 to 0.9 kg per tree site) resulted in greater trunk diameter increase and lower disease ratings compared to the non-fumigated control (Table 2). However, severity of RD in the control was relatively low; 70% of the trees were commercially acceptable. Greatest tree growth and lowest disease ratings occurred following Pic at 0.2 to 0.5 kg per tree site, but Pic at 0.9 kg per tree site caused phytotoxicity (mean disease severity 1.7).

In Experiments 3 and 4, which involved tree site fumigation in Orchards 2 and 3, respectively, severe RD resulted in almond trees planted in non-fumigated control plots in the first season of tree growth (mean trunk diameter increases 3 to 7 mm, disease ratings 3.3 to 3.5, commercially acceptable trees 0 to 17%; Table 1). By the end of the second growing season, tree trunk diameters in control plots had still increased much less than those in fumigated plots, although the control trees had only moderate mean disease ratings (1.0 to 2.1). In both experiments, pre-plant fumigation with MB at 0.5 kg per tree site resulted in large increases in trunk growth and tree height and lowered disease ratings compared to the control. The improvements in tree growth and health persisted in the second growing season. The other pre-plant fumigation treatments, Pic, IM (Experiment 4 only), IM:Pic, 1,3-D, and 1,3-D:Pic at 0.2 and 0.5 kg per tree site improved tree performance to similar or greater extents, compared to the MB treatment in both growing seasons. In Experiment 5, all rates of Pic tested (0.12, 0.24, 0.45, 0.9 kg per tree site) resulted in large and equivalent increases in tree trunk growth, compared the control (Fig. 1).

In the Experiments 6 to 8 with Nemaguard peach seedlings in microplots filled with soil from a RD-affected peach orchard, pre-plant fumigation treatments consistently had highly

significant effects on growth of Nemaguard peach seedlings (P<0.0001; Fig. 2, A-C). The plants in control plots accumulated relatively small top fresh weights. They had relatively small, chlorotic leaves and small root systems (data not shown). The low rate of MB (7 g per plot) generally had no significant effect on the plant top weights, whereas the low rate of Pic increased shoot weights by 1.5 to 14.5 × compared to the control, depending on the experiment and date of measurement. Both fumigants were effective at the high rate (50 g per plot) and increased shoot weights by 2.5 to 20 × compared to the control (Fig. 2). The favorable growth responses to fumigation became evident by early summer and persisted through the growing season.

Effects of rootstocks. In Experiment 9, rootstock and pre-plant fumigation had highly significant main effects on all tree response variables (P<0.0001 to 0.007; Table 3), but there was no significant interaction between the treatment factors (P=0.15 to 0.19). Neither rootstock performed well in the non-fumigated soil, although almond trees on Lovell were marginally healthier than those on Marianna 2624 (mean disease ratings 1.7 and 2.9, respectively). Pre-plant fumigation with either MB:Pic or Pic (0.5 kg per tree site) resulted in large increases trunk diameters and tree heights for both rootstocks, although trees on Nemaguard rootstock generally increased more in trunk diameter and height than those on Marianna 2624 rootstock (Table 3).

Results of Experiments 10 and 11 were combined for all treatment variables except "acceptable trees (%)", due to lack of significant experiment × treatment interaction for all variables except the latter (Table 3, Experiments 10,11). Rootstock and fumigation had significant main effects for all variables (P<0.0001 to 0.008). Interaction of rootstock × fumigation was significant only for disease ratings (P=0.01). Almond trees in non-fumigated soil grew poorly regardless of rootstock, but those on Lovell or Nemaguard (mean disease ratings 2.1 and 2.6, respectively) did marginally better than those on Marianna 2624 (rating 3.4) (Table 2, Experiments 10,11). Pre-plant fumigation with either MB:Pic or Pic (0.5 kg per tree site) resulted in large and significant increases trunk diameters and tree heights for all rootstocks.

Effects of pre-plant fumigation on root length density. Pre-plant soil fumigation with Pic increased root length density on almond trees in Orchards 2 and 3 (Fig. 3). For the trees on Lovell rootstock, results from the two orchards were combined due to lack of significant interaction of orchard location with other factors (P=0.10, Fig. 3A). On Lovell, there was significant interaction between pre-plant fumigation treatment and root diameter class (P<0.0001). Most roots were in the 0 to 0.5 mm root diameter class, and pre-plant fumigation with Pic more than doubled the length density in this class. For the trees on Marianna 2624 rootstock, there was a significant three-way interaction among fumigation treatment, orchard location, and root diameter class (P<0.0001), so results are presented by orchard. In Orchard 2, most root length occurred in roots ≤ 1.0 mm diameter, and trees in Pic plots had an average of approximately six times more total root length, compared to the control (Fig. 3B). In Orchard 3, results were similar, except Pic only increased density in the 0 to 0.5 mm class where it approximately doubled root length density compared to the control.

In Nemaguard peach root samples from microplots, both of the Pic treatments (7 and 50 g per plot) significantly increased root length densities in the 0 to 0.5 mm diameter class compared to the other treatments. Samples from both the MB treatments had root length densities equal to or smaller than the control (Fig. 4).

Lack of significant populations of plant parasitic nematodes. In Orchard 1, Experiment 9, the sugar floatation method extracted 0 to 1 lesion nematode (*Praytelenchus* sp.) per 250 cc of soil, regardless of pre-plant fumigation treatment. No other plant parasitic nematodes were detected. Similarly, no plant parasitic nematodes were detected by sugar

floatation or mist chamber extraction from soil and root samples collected from Orchard 2 in 2003 and 2004 and Orchard 3 in 2003.

In the 2002 microplots, numerically significant populations of the pin nematode (*Paratylenchus* sp.) were detected in samples from the non-fumigated treatment, but there was no clear association between the populations and incidence or severity of RD. An average of 424 and 122 pin nematodes per 250 cc were extracted by sugar flotation from non-fumigated control samples collected on 14 Aug. and 24 Sep., respectively. Fewer than 8 pin nematodes per 250 cc were detected in the other treatments in 2002, and none were in plots fumigated with 5 g MB. The lesion nematode (*Praytelenchus* sp.) was detected in only one control plot (2 per 250 cc; 14 Aug. 2002).

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

DISCUSSION

We have characterized symptoms of a particular replant problem, RD on almond and peach in the Central Valley of California, determined it is not associated with nematode infestation yet has severe impacts on three important rootstocks, and demonstrated it can be prevented by pre-plant fumigation with several MB alternatives. The results address a need for improved management strategies for replant problems. The results are relevant to California peach production as well as almond production because the crops have their most prevalent rootstocks in common (i.e., Nemaguard and Lovell peach) (8) and are grown in overlapping areas of the state. At sites severely affected by RD, pre-plant soil fumigation with Pic, applied as a tree-site or broadcast treatment, consistently prevented the disease and often was more effective than fumigation with MB. Additionally, tree site fumigation with 1,3-D, IM, or combinations of them with Pic, prevented the disease and matched or exceeded the efficacy of tree site treatments with MB.

The effectiveness of the tree site treatments demonstrated that RD can be prevented without applying fumigants to entire areas or wide strips of land. This is important because a reduction of treated area potentially reduces environmental impacts and fumigant costs. In repeated experiments, 0.2 and 0.5 kg Pic per tree site were equally effective and consistently prevented RD, and in Experiment 5, 0.12 kg per tree site was also effective. At commercial planting densities of 200 to 350 trees/ha, use of 0.2 kg of chloropicrin per tree site requires 46 to 80 kg/ha (orchard basis), which is significantly less than the amount required for a typical broadcast treatment with Pic (approximately 336 kg/ha).

Using the 60-cm-diameter tractor-powered auger to loosen soil in the tree planting holes before fumigation was considered essential for effective fumigation of tree sites with the probe. In exploratory experiments, poor results were obtained when an 8-cm-diameter auger was used. Use of the larger auger not only facilitated penetration of the fumigation probe, but it also probably facilitated diffusion of the fumigant to where it was most needed by lowering the bulk density of soil to be explored by the new tree roots in the first few months after planting. Concentrating a relatively large fumigant dose in a small volume of soil probably also contributed to the efficacy of tree site fumigation treatments. For example, under the reasonable

assumption that most a 0.2-kg dose of fumigant applied to a tree site is retained within a 0.7-m radius of a single injection point, the average application rate in the area would be 1300 kg/ha, about four times that of conventional broadcast rates.

Data were collected only for 1 to 2 growing seasons from the almond orchard and peach microplot experiments. Nonetheless, field observations suggest that this time span was sufficient for evaluating the control measures. In California, RD of almond is most clearly evident in the first year after planting, and we have not observed severe cases (i.e., high incidence of tree death or failure to grow) on almond or peach trees >1 year old, assuming the trees have grown satisfactorily in the first year after planting. In contrast, plant parasitic nematodes such as *Meloidogyne* spp., *Mesocriconemella xenoplax*, and *Praytylenchus vulnus* can cause progressive decline of trees for the life of an orchard, and ring nematode can predispose stone fruits to bacterial canker disease for several years after planting (9,27,32). Our findings apply only to almond and peach RD in absence of other replant problems.

Our results suggest practical problems worthy of further investigation. Long-term research is needed and underway to determine relative efficacy of broadcast, row-strip, and tree-site pre-plant applications of fumigant for various replant situations (i.e. with and without high populations of plant parasitic nematodes, following different crops and periods of fallowing, etc.). Also, although tree site treatments using hand-held probes are currently approved under some conditions for certain fumigants, they may involve more worker exposure to fumigant than applications using tractor-mounted shanks or drip systems. We are involved in adapting global positioning system technology to facilitate focused shank applications of fumigant to rectangular areas around tree sites. Finally, temporary subsurface drip systems have been used experimentally for "broadcast" fumigation of orchard areas (29), but the method involves design, materials, and expense for a drip system that is abandoned in the soil after fumigation. Our results suggest that, at least for RD, drip fumigation could be focused on areas near tree sites, which would make it possible to fumigate through a permanent drip system designed for future irrigation of the orchard, but additional research is needed to evaluate the focused drip treatments.

Although the orchard and microplot trials involved different locations and procedures (i.e., Sacramento Valley vs. San Joaquin Valley, orchard plots vs. microplots, almond trees vs. peach seedlings, etc.), there were important similarities in results. At both locations the experimental trees grew satisfactorily for several weeks, but within the first growing season those planted in non-fumigated soil exhibited varying degrees of stunting, chlorosis, wilting, and defoliation. At both locations, chloropicrin was generally more effective than MB for prevention of RD. These results suggest that RD has widespread geographical significance on these crops in California and that chloropicrin may be widely effective in preventing the disease. These findings are consistent with previous reports involving peach and almond (4,22,23,30).

The efficacy of the diverse fumigants in repeated orchard and microplot trials is evidence for biological mediation of RD. Although fumigants vary in toxicity to various pests and pathogens, Pic, MB, IM, and 1,3-D are all broad-spectrum biocides (5,20). The repeated negative results from nematode sampling indicate that plant parasitic nematodes did not play an important role in RD at the test locations. Significant populations of the pin nematode (*Paratylenchus* sp.) were detected in control plots containing RD-affected peach seedlings in the 2002 microplot experiment, but the association between RD and the nematode did not hold-- in plots treated with 5 g of MB, RD but not the nematode, was present. The pin nematode is known to be parasitic on *Prunus* spp. (2), but it is not regarded as an economic pest on these crops (17). It is interesting to

note that Pic, which was highly effective for prevention of RD, is 8.5% N and is relatively toxic to nitrifying bacteria (28). In a previous report, soil fumigation with either Pic or 1,3-D resulted in a net increase in N availability, despite an accompanying decrease in the rate of nitrogen transformations (i.e., mineralization, nitrification) (19). Similarly, soil sterilization with steam or soil fumigation with Pic resulted greater ammonium accumulation and nitrogen availability for several months compared to soil fumigation with MB (28). In our experiments, the amount of nitrogen applied in the tree site fumigations with Pic (approximately 9 to 36 g/site, depending on treatment) may have stimulated plant growth, but this was not investigated. However, it is doubtful whether amounts of nitrogen applied during broadcast fumigation in Orchard 1 (26 kg/ha) or the low rate in the microplots (5 g/plot) were alone responsible for much of plant growth response that resulted from the treatments.

In Orchards 2 and 3, decreased root length density was associated with RD incidence. The relationship was less clear in the 2004 microplots. The orchard results suggest that the disease may be initiated on fine roots, whereas the microplot results suggest that the root sampling protocol for peach plants was inadequate. We have preliminary evidence for contributions of culturable fungi to etiology of RD (3). Research is clearly needed and underway on microbial contributions to RD etiology.

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

		Tree performance in first growing season[b]			
Fumigant[a]	Rate of application (kg/ha)	Increase in trunk diameter (mm)	Disease rating (0 to 5 scale)	Tree height (m)	Acceptable trees (%)
Control	none	1	3.4	1.0	3
MB	400	4	2.1	1.2	42
Pic	400	10	0.3	1.7	96
1,3-D	400	2	2.9	1.1	8
95 % C.I. (+/-):		1	0.3	0.1	9

[a]All fumigants, methyl bromide (MB), chloropicrin (Pic), and 1,3-dichloropropene (1,3-D) were injected into soil by tractor-mounted shanks spaced 50 cm apart at a soil depth of 45 to 50 cm. MB included 2% Pic.

[b]Increase in trunk diameter measured from the time of planting, 22 Jan., to 13 Aug. 2001. Disease rating based on a scale of 0=healthy tree, 5=dead tree, and 1, 2, 3, and 4 were progressive increments of disease within the extremes. Tree height and acceptable trees determined on 13 Aug. 2001. Acceptable trees, based on commercial standards, had height >1.2 m and disease ratings <3.

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

			By end of first growing season[b]				By end of second growing season[c]	
Expt. no.	Fumigant[a]	kg/site	Growth in tree trunk diameter (mm)	Disease rating	Tree height (m)	Acceptable trees (%)	Increase in trunk diameter (mm)	Disease rating
2	Control	0.0	8	2.0	1.4	70	--	--
	MB	0.5	12	1.0	1.8	85	--	--
	1,3-D	0.8	12	1.0	1.8	89	--	--
	Pic	0.2	17	0.4	2.0	93	--	--
	Pic	0.5	17	0.4	2.0	93	--	--
	Pic	0.9	12	1.7	1.6	63	--	--
	95% C.I (+/-):		3	0.6	0.2	15	--	--
3	Control	0.0	7	3.3	1.1	0	16	2.1
	MB	0.5	18	1.0	1.7	92	48	0.0
	Pic	0.2	25	0.3	2.0	100	55	0.0
	Pic	0.5	22	0.4	1.9	100	50	0.0
	IM:Pic	0.2	22	0.5	1.9	92	56	0.1
	IM:Pic	0.5	21	0.7	1.9	92	53	0.0
	1,3-D	0.2	17	1.1	1.6	92	46	0.0
	1,3-D	0.5	20	0.7	1.7	92	50	0.0
	1,3-D:Pic	0.2	20	0.9	1.7	83	51	0.0
	1,3-D:Pic	0.5	24	0.3	1.9	92	54	0.0
	95% C.I (+/-):		3	0.5	0.2	15	6	0.7
4	Control	0.0	3	3.5	1.2	17	19	1.0
	MB	0.5	11	0.8	1.6	75	35	0.0
	Pic	0.2	17	0.1	2.0	100	40	0.0
	Pic	0.5	17	0.3	2.0	92	42	0.1
	IM	0.2	12	0.8	1.8	100	37	0.1
	IM	0.5	14	0.4	1.9	100	37	0.0
	IM:Pic	0.2	16	0.1	2.0	100	41	0.0
	IM:Pic	0.5	16	0.2	2.0	100	40	0.0
	1,3-D	0.2	13	0.8	1.7	100	33	0.5
	1,3-D	0.5	15	0.3	2.0	100	38	0.0
	1,3-D:Pic	0.2	14	0.3	1.9	100	37	0.3
	1,3-D:Pic	0.5	15	0.4	1.9	100	39	0.0
	95% C.I (+/-):		2	0.4	0.1	12	3	0.2

[a]All fumigants, methyl bromide (MB), chloropicrin (Pic), iodomethane (IM), IM:Pic (50:50 wt:wt), 1,3-dichloropropene (1,3-D), and 1,3-D:Pic (61:35 wt:wt, Telone C35) were injected by a hand-held probe at one point at a soil depth of 40 to 50 cm in the center of sites where trees were to be planted.

[b]For Experiments 2, 3, and 4, respectively: increase in trunk diameter measured from 27 Feb. to 11 Oct. 2002, 28 Feb. to 9 Dec. 2003, and 3 Mar. to 9 Dec. 2003; disease ratings made on 11 Oct. 2002, 30 Sep. 2003, and 30 Sep. 2003 based on a scale of 0=healthy tree, 5=dead tree, and 1, 2, 3, and 4 were progressive increments of disease within the extremes; and tree heights were measured on 11 Oct. 2002, 9 Dec. 2003, and 9 Dec. 2003. Acceptable trees, based on commercial standards had height >1.2 m and disease ratings <3.

[c]Experiment 2 was concluded at the end of the first growing season. For Experiments 3 and 4, increases in trunk diameter were measured from the dates of planting to 9 Feb. 2005 and disease ratings were made 31 Aug. 2004.

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

				By end of first growing season[b]					By end of second growing season[c]	
Expt. no.	Rootstock	Fumigant[a]	kg/site	Growth in tree trunk diameter (mm)	Disease rating	Tree height (m)	Acceptable trees (%)		Increase in trunk diameter (mm)**	Disease rating (0 to 5 scale)
9	Mar. 2624	Control	0.0	4	2.9	1.1	70		--	--
		MB:Pic	0.5	15	0.4	1.9	85		--	--
		Pic	0.5	14	0.6	1.9	89		--	--
	Nemaguard	Control	0.0	7	1.7	1.6	93		--	--
		MB:Pic	0.5	15	0.3	2.1	93		--	--
		Pic	0.5	17	0.0	2.3	63		--	--
	95% C.I (+/-):			2	0.5	0.2	21		--	--
							Ex.10	Ex.11		
10, 11	Mar. 2624	Control	0.0	5	3.4	1.2	20	0	23	1.3
		MB:Pic	0.5	20	0.5	1.9	100	90	48	0.0
		Pic	0.5	22	0.3	2.0	100	100	52	0.0
	Lovell	Control	0.0	9	2.1	1.5	90	60	34	0.1
		MB:Pic	0.5	21	0.1	2.3	100	100	49	0.0
		Pic	0.5	22	0.2	2.2	100	100	52	0.0
	Nemaguard	Control	0.0	7	2.6	1.4	100	20	31	0.3
		MB:Pic	0.5	18	0.3	2.0	100	100	46	0.0
		Pic	0.5	20	0.2	2.2	100	100	47	0.0
	95% C.I (+/-):			2	0.3	0.1	14	14	3	0.2

[a]Methyl bromide + chloropicrin (MB:Pic, 75% MB 25% Pic) and chloropicrin (Pic) were injected to a soil depth of hand-held probe at one point at a soil depth of 40 to 50 cm in the center of sites where trees were to be planted.

[b]For Experiments 9, 10, and 11, respectively: increases in trunk diameter were measured from 27 Feb. to 11 Oct. 2002, 28 Feb. to 9 Dec. 2003, and 3 Mar. to 9 Dec. 2003; disease ratings were made on 11 Oct. 2002, 30 Sep. 2003, and 30 Sep. 2003 based on a scale of 0=healthy tree, 5=dead tree, and 1, 2, 3, and 4 were progressive increments of disease within the extremes; and tree heights were measured on 11 Oct. 2002, 9 Dec. 2003, and 9 Dec. 2003. Acceptable trees, based on commercial standards, had height >1.2 m and disease rating <3.

[c]Experiment 9 was concluded at the end of the first growing season. For Experiments 10 and 11, increases in trunk diameter were measured from the dates of planting to 9 Feb. 2005.

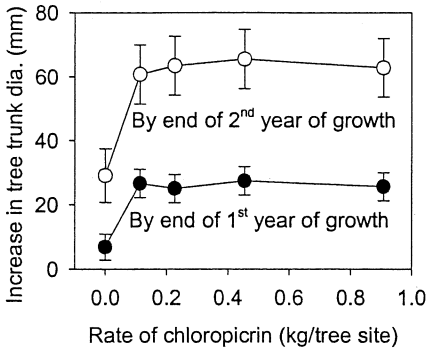

Fig. 1. Effect of different pre-plant doses of chloropicrin, injected at tree sites at soil depth of 40 to 50 cm, on growth of almond trees on Marianna 2624 rootstock in Experiment 5. Increase in tree trunk diameter was measured from planting on 28 Feb. 2003 to 9 Dec. 2003 (first season) and to 9 Feb. 2005 (second season). There were five replicate trees per treatment. Vertical bars are 95% confidence intervals.

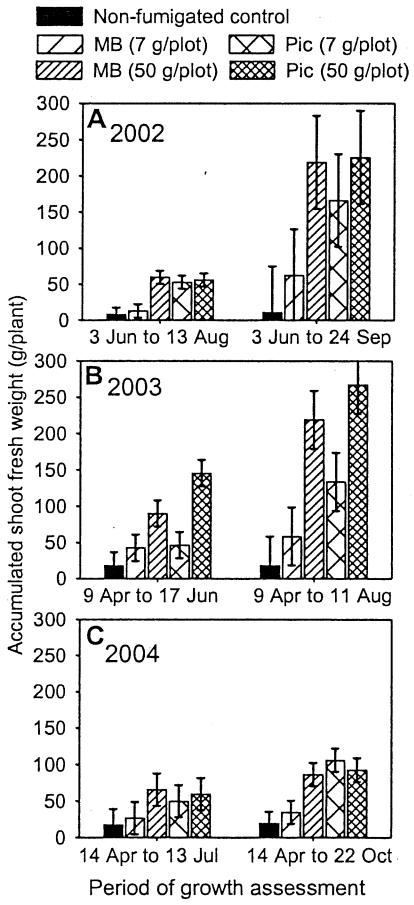

Fig. 2. Effect of pre-plant doses methyl bromide (MB) and chloropicrin (Pic), injected at soil depth of 40 to 50 cm, on growth of Nemaguard peach seedlings in microplots near Parlier, CA. The microplots were filled with soil from an adjacent established peach orchard. Fumigation and planting occurred: 30 Apr. and 3 June (A, 2002 experiment), 20 Nov. 2002 and 9 Apr. 2003 (B, 2003 experiment) and 19 Nov. 2003 and 14 Apr. 2004 (C, 2004 experiment). Three peach seedlings were planted in each microplot. There were four microplots per treatment per period of growth assessment, arranged in randomized complete blocks. At the end of each period of growth assessment, total shoot fresh weights were determined for the peach seedlings in four randomly selected blocks of microplots. Vertical bars are 95% confidence intervals.

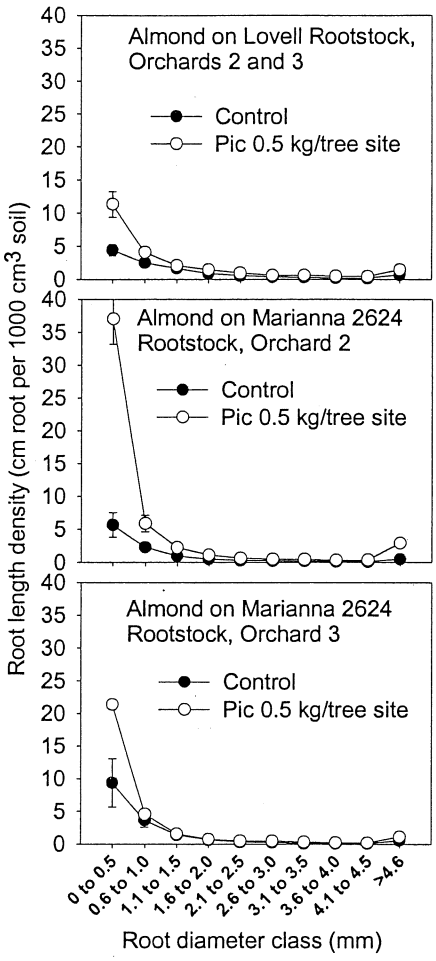

Fig. 3. Effect of pre-plant fumigation with chloropicrin, injected at tree sites at soil depth of 40 to 50 cm, on root length density on almond trees on Lovell peach and Marianna 2624 rootstocks in commercial orchards affected by replant disease near Chico, CA. The trees were planted in Feb. 2004. On 20 Oct. 2004, root systems were sampled from three randomly selected trees on each rootstock in each of the orchards. For each sampled tree, all roots and adhering soil within a 60-cm diameter × 45 cm deep cylinder of soil centered around its tree trunk were excavated by digging with hand shovels. The roots were gently washed free from soil. Root length densities were determined using an Epson 1640 XL scanner and WinRHIZO v.2004b software. Vertical bars are 95% confidence intervals.

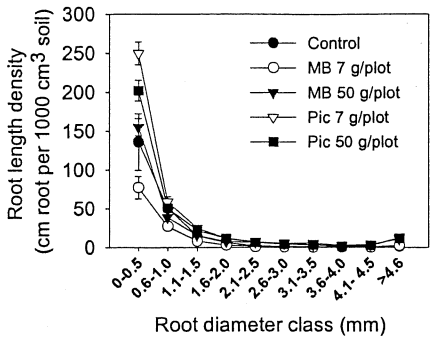

Fig. 4. Effect of pre-plant doses methyl bromide (MB) and chloropicrin (Pic), injected at soil depth of 40 to 50 cm, on root length densities on Nemaguard peach seedlings in microplots near Parlier, CA. The microplots were filled with soil from an adjacent established peach orchard. Fumigation and planting occurred 19 Nov. 2003 and 14 Apr. 2004, respectively. In the first week of Nov. 2004, 13-cm diameter × 30 cm deep cores of soil and the enclosed roots were collected from four randomly selected blocks of the microplots. Each soil-root core was centered around the stem of one peach plant, two plants were sampled per microplot. The roots were gently washed free from soil. Root length densities were determined using an Epson 1640 XL scanner and WinRHIZO v.2004b software. Vertical bars are 95% confidence intervals.

SECTION II. (Summary of on ongoing work, Objectives 1a-c, 2c,d).

Objective 1a. Efficacy of alternative pre-plant soil fumigants and alternative rootstocks for control of RD.

Procedures. Work completed in 2005 for Objective 1a included a repeat experiment to examine efficacy of different tree site rates of chloropicrin (Pic) for prevention of RD and a new experiment examining tolerance to RD in two rootstocks new to the California almond industry, Ishtara (from INRA, France) and Kuban 86 (from Russia). The trials were conducted in a Chico-area orchard not previously used for replanting experiments (Orchard 4). It had a long term history of almond production, and after it was replanted in early spring 2003 many of its trees developed RD symptoms that summer. In fall 2003, we prepared experimental tree sites in the orchard for fumigation by augering a 6-cm dia. × 50- to 60-cm-deep hole at each site. The experimental tree sites were between the grower's permanent tree sites. On 13 Nov. 2003, the holes were refilled with soil and received fumigation treatments. For the Pic rate study, treatments were: 0, 0.11, 0.22, 0.45 or 0.91 kg (0, 0.25, 0.5, 1.0, or 2.0 lb) of Pic per tree site. There were five tree sites per treatment in a randomized complete block design. Each tree site was planted with a Carmel almond tree on Marianna 2624 rootstock on 19 March 2004. For the rootstock trial, 20 Nonpareil almond trees on each rootstock were planted per pre-plant fumigation treatment (non-fumigated control, and 0.5 kg Pic per tree site) in a randomized complete block design on 19 March 2004. At the time of soil fumigation (both trials) soil temperature at 15 to 61 cm depth was 15 to 18° C and soil moisture content was .08 to 0.27 g water per g oven dry soil (8 to 27% moisture). Efficacy of the pre-plant treatments and tolerance of the rootstocks was determined by measuring increases in trunk diameter (from planting to 9 Feb. 2005) and assigning disease ratings (31 Aug. 2004, using methods described in Section I, Materials and Methods, Effects of alternative fumigation treatments, above).

Results and discussion. All of Pic rates, ranging from 0.11 to 0.91 kg per tree site (0.25 to 2.0 lb per tree site) significantly increased tree trunk diameter growth, compared to the non-treated control (Fig. 5). Diameter increases did not differ significantly among Pic treatments. Similarly, all Pic rates resulted in mean disease ratings of 0, whereas the non-treated control had a mean disease rating of 1.7 (0 to 5 scale, where 0=healthy and 5= dead tree; details of rating scale in Section I, Disease assessment). Although the magnitude of growth response to Pic in Orchard 4 was not as great as that in the first run of this experiment (Orchard 2, Fig. 1, Section I, above), the results were generally consistent, i.e., in that all of the Pic rates provided similar and effective suppression of RD. The results confirm an environmentally and economically important finding, that RD can be prevented by using very low rates of Pic per orchard acre.

Results of the rootstock trial were inconclusive. Severity of RD was not as great in control plots as in previous trials, and it appeared that phytotoxicity of residual chloropicrin also was a problem (Table 4). The preparatory tree site augering was suboptimal; because of an interfering trellis system in Orchard 4, we had to use a 6-cm diameter auger instead of the 60-cm auger used in previous trials. Also, the fumigation date, 13 Nov. in 2003 was later than optimum. It is likely that these factors interfered with distribution and dissipation of fumigant. The results suggest that it is prudent to use a large diameter auger (i.e., 60 cm) for tree site preparation and to complete soil fumigation before Nov.

Objective 1b. Efficacy of short-term fallowing and crop rotations for control of RD.

Procedures. A series of two successive experiments examining effects of 1-year pre-plant fallowing and cover crop rotations on severity of RD was completed in late 2004 in microplots near Parlier, CA. The microplots were sections of concrete pipe (60 x 120 cm) inserted into the ground and filled with soil from a RD-affected orchard in Apr 2002. In the first experiment pre-plant treatments were applied in 2002 and included: 1) almond on Nemaguard peach rootstock (A/NG) Jun-Nov; 2) A/NG Jun-Nov + MB:Pic (50:50 w:w, 448 kg per hectare, 19 Nov); 3) bare fallow Apr-Nov; 4) fallow Apr-Nov + MB:Pic (as above); 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 5). After each crop's growth period, the roots (sudan, A/NG) or roots and shoots (corn, wheat) were chopped and incorporated into the soil. Effects of the pre-plant treatments were assessed by planting each microplot with four 10-cm-tall Nemaguard peach seedlings on 9 Apr 2003 and measuring total top fresh weight (shoots + leaves) produced by the plants by 18 November 2003. In the second experiment, completed in late 2004, pre-plant treatments were applied and assessed as in the first test (Table 5), except 1 year later.

Results and Discussion. Without fumigation, only sudan and wheat rotations (Trts. 6 and 7, Fig. 6) 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. 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. 6). The effective rotations appear worthy of testing commercial orchards and have potential as cultural alternatives to pre-plant fumigation.

Objective 1c, Specificity between RD on peach (rootstock for almond) and RD on grape.

Procedures. To examine specificity between peach and grape RDs, microplots (46 x 120 cm) near Parlier, CA were filled in summer 2003 with non-fumigated soil sampled from 1) a peach orchard, and 2) an adjacent grape vineyard. In Nov. 2003, the soils were either left non-fumigated or pre-plant fumigated with MB (448 kg/ha), Pic (448 kg/ha), Telone (448 kg/ha), or Telone C35 (627 kg/ha). In Apr 2004, the microplots were planted with Nemaguard peach seedlings (10 cm tall, two per plot) or rooted Thompson Seedless grape cuttings (20 cm tall, two per plot); each crop was replanted in 6 replicate plots per combination of soil source and fumigation treatment. Effects of the pre-plant crop history and fumigation treatments were assessed by measuring top fresh weights of the seedlings and vines on 28 Oct. 2004.

Results and discussion. In soil from the peach orchard, pre-plant fumigation treatments significantly increased top fresh weights of Nemaguard peach seedlings (+76 to 144%, compared to the non-fumigated control; Fig. 7A), whereas they did not significantly increase top fresh weights of grapevines (-4 to +16%, compared to the control; Fig. 7B). In soil from the grape vineyard, pre-plant fumigation increased the growth of peach seedlings marginally to significantly, depending on the fumigant (+16 to 31%, Fig. 7A), and increased growth of grapevines significantly (+26 to 43%, Fig. 7B). By fall 2004, there were significant counts of root knot nematodes in the non-fumigated grape soil (data of S.M. Schneider), which may have caused some or all of the growth response to fumigation in grape. In contrast, the peach soil did not contain significant counts of plant parasitic nematodes (data of S.M. Schneider). Overall, the results suggest that almond or peach trees on Nemaguard rootstock have relatively low risk of

severe RD at old vineyard sites. Field observations of the investigators and others are consistent with these results. Our results do not provide evidence for a grape RD with strong host specificity. The experiment will be repeated.

Objective 2b. Examining possible roles of fungi in RD.

Procedures. In 2005 we used discriminant analysis, a statistical regression method, to examine associations between incidence of fungi in or on fine roots and incidence of RD. Approximately 20,000 fine root segments ($\leq$ 1 mm diameter $\times$ 1 cm length) were sampled over a period of 4 years from a total of 84 RD-affected trees (in replicate non-fumigated plots) and 96 healthy trees (in replicate pre-plant fumigated plots) among three commercial orchards near Chico, CA and two microplot experiments near Parlier, CA. The isolates were collected and cultured in a manner so that effects of isolation medium, root surface sterilization, tree health status, and other factors could be evaluated; statistical replication was maintained at each step of collection, isolation, and preservation. All fungi and oomycetes isolated from these roots (approximately 9000 isolates) were morphologically identified to genus level when possible and grouped into similar unknown types otherwise. Discriminant analysis ("Stepwise Proc Discrim", SAS Version 9; Cary, NC) was performed separately for appropriate groups of orchard and microplot experiments.

Results and discussion. Among Marianna 2624 root segments collected near Chico from Orchards 1 (in 2001 and 2002) and Orchards 2 and 3 (in 2003 and 2004) and surface sterilized for 2 min in 10% commercial bleach before culturing, incidence of *Cylindrocarpon* was consistently the strongest positive predictor for discriminating between RD-affected and healthy trees (Tables 6-9). Among root segments collected from the same orchards and only rinsed with sterile water before culturing, "*Fusarium*-all" was the strongest single predictor retained in the stepwise model in Orchard 1 (*Fusarium* subgroups were not delineated as in 2003 and 2004) and "*Fusarium* C" was the strongest single positive predictor for Orchards 2 and 3 (Tables 6,7,10,11). Although some other fungi appeared as significant positive or negative predictors in some of the discriminant analyses for Chico, they were not consistent predictors. Among Nemaguard peach root segments collected from microplots near Parlier and surface sterilized before culturing, incidence of *Rhizoctonia* was the strongest significant positive predictor for the disease (Tables 12,13). Among roots collected from the same plots but only rinsed with sterile water before culturing, incidence of "*Fusarium* all" was the strongest significant predictor (Tables 10,12). In previous Almond Board reports isolates of *Cylindrocarpon*, *Fusarium*, *Rhizoctonia* species (and some but not all other isolates of fungi) from roots of RD-affected Nemaguard peach caused root cortex necrosis in Nemaguard peach seedlings in greenhouse experiments. The results of the discriminant analyses provide a basis for in-depth characterization of isolates associated with disease; DNA-based species identifications and additional tests of pathogenicity will be completed.

Objective 2c. Examining possible roles of bacteria in RD.

Procedures. Soil and root samples for examining bacterial roles in RD were collected from all plots and trees sampled for fungi (Objective 2b, above). In 2005, we started characterizing the samples' bacterial populations to see whether changes in their composition were associated with RD incidence. In 2005, culture- and non-culture-based (i.e., DNA-based) approaches were used to characterize populations collected from four RD-affected trees and four healthy trees in Chico Orchard 2 on 22 Apr. 2003, and culture-based approaches were used to

characterize populations from four RD-affected and eight healthy trees in Parlier microplots on 17 June 2003.

The cultured populations from Chico have included 114 bacterial isolates from rhizospheres of the four replicate RD-affected trees (in non-fumigated plots) and 96 isolates from the rhizospheres of the four replicate healthy trees (in Pic-fumigated plots). The isolates were selected randomly after dilution plating on 0.1× TSA agar from roots ≤ 1 mm diameter, streaked to purity, preserved at -80 C, and ultimately identified to genus based on 16s rDNA base sequences (fragment sizes 250 to 600 bp). The 16s rDNA was amplified using primers fD1 and rP1 (Ross et al, 2000), and NCBI blast searches were used to link the 16s rDNA sequences to a bacterial genera.

Non-culture based characterizations of bacteria from the Chico samples were conducted with 180 cloned bacterial fragments of rDNA (each theoretically from a different randomly selected bacterium) from non-fumigated bulk soil close to the roots of the RD-affected trees (same trees as used for bacterial culturing, above) and 176 cloned rDNA bacteria from the healthy trees (same trees as used for culturing). Total DNA was extracted from each replicate soil sample and subjected to PCR amplification using 63F and 1401R primers that are specific for rDNA of bacteria (Marchesi, 1997). Resulting rDNA fragments from bacteria were separated by cloning using Promega Easy-T vector and competent cells. Approximately 300 to 700 bp of the cloned fragments were sequenced, 5' to 3' end, using the M9 vector sequencing primer. The base sequences were used in NCBI blast searches to determine the soil bacteria from which they originated.

To date, only cultured bacteria have been identified from the Parlier microplots. They included 74 isolates from the rhizospheres of RD-affected Nemaguard seedlings in four replicate non-fumigated microplots, 84 isolates from healthy Nemaguard in methyl bromide-fumigated soil (50 g/plot, four plots), and 108 isolates from healthy Nemaguard in chloropicrin-fumigated soil (50 g/plot, four plots). The isolates were selected and identified as described for the cultured bacteria from Chico plots.

Results and discussion. The bacterial identifications completed to date have revealed limited variation that may be associated with RD incidence and method of bacterial detection (i.e., culture-based vs. non-culture-based) (Tables 15,16), but it is necessary to increase the size of the described populations and include populations from additional samples before statistically based conclusions are justified. The required work is underway. At present it is clear that at both trial locations (Chico Orchard 2, Parlier microplots), *Pseudomonas* is a dominant genus, both in non-fumigated and non-fumigated soil. The dominance of this genus was apparent in Chico samples whether culture-based or non-culture-based methods were used. At both trial locations, quantitative dilution plating afforded estimates of colony forming units per gram of root, but those data are not presented in this report, and there were not large differences in absolute numbers of bacteria associated with pre-plant soil treatments.

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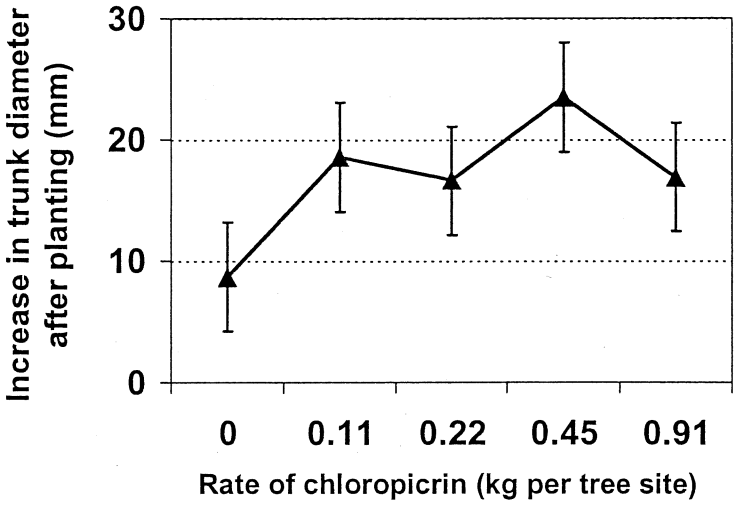

Fig. 5. Effect of rate of pre-plant chloropicrin treatment on growth of Carmel almond trees on Marianna 2624 rootstock in Orchard 4, near Chico. The orchard had exhibited incidence of replant disease in summer 2003. In fall 2003, tree site plots between permanent grower tree sites were prepared for fumigation by augering a 6-cm dia. × 50- to 60-cm-deep hole at each site. On 13 Nov. 2003, the holes were refilled with soil and received the fumigation treatments. There were five tree sites per treatment in a randomized complete block design. Each tree site was planted with a Carmel almond tree on Marianna 2624 rootstock on 19 March 2004. Efficacy of the pre-plant treatments was assessed by measuring increases in trunk diameter that occurred between planting and 9 Feb. 2005.

Table 4. Tolerance of three rootstocks to RD in Orchard 4, near Chico, CA; 2004[a]

Fumigation treatment	Rootstock	Growth in tree trunk diameter (mm)	Disease severity rating
Non-fumigated	Lovell peach	10	0.4
	Ishtara	11	0.4
	Kuban 86	14	0.1
Chloropicrin (0.5 kg/tree site)	Lovell peach	14	1.0
	Ishtara	13	0.5
	Kuban 86	16	0.6
95 % confidence intervals (+/-):		2	0.6

[a]The trial was conducted in an orchard that had incidence of replant disease in summer 2003. In fall 2003, tree sites were prepared for fumigation by augering a 6-cm-dia. × 50- to 60-cm-deep hole at each site. On 13 Nov. 2003, the holes were refilled with soil and received the fumigation treatments. Twenty Nonpareil almond trees on each rootstock were planted per pre-plant fumigation treatment in a randomized complete block design on 19 March 2004. Efficacy of the pre-plant treatments and tolerance of the rootstocks was determined by measuring increases in trunk diameter that occurred between planting and 9 Feb. 2005 and assigning disease rating (0 to 5 scale, where 0=no disease, 5=dead tree, and 1 to 4 = progressive intermediate increments of disease severity).

Table 5. 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.

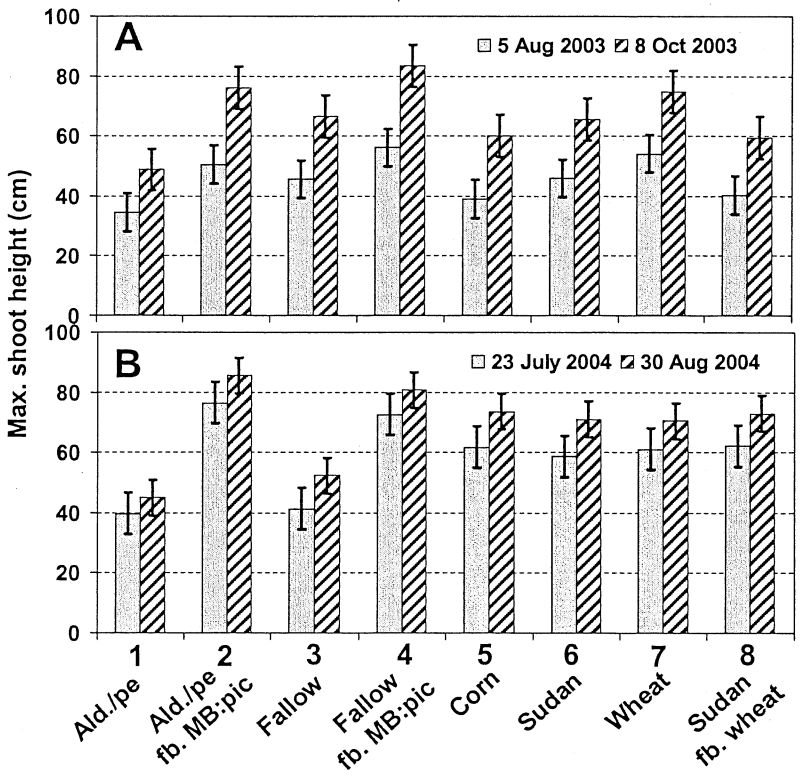

Fig. 6. Effect of short-term fallowing, short term crop rotation, and pre-plant fumigation on growth of Nemaguard peach seedlings planted in micro plots near Parlier, CA. **A**, Experiment 1 (2002/03) and **B**, Experiment 2 (2003/04). See Table 5.

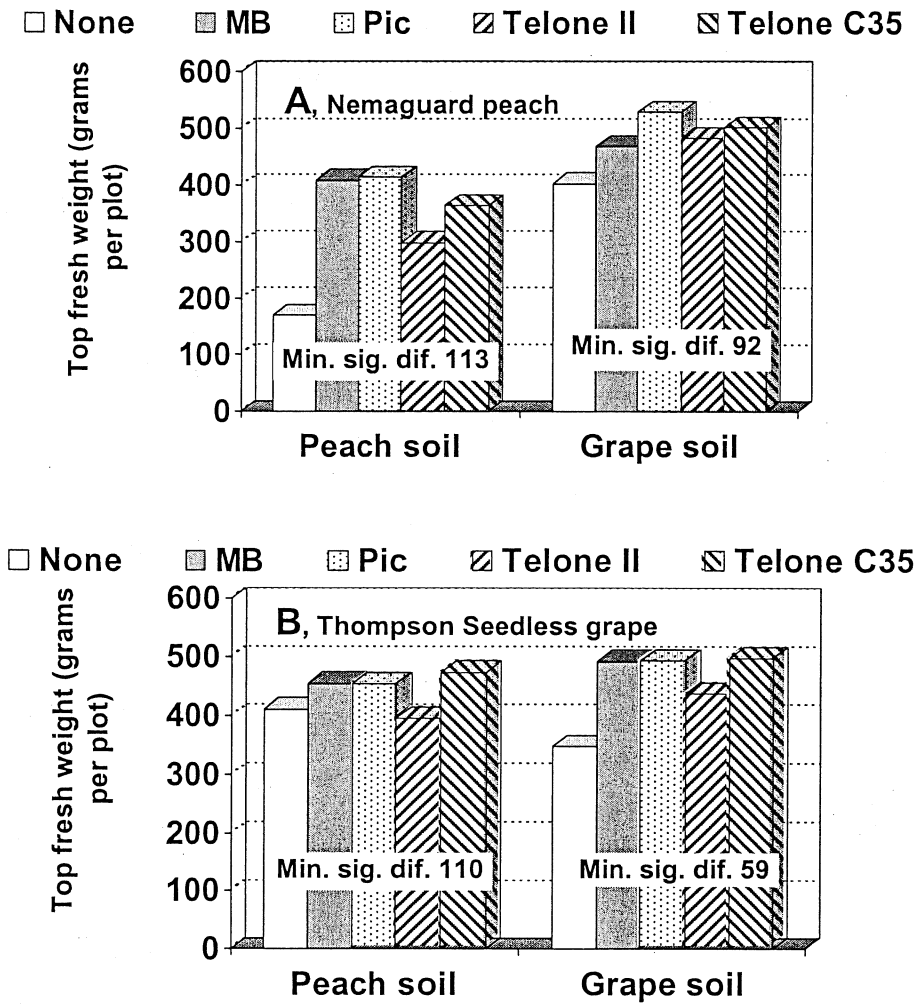

Fig. 7. Effect of pre-plant fumigation treatments on growth of **A**, Nemaguard peach seedlings and **B**, Thompson Seedless grapes in microplots filled with soils from a peach orchard and grape vineyard adjacent to the orchard. The soils were used to fill the microplots in summer 2003. In Nov 2003, the soils were either left non-fumigated or pre-plant fumigated with Telone (448 kg/ha), MB (448 kg/ha), Telone C35 (600 kg/ha), or Pic (448 kg/ha). In Apr 2004, the microplots were planted with Nemaguard peach seedlings (10 cm tall, two per plot) or rooted Thompson Seedless grape cuttings (20 cm tall, two per plot); each crop was replanted in 6 replicate plots per combination of soil source and fumigation treatment. Effects of the pre-plant crop history and fumigation treatments were assessed by measuring top fresh weights of the seedlings and vines on 28 October 2004.

Table 6. Association of fungi with incidence of replant disease in almond on Marianna 2624 rootstock, Orchard 1, near Chico, CA, 2001 and 2002 [a]

Fungal genus or group	Incidence of isolation from roots ≤ 1 mm dia. (%)			
	Surface sterilized roots		Surface sterilized roots	
	RD-affected	Healthy	RD-affected	Healthy
Acremonium	0.0	0.0	0.0	0.0
Alternaria	1.7	1.3	2.2	5.6
Arthrobotrys	0.0	0.0	0.0	0.0
Aspergillus	0.3	0.8	0.3	0.8
Basidomycete-unknown genus	1.3	5.9	0.0	2.1
Botrytis	0.0	0.0	0.0	0.0
Candida	0.0	0.0	0.0	0.4
Chaetomium	0.0	1.9	0.3	1.7
Chrysosporium	0.0	0.0	0.0	0.0
Cladobotryum	0.0	0.0	0.0	0.0
Cladosporium	0.0	0.3	0.0	0.0
Coniothyrium	0.0	0.8	0.0	0.2
Curvularia	0.0	0.2	0.0	0.5
Cylindrocarpon	17.8	7.5	3.9	2.9
Doratomyces	0.0	0.0	0.1	0.1
Drechslera	0.1	0.0	0.0	0.0
Fusarium-ALL	21.9	12.9	33.2	17.3
Geotrichum	0.0	0.2	0.0	0.0
Gliocladium	0.2	0.0	0.6	1.4
Helminthosporium	0.0	0.0	0.0	0.2
Heterosporium	0.0	0.0	0.0	0.2
Macrophomina	0.0	0.0	0.0	0.0
Mortierella	0.3	1.2	20.5	33.6
Mucor	0.0	0.2	1.1	0.4
Mycotypha	0.3	0.4	0.0	0.2
Papulospora	0.0	0.6	0.3	0.2
Penicillium	1.4	1.5	3.1	5.5
Peyronellaea	0.3	0.0	0.3	0.0
Phytophthora	0.0	0.0	0.2	0.0
Pythium	0.1	0.7	2.6	1.4
Rhizoctonia	2.2	2.1	3.4	3.1
Stemphyllium	0.0	0.0	0.0	0.0
Trichoderma	0.0	0.0	0.0	0.1
Trichurus	0.0	0.1	0.2	0.0
Ulocladium	0.6	0.5	0.3	0.4
Unk-ALL	0.0	0.0	0.0	1.3
ALL fungi	48.5	39.2	72.8	79.5

[a] Fungal groupings based on morphological characteristics; capital letters designate fungi that have similar characteristics but were not identified to species level. "Unk" indicates unknown genera. RD-affected and healthy roots were collected from trees in non-fumigated and chloropicrin-fumigated plots, respectively, and cultured on water agar and amended PDA medium for true fungi and PARP medium for oomycetes. Approximately 1000 roots were cultured from a total of 20 trees for each status of tree health.

Table 7. Association of fungi with incidence of replant disease in almond on Marianna 2624 rootstock, Orchards 2 and 3, near Chico, CA, 2003 and 2004[a]

Fungal genus or group	Incidence of isolation from roots ≤ 1 mm dia. (%)			
	Surface sterilized roots		Water-rinsed roots	
	RD-affected	Healthy	RD-affected	Healthy
Acremonium	0.1	0.0	0.2	0.3
Alternaria	0.2	0.1	2.4	2.8
Arthrobotrys	0.0	0.0	0.1	0.0
Aspergillus	0.3	0.1	0.7	1.1
Basidiomycete unknown	0.3	0.1	0.0	0.0
Botrytis	0.1	0.0	0.0	0.1
Chaetomium	0.0	0.1	0.0	0.4
Cladobotryum	0.7	1.5	0.3	2.2
Cladosporium	0.0	0.2	0.1	0.1
Curvularia	0.1	0.1	0.3	0.4
Cylindrocarpon	12.4	3.5	3.6	2.6
Doratomyces	0.0	0.0	0.0	0.2
Fusarium A	4.1	0.6	4.5	1.2
Fusarium B	0.2	0.2	1.8	0.5
Fusarium C	4.4	0.5	6.7	1.6
Fusarium D	2.4	1.6	1.8	2.7
Fusarium E	1.4	0.6	2.4	1.1
Fusarium F	0.0	0.0	0.1	0.0
Fusarium H	2.2	1.1	7.9	4.4
Fusarium I	1.8	1.8	0.7	2.4
Fusarium P	0.0	0.0	0.1	0.0
Fusarium-other	2.0	1.8	4.5	2.6
Fusarium ALL	18.4	8.2	30.4	16.5
Gliocladium	1.1	1.5	2.2	7.3
Macrophomena	0.2	0.1	0.4	0.1
Mortierella	1.7	0.6	29.8	18.1
Mucor	0.1	0.2	0.9	0.5
Papulospora	0.2	0.3	0.0	0.0
Penicillium	0.8	0.5	1.2	1.7
Pythium	3.0	0.9	9.9	4.4
Rhizoctonia	1.8	0.9	2.3	0.6
Stemphyllium	0.0	0.0	0.0	0.1
Thrichoderma	0.3	0.4	1.1	2.8
Ulocladium	0.1	0.0	0.4	0.2
Unk-other	4.5	3.4	7.2	7.7
Unk A	2.2	2.3	1.3	4.0
Unk B	0.8	0.4	0.3	0.5
Unk C	0.2	0.2	0.2	0.0
Unk D	0.0	0.0	0.2	0.1
Unk E	0.0	0.0	0.0	0.1
Unk G	0.2	0.3	0.1	0.4
Unk H	1.1	1.4	0.0	0.7
Unk I	0.1	0.1	0.0	0.1
Unk M	0.1	0.1	0.0	0.2
Unk N	0.1	0.3	0.5	0.5
Unk O	0.1	0.2	0.0	0.1
Unk P	0.1	0.1	0.0	0.0
All fungi	51.0	27.8	95.7	76.3

[a] Fungal groupings based on morphological characteristics; capital letters designate fungi that have similar characteristics but were not identified to species level. "Unk" indicates unknown genera. RD-affected and healthy roots were collected from trees in non-fumigated and chloropicrin-fumigated plots, respectively, and cultured on water agar and amended PDA medium for true fungi and PARP medium for oomycetes. Approximately 5000 roots were cultured from a total of 40 trees for each status of tree health.

Table 8. Results of stepwise discriminant analysis, in which fungal variables in Table 6 (incidences of isolation for each fungal group) were examined as predictors of replant disease; Orchard 1, Chico, 2001,02; surface sterilized roots[a]

Step	Incidence Variable entered	Incidence variable removed	Partial r^2	F	P>F
1	*Cylindrocarpon*		0.1081	5.94	0.02
2	*Geotrichum*		0.0678	3.49	0.07
3	*Aspergillus*		0.1015	5.31	0.03
4	*Papulospora*		0.0637	3.13	0.08
5	*Chaetomium*		0.0656	3.16	0.08

[a]Stepwise discriminant analysis ("Stepwise Proc Discrim", SAS Version 9) was used to examine the significance of fungal incidence variables in predicting health status of almond trees.

Table 9. Results of stepwise discriminant analysis, in which fungal variables in Table 7 (incidence of isolation for each fungal group) were examined as predictors of replant disease; Orchards 2 and 3, Chico, 2003,04; surface sterilized roots[a]

Step	Incidence variable entered	Incidence variable removed	Partial r^2	F	P>F
1	*Cylindrocarpon*		0.24	25.1	<.0001
2	*Fusarium C*		0.13	11.4	0.001
3	*Unknown I*		0.07	5.4	0.02
4	*Penicillium*		0.07	5.4	0.02
5	*Acremonium*		0.06	4.7	0.03
6	*Ulocladium*		0.07	5.1	0.03
7	*Botrytis*		0.05	4.1	0.05
8	*Unknown A*		0.05	3.5	0.07
9	*Fusarium I*		0.03	2.4	0.12
10	*Fusarium A*		0.03	2.5	0.12

[a]Stepwise discriminant analysis (STEPDISC procedure, SAS statistical software, Cary, NC) was used to examine the significance of fungal incidence variables in predicting health status of almond trees. Only first 10 of 11 steps shown.

Table 10. Results of stepwise discriminant analysis, in which fungal variables in Table 6 (incidence of isolation for each fungal group) were examined as predictors of replant disease; Orchard 1, Chico, 2001,02; water rinsed roots[a]

Step	Incidence Variable entered	Incidence variable removed	Partial r^2	F	P>F
1	*Mortierella*		0.1691	9.77	0.003
2	*Fusarium ALL*		0.1313	7.11	0.01
3	*Thrichoderma*		0.0752	3.74	0.06
4	*Candida*		0.0787	3.84	0.06
5	*All fungi*		0.0532	2.47	0.12
6		*Mortierella*	0.028	1.27	0.27
7	*Phytophthora*		0.0596	2.79	0.10
8	*Peyronellaea*		0.053	2.41	0.13
9	*Mucor*		0.0574	2.56	0.12

[a]Stepwise discriminant analysis (STEPDISC procedure, SAS statistical software, Cary, NC) was used to examine the significance of fungal incidence variables in predicting health status of almond trees.

Table 11. Results of stepwise discriminant analysis, in which fungal variables in Table 7 (incidence of isolation for each fungal group) were examined as predictors of replant disease; Orchards 2 and 3, Chico, 2003,04; water-rinsed roots[a]

Step	Incidence variable entered	Incidence variable removed	Partial r^2	F	P>F
1	*Fusarium C*		0.30	32.8	<.0001
2	*Cladobotryum*		0.11	9.9	0.002
3	*Fusarium I*		0.08	6.9	0.01
4	*Fusarium ALL*		0.09	7.7	0.007
5	*Doratomyces*		0.11	8.8	0.004
6	*Unknown O*		0.12	9.9	0.002
7	*Rhizoctonia*		0.05	3.7	0.06
8	*Cylindrocarpon*		0.06	4.2	0.04
9	*Unknown A*		0.04	2.9	0.09
10	*Fusarium H*		0.06	4.2	0.05

[a]Stepwise discriminant analysis (STEPDISC procedure, SAS statistical software, Cary, NC) was used to examine the significance of fungal incidence variables in predicting health status of almond trees. Only first 10 of 20 steps shown.

Table 12. Association of fungi with incidence of replant disease on Nemaguard peach in microplots near Parlier, CA, 2003-04[a]

Fungal variable	Incidence of isolation from roots ≤ 1 mm dia. (%)			
	Surface sterilized roots		Water-rinsed roots	
	RD-affected trees	Healthy trees	RD-affected trees	Healthy trees
Acremonium	0.2	0.1	0.1	0.3
Alternaria	0.0	0.0	0.2	0.2
Arthrobotrys	0.0	0.0	0.2	0.0
Aspergillus	0.3	0.2	1.4	0.8
Basidiomycete unknown	0.0	0.0	0.1	0.1
Chaetomium	0.4	0.1	0.3	0.2
Chrysosporium	0.0	0.0	0.6	0.0
Cladobotryum	0.2	0.2	0.8	1.1
Cylindrocarpon	4.8	3.9	4.5	2.4
Doratomyces	0.3	0.1	0.1	0.1
Fusarium A	3.3	1.4	7.4	4.3
Fusarium B	0.0	0.0	0.7	0.1
Fusarium C	1.0	0.8	2.9	1.8
Fusarium D	0.7	0.9	2.0	2.5
Fusarium E	0.0	0.2	0.9	0.3
Fusarium H	0.0	0.1	2.3	0.6
Fusarium I	0.0	0.1	0.2	0.1
Fusarium-other	0.5	0.6	5.7	1.9
Fusarium ALL	5.6	4.1	22.0	11.4
Gliocladium	0.6	0.8	2.5	6.8
Macrophomena	0.2	0.5	0.0	0.1
Mortierella	1.1	0.9	14.2	9.7
Mucor	0.0	0.1	0.6	0.5
Papulospora	1.0	0.7	0.1	0.3
Penicillium	0.8	0.3	1.7	2.3
Pythium	1.5	0.5	7.2	4.3
Rhizoctonia	4.3	0.7	5.6	1.6
Thrichoderma	0.4	3.6	3.9	10.2
Ulocladium	0.0	0.2	0.4	0.2
Unk-other	4.3	2.5	6.1	4.9
Unk G	1.0	1.2	3.7	3.2
Unk H	1.8	1.3	0.2	0.2
Unk I	1.8	0.5	0.4	0.3
Unk J	0.2	0.0	0.8	0.3
Unk K	0.2	0.0	0.1	0.1
Unk L	0.0	0.2	0.0	0.2
Unk M	0.3	0.4	1.6	1.9
Unk N	1.2	0.8	0.5	0.1
Unk P	0.1	0.5	0.0	0.1
Unk Q	0.5	0.3	0.6	0.5
Unk R	0.1	0.1	0.0	0.7
Unk S	0.8	0.3	0.1	0.3
Unk T	0.2	0.1	0.2	0.3
Unk U	0.2	0.1	1.1	0.3
Unk V	0.0	0.8	0.0	0.0
All fungi	33.9	26.0	81.9	66.2

[a]Fungal groupings based on morphological characteristics; capital letters designate fungi that have similar characteristics but were not identified to species level. "Unk" indicates unknown genera. Roots ≤ 1 mm diameter were collected from 24 RD-affected trees (12 trees per treatment in non-fumigated plots and plots fumigated with a low rate of methyl bromide plots [5g/plot]) and 36 healthy trees (12 trees per treatment in plots fumigated with low and high rates of chloropicrin [5 and 50 g/plot, respectively] and a high rate of methyl bromide [50 g/plot]. Segments of the roots were cultured on water agar and amended PDA medium for true fungi and PARP medium for oomycetes. Approximately 8000 roots segments were cultured, distributed proportionally among the sampled trees.

Table 13. Results of stepwise discriminant analysis, in which fungal variables in Table 12 (incidence of isolation for each fungal group) were examined as predictors of replant disease, microplots near Parlier, CA, 2003,04; surface sterilized roots[a]

Step	Incidence variable entered	Incidence variable removed	Partial r^2	F	P>F
1	*Rhizoctonia*		0.1133	7.4	0.01
2	*Pythium*		0.0698	4.3	0.04
3	*Unknown I*		0.0832	5.1	0.03
4	*Unknown J*		0.0567	3.3	0.07
5	*Macrophomena*		0.0524	3.0	0.09
6	*Unknown K*		0.0479	2.7	0.11
7	*Thrichoderma*		0.0415	2.3	0.14

[a]Stepwise discriminant analysis was used to examine the significance of fungal incidence variables in predicting health status of almond trees.

Table 14. Results of stepwise discriminant analysis, in which fungal variables in Table 12 (incidence of isolation for each fungal group) were examined as predictors of replant disease, microplots near Parlier, CA, 2003,04; water-rinsed roots[a]

Step	Incidence variable entered	Incidence variable removed	Partial r^2	F	P>F
1	*Fusarium-all*		0.1782	12.57	0.0008
2	*Thrichoderma*		0.0888	5.55	0.0219
3	*Fusarium H*		0.1105	6.96	0.0108
4	*Rhizoctonia*		0.0878	5.29	0.0252
5	*Chrysosporium*		0.072	4.19	0.0455
6	*Fusarium E*		0.0671	3.81	0.0561
7	*Arthrobotrys*		0.0747	4.2	0.0455
8	*Unknown H*		0.0659	3.6	0.0635
9	*Mortierella*		0.0519	2.74	0.1042
10	*Penicillium*		0.0511	2.64	0.1108

[a]Stepwise discriminant analysis was used to examine the significance of fungal incidence variables in predicting health status of almond trees. Only first 10 of 12 steps shown.

Table 15. Culture- and non-culture based assessments of bacterial populations associated with almond trees affected by replant disease in non-fumigated soil and healthy almond trees in chloropicrin-fumigated soil, Orchard 2, near Chico, CA

Genus	Percentage of the bacterial population			
	Culture-based assessment[a]		Non-culture-based assessment[b]	
	Non-fumigated soil	Chloropicrin-fumigated soil	Non-fumigated soil	Chloropicrin-fumigated soil
Acidobacterium	0.0	0.0	1.0	1.0
Arthrobacter	1.2	0.0	0.0	0.0
Bacillus	1.2	0.0	2.0	1.0
Caulobacter	0.0	0.0	0.5	0.0
Cellvirbio	0.0	0.0	0.5	0.0
Clostridium	0.0	0.0	1.0	0.0
Conexibacter	0.0	0.0	1.0	0.0
Deltaproteobacteria	0.0	0.0	2.0	0.6
Devosia	0.0	0.0	0.0	0.6
Enterobacter	0.0	0.0	17.0	9.2
Flavobacter	22.6	15.0	0.0	0.0
Herbasprillum	1.9	1.2	0.0	0.0
Janthinobacter	1.2	1.9	0.0	0.0
Mesorhizobium	0.0	0.0	0.0	6.1
Novosphingobium	3.0	2.2	0.0	0.0
Pseudomonas	61.9	74.6	75.0	85.8
Ralstonia	0.0	0.0	0.0	1.1
Rhizobium	3.0	3.2	0.0	0.6
Rhodoferax	0.0	0.9	0.0	0.0
Stenotrophomonas	4.0	1.0	0.0	0.0

[a] Based on 114 and 96 isolates of bacteria sampled from non-fumigated soil in the rhizosphere of RD-affected trees and chloropicrin-fumigated soil in the rhizosphere of healthy trees, respectively; four single-tree plots were sampled per treatment on 22 Apr 2003. The isolates were selected randomly after extraction from the rhizosphere (roots $\leq$ 1 mm diameter) and dilution plating on 0.1× TSA agar. The colonies were streaked to purity and preserved at -80 C. The isolates were identified based on 16s rDNA base sequences (fragment sizes 250 to 600 bp) amplified using primers fD1 and rP1 (Ross et al, 2000). The base sequences were used in NCBI blast searches to identify the bacteria from which they originated.

[b] Based on 180 and 176 cloned bacterial rDNA fragments from non-fumigated bulk soil close to the roots of RD-affected trees and from chloropicrin-fumigated bulk soil close to the roots of healthy trees, respectively; four single-tree plots were sampled per treatment on 22 Apr 2003. The soil samples were collected on dry ice and stored at -80 C. Total DNA was extracted from the soil samples and subjected to PCR amplification using 63F and 1401R primers that are specific for rDNA of bacteria (Marchesi, 1997). Resulting rDNA fragments from bacteria were separated by cloning using Promega Easy-T vector and competent cells. Approximately 300 to 700 bp of the cloned fragments were sequenced, 5' to 3' end, using the M9 vector sequencing primer. The base sequences were used in NCBI blast searches to identify the bacteria from which they originated.

Table 16. Culture-based assessment of bacterial rhizosphere populations associated with Nemaguard peach seedlings (rootstock for almond) affected by replant disease in non-fumigated soil and healthy Nemaguard seedlings in chloropicrin-fumigated and methyl bromide-fumigated soil in microplots near Parlier, CA

Genus	Percentage of the bacterial population		
	Non-fumigated soil	Chloropicrin-fumigated soil	Methyl bromide-fumigated soil
Agrobacterium	0	0	1.2
Arthrobacter	0	2.8	13.1
Bacillus	11.4	7.4	9.5
Cellulomonas	0	0.9	0
Curtobacterium	0	0.9	0
Enterobacter	0	2.8	1.2
Flavobacterium	5.7	5.6	0
Janthinobacterium	0	1.9	0
Microbacterium	1.4	2.8	4.8
Micrococcus	0	1.9	1.2
Novosphingobium	1.1	0.9	0
Oxalbacteriaceae	1.4	0	0
Paenbacillus	0	0.9	0
Pantoea	0	1.9	0
Pseudomonas	32.9	38	56
Ralstonia	1.4	0	0
Rhizobium	25.7	11.1	4.8
Rhodococcus	0	0.9	0
Sinorhizobium	10	0	0
Stenotrophomonas	0	0.9	0
Variovorax	8.6	18.5	8.3

[a] Based on 74, 108, and 84 isolates of bacteria cultured from the rhizospheres of RD-affected Nemaguard plants in non-fumigated soil, healthy Nemaguard plants in chloropicrin-fumigated soil (50 g/plot), and healthy Nemaguard plants in methyl bromide-fumigated soil (50 g/plot). The bacteria originated from four microplots per soil treatment, all sampled xx xxx 2003. The isolates were selected randomly after extraction from the rhizosphere (roots $\leq$ 1 mm diameter) and dilution plating on 0.1× TSA agar. The colonies were streaked to purity and preserved at -80 C. The isolates were identified based on 16s rDNA base sequences (fragment sizes 250 to 600 bp) amplified using primers fD1 and rP1 (Ross et al, 2000). The base sequences were used in NCBI blast searches to identify the bacteria from which they originated.