Developing Improved Strategies for Management of Replant Problems

Project No.: **11-PATH1-Browne**

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Objectives:

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

Interpretive Summary:

The overall goal of this project is to improve almond orchard replacement strategies, maximizing their economy while reducing their dependence on soil fumigation. Specific project objectives are: 1) determining causes of almond replant disease (RD), a poorly understood soilborne complex that suppresses growth and productivity in successive almond plantings, even in the absence of plant parasitic nematodes, and 2) developing effective approaches to manage RD and other biological replant problems (e.g., nematode parasitism, root and crown rots, etc.) with little or no fumigant. In 2011-12, specific project activities included: 1) identifying microorganisms associated with RD in: a) our 2011-planted rootstock trial at the USDA station near Parlier and b) our bioassay trials in a greenhouse (done by sampling roots healthy and RD-affected trees/plants in the trials, isolating microorganisms from the roots, and identifying the microorganisms by DNA sequencing); 2) testing pathogenicity of fungi and *Pythium* species associated with RD (done by inoculating potting soil with the test organisms in a greenhouse and measuring resulting impacts on Nemaguard peach growth and health); 3) assessing greenhouse bioassay methods to predict and study occurrence of RD in orchard replant soils (done by subjecting soil form three orchard locations to informative pre-plant treatments (fumigation, pasteurization, autoclaving, and a control) and measuring responses of Nemaguard peach to the treatments and assay conditions; also, different plant "formats" and soil mixtures with sand were tested for effects on assay results); 4)

continuing evaluations of 22 Prunus rootstocks for resistance to RD and *Phytophthora* species (being done by challenging the rootstocks with the RD complex in field trials and with *Phytophthora* species in greenhouse trials) and 5) collaborating with David Doll and Brad Hanson in evaluation of non-fumigant pre-plant treatments (steam, Brassica seed meal) for management of orchard replant problems (this is being done by monitoring effects of the treatments on almond tree growth in orchard replant trials).

In the work described above, microorganisms associated with RD in the rootstock trial included: *Pythium* species (*P. ultimum*, *P. vexans*, *P. irregulare*, *P. pareocandrum*, and two additional unidentified but distinct *Pythium* sp.) and several fungi (*Cylindrocarpon macrodidymum*, *Fusarium solani*, *F. oxysporum*, and *F. equiseti*, *Macrophomina* sp., *Mucor* sp., and an unknown fungal endophyte). These organisms were isolated more frequently from RD-affected rootstocks in non-fumigated soil than from healthy rootstocks in fumigated soil. In contrast, the fungi *Trichoderma* sp., *Chaetomium* sp., and *Psathyrella candolleana* were isolated more frequently from roots of the healthy plants than from roots of the RD-affected plants. In the bioassay trial, a *Pythium* sp. (identical to a *Pythium* sp. from the rootstock trial), *Cylindrocarpon* spp., and *Fusarium* spp. generally were detected at higher counts from roots of RD-affected Nemaguard assay plants (in non-treated orchard soil) than from healthy Nemaguard assay plants (in fumigated orchard soil). Pathogenicity was demonstrated among some isolates of *Cylindrocarpon macrodidymum*, *C. destructans*, *Pythium* sp., and *Thielaviopsis sp.* Our findings to date are consistent with a hypothesis that multiple *Pythium* species and fungi contribute to RD. The bioassay method assessments revealed that soil fumigation (SF) and soil pasteurization (SP) were more effective in preventing symptoms associated with RD than soil autoclaving. Also, use of sprouting Nemaguard seedlings afforded better RD assay capability than use of potted clonal Nemaguard plants from tissue culture. The bioassay generally yielded similar results whether soil was collected from the 0.5 to 1 ft depth or 1.0 to 2-ft depth, although when soil was collected from a standing orchard as opposed to a cleared orchard, the deeper soil resulted in greater expression of RD for some parameters. The bioassay distinguished effectively between fumigated and non-fumigated soil collected at Parlier; i.e., unless subjected to pre-plant SP or SF, soil collected from a non-fumigated plot at Parlier stunted Nemaguard seedling growth, whereas soil collected from a field fumigated plot at Parlier resulted in optimal Nemaguard seedling growth, regardless of post collection treatment. The results indicate that the bioassay, with further optimization and validation, could be a practical tool for RD prediction as well further examination of RD etiology in multiple soils. In our first rootstock trial (planted 2011) evaluating genetic resistance to RD among 22 rootstocks for almond and stone fruits, rootstocks with only peach parentage were relatively susceptible to RD (from Apr to Oct 2011, stem diameter increases in non-fumigated plots divided by the increases in fumigated plots [i.e., NF/F proportions] were 0.30 to 0.53); while rootstocks with peach x almond parentage generally did better (NF/F proportions 0.53 to 0.71) and rootstocks with plum parentage were variable (NF/F proportions 0.37 to 0.74). A repeat of the rootstock trial was planted in May 2012 and will first provide data in winter 2013. The preliminary results from the 2011 rootstock trial suggest that, at least following removal of almond on Nemaguard rootstock in Hanford Sandy Loam soil, replanting on Empyrean 1 and some peach x almond hybrid

rootstocks may result in less severe RD than replanting on Nemaguard peach rootstock. Continued rootstock selection and development could be a valuable component in preventing RD with minimal or no dependence on soil fumigation. The same complement of 22 rootstocks was obtained for repeat evaluations of genetic resistance to *Phytophthora* species, but the round of evaluations will require another year to complete. Compared to the non-treated control, Brassica seed meal application through the tree-site auger improved stem diameter growth by 27%, augering alone without the seed meal improved stem diameter growth by 12%, and pre-plant fumigation increased stem diameter growth by 47%. It appears spot treatments with Brassica seed meal have potential but will require improvement before they approach the effectiveness of strip or spot fumigation.

Materials and Methods:

Objective 1. Determine the biological causes of replant disease (RD).

Identifying microorganisms associated with RD. We identified microorganisms in root samples from RD-affected and healthy rootstock trees of Nemaguard, Lovell, Hansen 536 and Bright's Hybrid 106. The trees were part of a field experiment to evaluate resistance to RD in a total of 22 almond and stone fruit rootstocks at the USDA station near Parlier, CA. The test site had been cleared from almonds on Nemaguard rootstock in summer 2010. In Oct 2010, soil plots were shank fumigated with Telone C35 (540 lb $acre^{-1}$) or shanked without fumigant (the control). The rootstocks were planted in both soil treatments in Apr 2011, and the root samples were collected in Oct 2011. For each rootstock sampled, roots were collected from four replicate healthy trees (in the fumigated plots) and four replicate RD-affected trees (in the non-fumigated plots). The roots were collected from 6 to 24" depth in soil and kept cool during transport back to the lab. The root samples were subdivided into two portions, one used for immediate culture-based isolations and the other frozen at -80 C for subsequent culture-independent examinations. Media used for the culture isolations included: Tryptic Soy Agar (TSA) and Nutrient broth yeast extract medium (NBY) for bacteria; water agar + ampicillin (WAamp) for fungi; and PARP for oomycetes (i.e., *Pythium*, *Phytophthora*). Fungal and oomycete isolation plates were incubated at 20 to 24 C, and all isolates from them were subcultured to 0.2-X Potato dextrose agar + ampicillin. Bacterial isolation plates were incubated at 28 C; all isolates were mixed and the mixture was transferred to LB broth with 15% glycerol for storage at -80 C. The 1172 fungal and oomycete isolates were grouped according to morphology, and representative isolates of each group (>400) were purified (i.e., by single spore selection or hyphal tipping) then identified based on sequencing of ITS 1 and 2 regions of rDNA.

Testing pathogenicity of microorganisms associated with RD. Due to their apparent associations with RD, a pathogenicity trial was completed with isolates of *Cylindrocarpon*, *Pythium*, *Thielaviopsis* from multiple replant trials, as follows: Inocula of purified, identified isolates of the organisms were produced on V8 juice-oat seed medium. The inocula were mixed into UC mix potting soil at two rates, 5% and 10% (by

volume). Nemaguard peach seedlings were transplanted into 20-oz pots of the inoculated soils. Controls were included and consisted of 20 oz pots filled with soil amended at 5% and 10% rates with sterile V8 juice-oat seed medium. There were five replicate pots for each inoculum treatment and control, randomized in a split plot design (main plots were inoculants, subplots were inoculum rates). Effects of the treatments were assessed 3 months after transplanting by measuring plant top weights, root fresh weights, and estimating percentage of root cortex necrosis (referred to as % root rot for simplicity). Analysis of variance was completed with SAS statistical software. The same methods are being used in a series of experiments to test pathogenicity in a large collection of *Pythium* isolates and other fungi from our 2011 rootstock trial.

Objective 2. Support development of new management strategies for RD and other replant problems.

Developing a bioassay to predict and study RD. On 17 January 2012 Hanford sandy loam soil was collected from three orchard sites in Fresno County for use in development of a greenhouse bioassay to predict and study causes of RD. At collection time, soil from orchard 1, south of Kingsburg, was still planted with peach trees on Nemaguard rootstock, but the orchard was to be replanted with almond in winter 2013. Soil from orchard 2, east of Sanger, had been cleared from almond on Nemaguard rootstock in 2010/11 and was to be replanted to almond in 2013. Soil from orchard 3 had been cleared from almond on Nemaguard rootstock in 2011 and was to be used for our 2012 repeat of the Prunus rootstock trial. For orchard 3, soil was collected from non-fumigated as well as Telone C35-fumigated (540 lb acre^{-1}) areas. At each orchard collection site, soil was collected from two soil depth ranges, 0.3 to 1.0 ft and 1.0 to 2.0 ft.

Two experiments were completed to learn about effects of soil sample depth, post-collection soil treatment (i.e., pasteurization, autoclaving, or bucket fumigation) assay plant “format” (i.e., sprouting Nemaguard vs. several-month-old clonal Nemaguard from tissue culture, from trays with ca. 0.8” x 2.0” cells), and ratio of field soil to sand (sand is used in bioassay experiments to add porosity to the soil mix so that soil drainage is adequate, approximating that in a field soil). In each experiment, the test soils received their post-collection treatments during March and April 2012 and were planted with Nemaguard on 25 April 2012. Treatment effects were assessed 22 June 2012 according to shoot and root fresh weights, percentage of root cortex rotted, and a visual disease severity rating of Nemaguard plant tops.

Evaluations of genetic resistance to RD. As described in part above (Objective 1), a field experiment was established at the USDA station near Parlier, CA to evaluate resistance to RD in a total of 22 almond and stone fruit rootstocks (**Table 1**). The test site had been cleared from almonds on Nemaguard rootstock in summer 2010. In Oct 2010, soil plots were shank fumigated with Telone C35 (540 lb acre^{-1}) or shanked without fumigant (the control). The rootstocks were planted in both soil treatments in Apr 2011. Resistance was assessed in Oct 2011 by determining NF/F growth proportions (GPs), i.e., GPs calculated by dividing increases in stem diameter (and plant height and

pruning weights) accumulated in non-fumigated plots by the increases in fumigated plots. A repeat experiment was established in 2012 an adjacent block using similar methods, except the 22 rootstocks were planted in May instead of April.

Evaluations of genetic resistance to *Phytophthora*. Rootstock plants were obtained from Duarte nursery and North American Plants for 2012 assessments of resistance to *Phytophthora* species (we reported on 2010/11 assessments last year), but they did not all grow equivalently and will require a cycle of chilling before they can effectively be evaluated for resistance to the pathogens. It is anticipated that results of next tests will be available in 2013.

Evaluation spot treatment with Brassica seed meal for control of RD. We evaluated almond tree growth responses to pre-plant tree site treatment with Brassica seed meal. The assessment was part of an orchard replant trial conducted near Delhi, CA. The Brassica seed meal was obtained as a fine-ground product (Mustard Products & Technologies, www.mptmustardproducts.com\) and applied 29 November 2010 at a rate of 6 tons/treated acre to a soil volume 2.5 ft in diameter and 2 ft deep; each application was centered on a tree planting site. For comparison, a non-treated control and a 2.5-ft-diameter auger control (augered as for Brassica amendment, but no amendment added), and a standard row-strip treatment with Telone C35 (540 lb / treated acre, treated 23 November 2010) were included. Treatments were arranged in seven randomized complete blocks with a total of 14 to 42 trees per treatment. Treatment efficacy was assessed according to growth of the replanted almond trees and counts of ring nematodes

Results and Discussion:

Objective 1. Determine the biological causes of replant disease (RD).

Identifying organisms associated with RD. In the 2011 rootstock trial, several *Pythium* species were isolated much more frequently from roots of RD-affected trees (in non-fumigated soil) than from roots of healthy trees (in fumigated soil) (**Figure 1**; *P. irregulare*, *P. parecandrum*, *P. ultimum*, *P. vexans*, and *Pythium* sp. '10'). Also, isolates of *Cylindrocarpon macrodidymum*, *C. olidum*, *Fusarium* species, *Macrophomina* sp., *Humicola* sp., and *Mucor* sp. were isolated more frequently from roots of RD-affected trees than from healthy trees, but the effect of fumigation on isolation counts of these fungi generally was less pronounced than for species of *Pythium*. Isolation counts for *Chaetomium* sp., *Psathyrella candolleana*, and *Trichoderma* sp. were higher from roots of healthy trees in fumigated plots than from roots RD-affected trees in non-fumigated plots.

In the 2012 bioassay trial, a *Pythium* sp. (with an identical ITS sequences to a *Pythium* sp. from the rootstock trial), *Cylindrocarpon* spp., and *Fusarium* spp. generally were isolated in greater number from roots of RD-affected Nemaguard assay plants (in non-treated orchard soil) than from healthy Nemaguard assay plants (in fumigated orchard soil) (**Figure 2**).

The rootstock and bioassay isolations must be considered only semi-quantitative at best, because culturing efficiency varies greatly among organisms and the number of samples that can be feasibly monitored is limited. Nevertheless, the organisms cultured and their counts give us a basis for prioritizing organisms to include in pathogenicity tests. Accordingly, we concentrated on species of *Pythium* and *Cylindrocarpon* in our 2012 pathogenicity tests, and will continue the testing with the other organisms that were associated with RD in this year's sampling.

Testing pathogenicity of microorganisms associated with RD. All tested Isolates of *Cylindrocarpon destructans*, *C. macrodidymum*, *Thielaviopsis sp.*, and *Pythium* sp. caused significant levels of root cortex necrosis (50-74%), compared to the control treatment (31%) (**Table 2**). Inoculation with some isolates resulted in smaller root and/or top fresh weights; root and top fresh weight mean values for inoculated plants ranged from 0.65 to 1.13 of mean weight values for the control (**Table 2**). The results indicated that although some isolates of each species tested are capable of inducing disease in Nemaguard peach, they are not highly aggressive pathogens, at least under the conditions of our test. It may be that environmental conditions, including factors that stress plants, contribute to development of RD. Also, additional organisms not included in the test probably contribute to RD, directly or indirectly. Nevertheless, the demonstrations of pathogenicity suggest that the organisms can contribute to RD.

Objective 2. Support development of new management strategies for RD and other replant problems.

Developing a bioassay to predict and study RD. In experiment 1, selected bioassay treatments with sprouting Nemaguard seedlings indicated potential for RD in the soil samples collected from non-fumigated sites. It was found that post-collection treatments soil fumigation (SF) and soil pasteurization (SP) were more effective in preventing symptoms associated with RD than soil autoclaving (**Figure 3**). Also use of sprouting Nemaguard seedlings afforded better RD assay capability (**Figure 3**) than use of potted clonal Nemaguard plants from tissue culture (data not shown). All plants from tissue culture developed high levels of root cortex necrosis and were generally less responsive to soil treatments than the sprouting Nemaguard seedlings. The bioassay generally yielded similar results whether soil was collected from the 0.3 to 1 ft depth or 1.0 to 2-ft depth, although when soil was collected from a standing orchard as opposed to a cleared orchard, the deeper soil resulted in greater expression of RD for some parameters. The bioassay distinguished effectively between fumigated and non-fumigated soil collected at Parlier; i.e., unless subjected to pre-plant SP or SF, soil collected from a non-fumigated plot at Parlier stunted Nemaguard seedling growth, whereas soil collected from a field fumigated plot at Parlier resulted in optimal Nemaguard seedling growth, regardless of post collection treatment.

In the experiment 2 bioassay, the post-collection soil treatment (i.e., non-treated control vs. autoclaving or fumigation) had large impacts on responses of the Nemaguard peach seedlings (**Table 3**). Soil autoclaving, and to a greater extent, soil fumigation increased top and root fresh weights and reduced severity of root cortex necrosis. The mixture

ratio of field soil to sand had relatively small effects on seedling top and root fresh weights (**Table 3**). The results indicate that the bioassay, with further optimization and validation, could be a practical tool for RD prediction as well further examination of RD etiology in multiple soils.

Evaluations of genetic resistance to RD. By Oct. 2011 in the rootstock trial planted in Apr. 2011, the increase in stem diameter growth or the rootstocks was affected by highly significant interaction of rootstock x soil fumigation treatments (P <0.0001 and =0.003 for experiments with rootstocks from California and Oregon nurseries; **Figure 4 A,B** and **Figure 5 A,B**, respectively). The significance of the interaction is evidence that the rootstocks vary in their relative susceptibility to the RD complex. Although all rootstocks tested suffered some degree of reduced growth due to a lack of pre-plant soil fumigation, the most rootstocks with peach parentage (Harrow Blood x Okinawa clones 1, 10, 28, 32, and 50; Lovell; Nemaguard; and Empyrean #1) were relatively susceptible (mean stem diameter NF/F stem diameter growth proportions 0.30 to 0.53); while most rootstocks with both peach and almond parentage (Bright Hybrid clones 5 and 106; Garnem; Hansen 536; Tempropac; and PAC 9808-02=Rootpac 20) did better (NF/F proportions 0.53 to 0.71), and rootstocks with plum parentage (Controller 5; Krymsk clones 1, 2, 9, 86; Replantpac; Marianna2624; and Myrobalan) were variable (NF/F proportions 0.37 to 0.74). Similar results to the stem diameter responses were obtained for tree height and pruning weights (meas. Oct 2011 and Feb 2012, respectively; data not shown).

Overall, our results suggest that resistance to the RD complex is 1) more than a simple matter of inherent rootstock vigor (although increased vigor does appear to be a factor contributing to the resistance) and 2) more than a simple matter of how genetically divergent a replanted rootstock is from the rootstock preceding it at an orchard replant site. For example, there was no significant correlation between NF/F stem growth proportions with corresponding tree heights in fumigated plots, illustrating the lack of a consistent relation between at least one measure of rootstock vigor and tolerance or resistance to RD. Similarly, results of previous field trials as well as the results of this trial indicate that tolerance or resistance to RD at an orchard replant site is not simply a matter of how genetically divergent a replanted rootstock is from the rootstock it is following-- for example, in almond replant trials in Butte County, almond RD was more severe when Marianna 2624 rootstock (highly divergent from Nemaguard genetically) was replanted after Nemaguard peach rootstock than when Nemaguard peach rootstock was replanted after itself (1). Furthermore, in the 2011 trial at Parlier where rootstocks were tested after removal of an orchard on Nemaguard peach rootstock, Empyrean 1 (peach parentage) grew as well in soil impacted by the RD complex as some rootstocks with parentage divergent from peach (i.e., some peach x almond hybrids and some plum hybrids) (**Figure 4**).

The results indicate that judicious development and use of rootstocks for *Prunus* spp. has potential to help manage RD. We are repeating the rootstock trial at Parlier to verify the results. At this time, our results suggest that peach x almond hybrids and some of the most vigorous peach rootstocks may be less impacted by RD than

Nemaguard peach, at least in a soil like Hanford Sandy Loam. However, not all of the stocks tested are acceptable for almond, and regardless of RD risk, growers should carefully consider the horticultural suitability of prospective rootstocks to all of the demands of a site before making a rootstock selection. For example, due to their other susceptibilities, some peach x almond hybrids are known as poor choices for replanting at sites subject to the ring nematode / bacterial canker complex or subject to poor drainage or problems with crown rot due to *Phytophthora*.

Evaluation spot treatment with Brassica seed meal for control of RD. In the almond replant trial located on a sandy soil near Merced, CA, the spot treatment with Brassica resulted in some improvement in replanted tree growth. Compared to the non-treated control, *Brassica* seed meal application through the tree-site auger improved stem diameter growth in the first growing season (2011) by 27%, whereas augering alone without the seed meal improved stem diameter growth by 12%, and pre-plant fumigation increased stem diameter growth by 47% (**Figure 6**). It appears spot treatments with Brassica seed meal have potential but will require improvement before they approach the effectiveness of strip or spot fumigation.

Research Effort Recent Publications:

Browne, G., Lampinen, B., Doll, D., Hanson, S., Schmidt, L., Bhat, R., Fennimore, S., B., Holtz, B., Upadhyaya, Gao, S., Klonsky, K., and Johnson, S. 2011. Integrated pre-plant alternatives to methyl bromide for almonds and other stone fruits. Pp. 32-1 to 32-4, Proceedings, Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, available online: http://mbao.org/2011/Proceedings/32BrowneG.pdf

Baumgartner, K., Fujioshi, P., Kluepfel, D., Browne, G., and Leslie, C. 2012. Idenfification of tree-crop rootstocks with resistance to Armillaria root disease. (Abstract, presented at the National Annual Meeting of the American Phytopathological Society, available on line at: http://www.apsnet.org/meetings/Documents/2012_Meeting_Abstracts/aps12abO23.htm).

Browne, G.T., Schmidt, L.S., Bhat, R.G., Gartung, J., Wang, D., and Kluepfel, D.A. 2012. Growth of new rootstocks for *Prunus* spp. in fumigated and nonfumigated replant soil. (Abstract, presented at the National Annual Meeting of the American Phytopathological Society, available on line at: http://www.apsnet.org/meetings/Documents/2012_Meeting_Abstracts/aps12abP301.htm)

Doll, D.A., Browne, G.T., Hanson, B., and Fennimore, S.A. 2012. First-year almond tree performance as affected by preplant soil steam, backhoe, and fumigation treatments in a replanted site with the presence of plant-parasitic nematodes. (Abstract, presented at the National Annual Meeting of the American Phytopathological Society, available on line at: http://www.apsnet.org/meetings/Documents/2012_Meeting_Abstracts/aps12abP183.htm)

Schmidt L.S., Bhat R.G., Kluepfel, D.A., and Browne, G.T. 2012. Resistance to Phytophthora in new rootstocks for almond and stone fruits. (Abstract, presented at the National Annual Meeting of the American Phytopathological Society, available on line at: http://www.apsnet.org/meetings/Documents/2012_Meeting_Abstracts/aps12abP258.htm)

Irrigation Water Relation to the Health of Deciduous Fruit and Nut Crops. 2012. Browne, G.T., Beede, R.H., and Schmidt, L.S. In: Waterborne Plant Pathogens: Biology, Detection and Management, C. Hong and G. Moorman, Eds. APS Press, MN. (Accepted, In Press)

Reference Cited:

Browne, G. T., Connell, J. H., and Schneider, S. M. 2006. Almond replant disease and its management with alternative pre-plant soil fumigation treatments and rootstocks. Plant Disease 90:869-876.

Table 1. Rootstocks included in 2011 and 2012 evaluations of resistance to replant disease complex and propagated for 2013 evaluations to *Phytophthora* species

Rootstock	Type	Genetic background
HBOK1	Pe	*HB x OK peach*
HBOK 10 (Controller 8)	Pe	*HB x OK peach*
HBOK 28	Pe	*HB x OK peach*
HBOK 32 (Controller 7)	Pe	*HB x OK peach*
HBOK 50 (Contoller 9.5)	Pe	*HB x OK peach*
Lovell	Pe	*P. persica*
Nemaguard	Pe	*P. persica x P. davidiana*
Empyrean#1 (Barrier 1)	Pe	*P. persica x P. davidiana*
Bright Hybrid-5	Pe x Al	*P. persica x P. dulcis*
Bright Hybrid 106	Pe x Al	*P. persica x P. dulcis*
GxN 15(Garnem)	Pe x Al	*P. dulcis x P. persica (Nemared)*
Hansen 536	Pe x Al	*[Okin.x (P. davidiana x Pe PI 6582)] x alm.*
Tempropac	(Pe x Al) x Pe	*(P. dulcis x P. persica) x P. persica*
PAC 9908-02	(Pe x Al) x Pe	*(P. dulcis x P. persica) x P. persica*
Controller 5 (=K146-43)	Pl hybrid	*P. salicina x P. persica*
Krymsk #1 (VVA 1)	Pl hybrid	*P. tomentosa x P. cerasifera*
Krymsk 2	Pl hybrid	*P. incana x P. tomentosa*
Krymsk 9	Pl hybrid	*P. armeniaca x P. ceracifera*
Krymsk#86 (Kuban 86)	Pl hybrid	*P. persica x P. cerasifera*
Replantpac	Pl hybrid	*P. ceracifera x P. dulcis*
Myrobalan	Pl hybrid	*P. ceracifera*
Marianna 2624	Pl hybrid	*P.munsoniana x P. cerasifera*

Table 2. Pathogenicity in Nemaguard peach by *Cylindrocarpon* species, *Thielaviopsis sp.*, and *Pythium* sp. associated with replant disease

Isolate	Location-orchard-yr collected	Top wt (g)	Root wt. (g)	Root rot (%)
Control		31.0	12.6	31
Cylindrocarpon destructans	Chico-z-2003	30.5	11.6	73
C. macrodidymum	Chico-z-2003	27.3	11.5	62
C. macrodidymum	Chico-d-2003	27.3	11.0	66
C. macrodidymum	Chico-z-2004	32.9	12.8	71
C. macrodidymum	Chico-d-2004	28.2	12.7	71
C. macrodidymum	Parlier-m-2004	27.9	8.6	69
C. macrodidymum	Chico-d-2004	28.3	13.0	66
C. macrodidymum	Firebaugh-t-2007	29.9	12.5	63
C. macrodidymum	Firebaugh-t-2007	27.5	11.1	74
C. macrodidymum	Parlier-14N-2008	30.7	11.4	74
C. macrodidymum	Parlier-24C-2008	30.5	10.1	**50**
C. macrodidymum	Firebaugh-t-2010	33.0	14.2	63
C. macrodidymum	Firebaugh-t-2010	31.0	12.2	65
C. macrodidymum	Firebaugh-t-2010	26.7	9.3	57
C. macrodidymum	Firebaugh-t-2010	26.0	10.1	64
C. macrodidymum	Wasco-t-2011	26.1	11.2	73
C. macrodidymum	Wasco-t-2011	26.4	11.6	73
C. macrodidymum	Wasco-t-2011	24.1	8.7	63
C. macrodidymum	Merced-n-2011	26.0	10.5	66
C. macrodidymum	Merced-n-2011	30.5	12.3	65
C. macrodidymum	Merced-n-2011	27.9	10.5	66
C. macrodidymum	Merced-n-2011	29.8	10.9	72
Thielaviopsis sp.	Wasco-t-2011	20.1	8.6	**74**
Pythium sp.	Wasco-t-2011	29.9	10.7	59
Pythium sp.	Wasco-t-2011	29.4	9.7	59
Pythium sp.	Wasco-t-2011	31.2	11.4	63
Pythium sp.	Merced-n-2011	25.6	8.4	57
Value of P:		0.008	0.04	<0.0001
95% C.I.:		(+/- 5.2)	(+/- 3.3)	(+/- 12)

Table 3. Effects of dilution with sand and biocidal treatments on expression of replant disease in a greenhouse bioassay[a]

Effect	Treatment	Top wt. (g)	Root wt. (g)	Root rot (%)
Soil mixing with course sand	1:0 field soil : sand ratio	11.6	5	29
	5:1 field soil : sand ratio	8.3	4.3	25
	4:1 field soil : sand ratio	9.1	4.7	25
	3:1 field soil : sand ratio	12.0	5.6	33
	2:1 field soil : sand ratio	11.4	5.2	31
	1:1 field soil : sand ratio	10.8	5.8	37
	P value:	0.13	0.04	0.04
	95% CI	--	1.1	6
Post-collection soil treatment	Control	6.0	2.9	70
	Autoclave	10.6	5.3	13
	Fumigation	15.1	7.1	7
	P value:	<0.0001	<0.0001	<0.0001
	95% CI	(+/- 2.7)	(+/- 1.1)	(+/- 4)

[a] Soil was collected from 0.3- to 2.0-ft depths in non-fumigated areas in three orchards situated on Hanford Sandy Loam in Fresno County. After collection, the soils were mixed. Subsamples of the soil were used for the treatments listed above and then distributed to replicate 20-oz pots. Nemaguard peach seedlings were grown in the potted soil for 2 months before assessing effects of the treatments on the variables listed above.

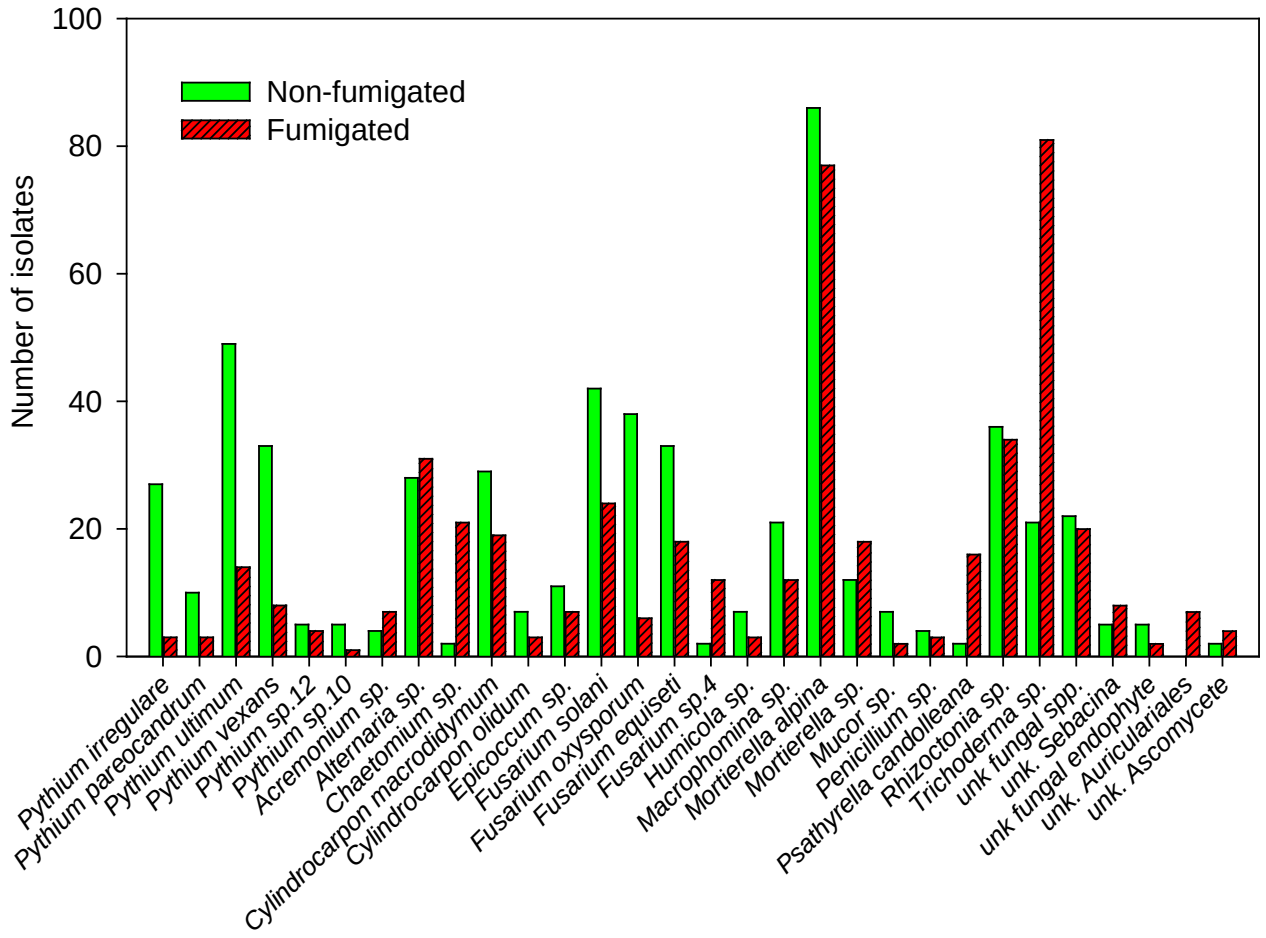

Figure 1. Total counts of isolates cultured from roots of Nemaguard, Lovell, BB106, and Hansen 536 rootstocks as a function of pre-plant soil treatment. All trees in non-fumigated plots were stunted in growth due to the replant disease complex, compared to the trees in pre-plant fumigated soil. For each rootstock and culture isolation medium, a total of 48 root pieces (12 per each of four replicate trees) 1 cm long were cultured per soil treatment. Roots were cultured on PARP medium to detect oomycetes such as Pythium species and on water agar + ampicillin medium to detect fungi.

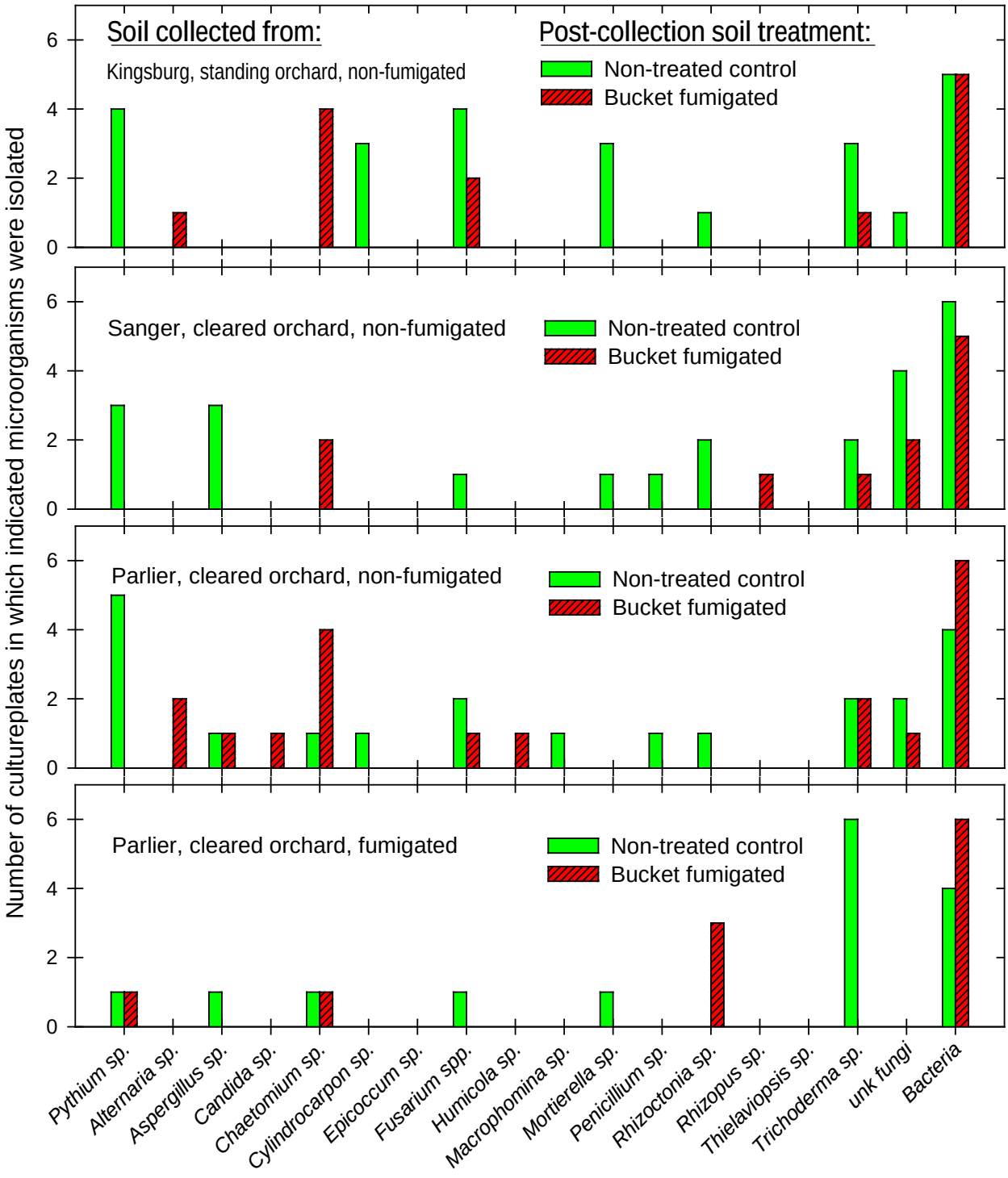

Figure 2. Results of culture-based isolations from Nemaguard peach roots at the conclusion of a bioassay experiment. Total number of culture plates out of eight total (four water agar + ampicillin and four PARP culture plates were used per treatment).

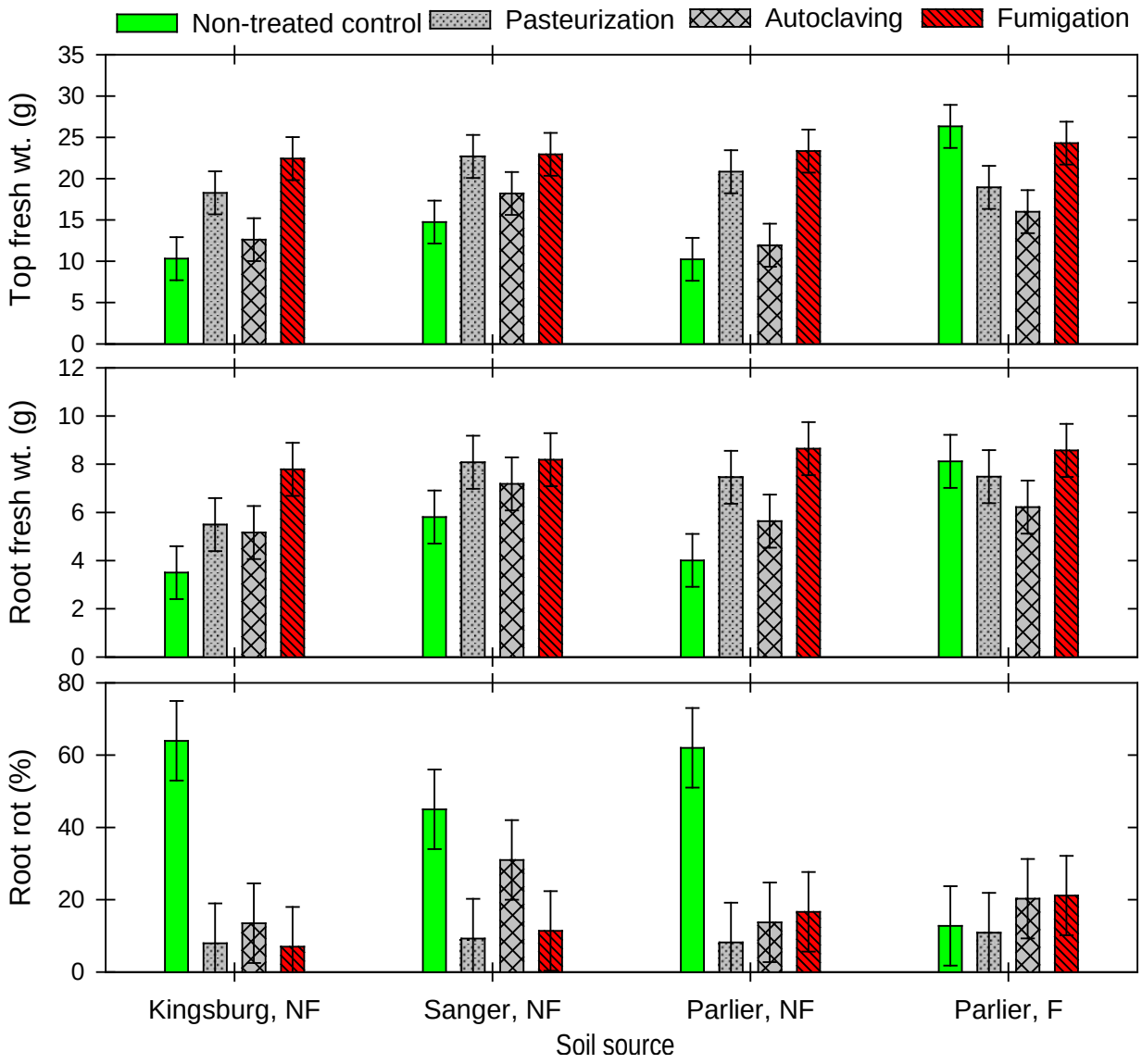

Figure 3. Effects of pre-plant bioassay treatments (i.e., soil pasteurization, autoclaving, and fumigation; administered in buckets after soil collection from field) on severity of replant disease symptoms in Nemaguard peach seedlings. The seedlings were used as bioassay plants in four soils (Kingsburg NF, Sanger NF, Parlier NF, and Parlier F, all Hanford Sandy Loam) to be used to replant almond orchards. Designations of "NF" indicate soil not fumigated before collection; "F" indicates soil fumigated in the field (Telone C35, 540 lb/ac) before collection. The seedlings were planted into the soil while sprouting and grown for 2 months before the variables above were measured. Values are means from six plants. Vertical bards indicate 95% confidence intervals. Note that growth suppression and root cortex necrosis symptoms were reduced by either fumigation in the field (as was done for "Parlier F" soil) or by soil pasteurization, soil autoclaving, or soil fumigation treatments, but soil pasteurization and fumigation were more effective in preventing growth suppression and root necrosis than soil autoclaving.

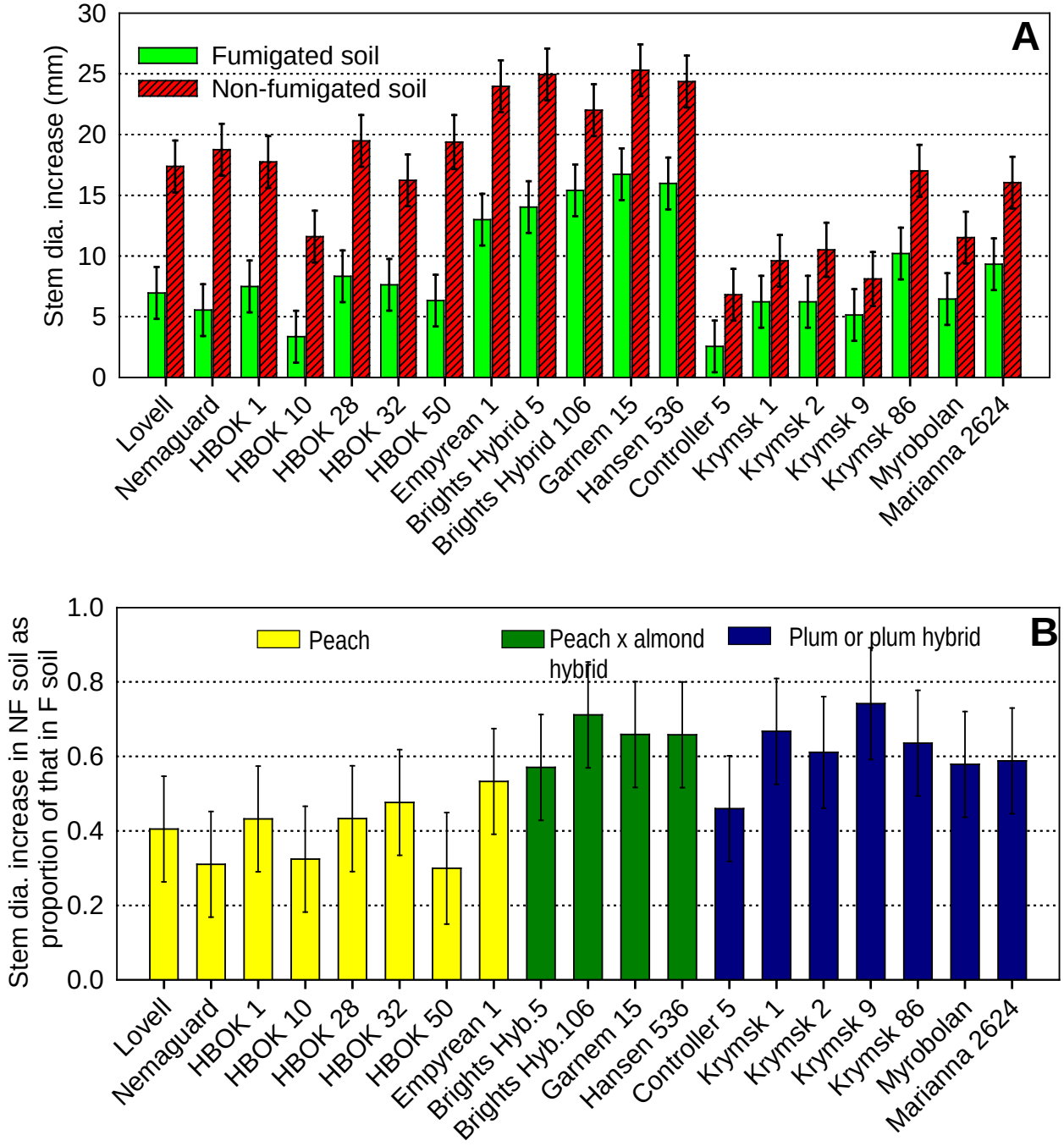

Figure 4. Rootstocks propagated in California: Increase in stem diameters in first growing season after planting (Apr-Oct 2011) in first rootstock trial at USDA-ARS Parlier. **A,** actual stem diameter increases, and **B,** stem diameter increases in non-fumigated soil expressed as a proportion of stem diameter increases that occurred in fumigated soil. For each rootstock and each fumigation treatment there were eight replicate plots of three trees. Vertical bars indicate 95% confidence intervals.

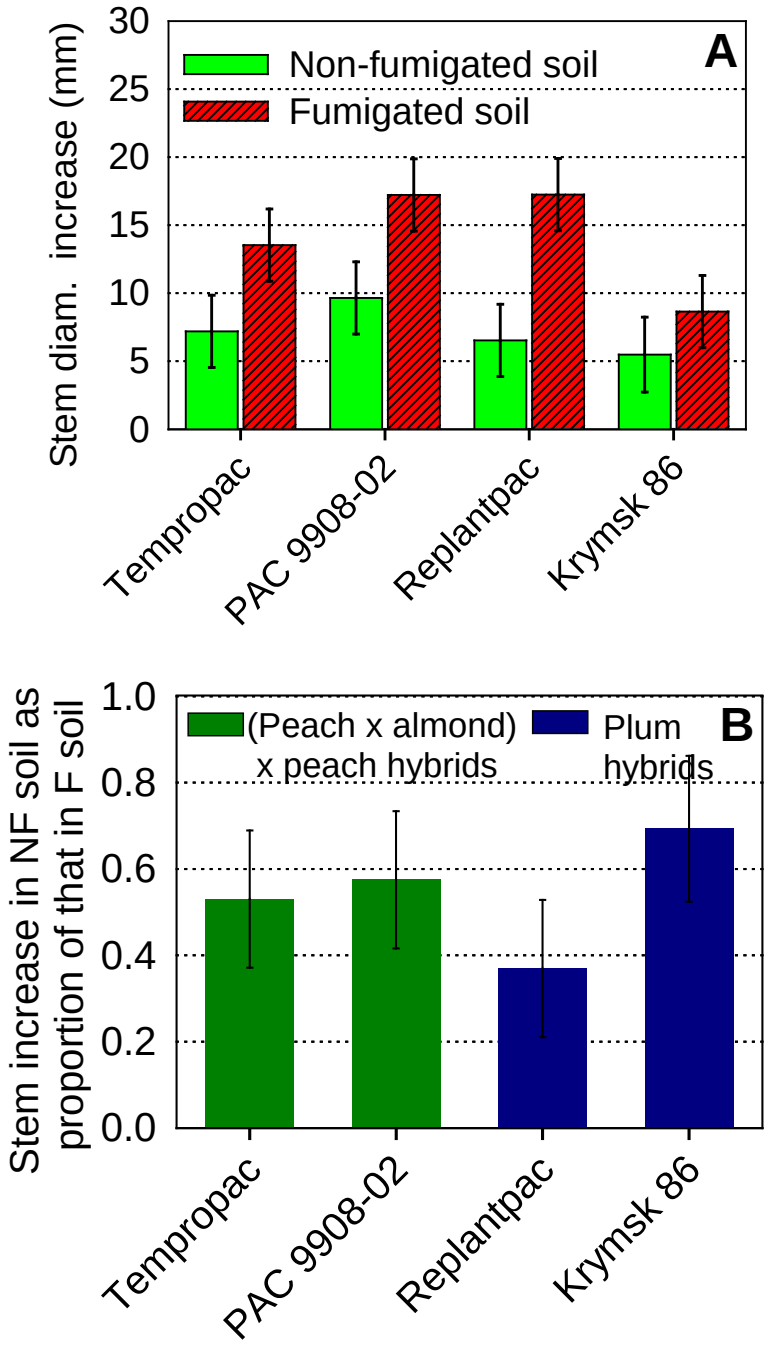

Figure 5. Rootstocks propagated in Oregon: Increase in stem diameters in first growing season after planting (Apr-Oct 2011) in first rootstock trial at USDA-ARS Parlier. **A,** actual stem diameter increases, and **B,** stem diameter increases in non-fumigated soil expressed as a proportion of stem diameter increases that occurred in fumigated soil. For each rootstock and each fumigation treatment there were eight replicate plots of three trees. Vertical bars are 95% confidence intervals.

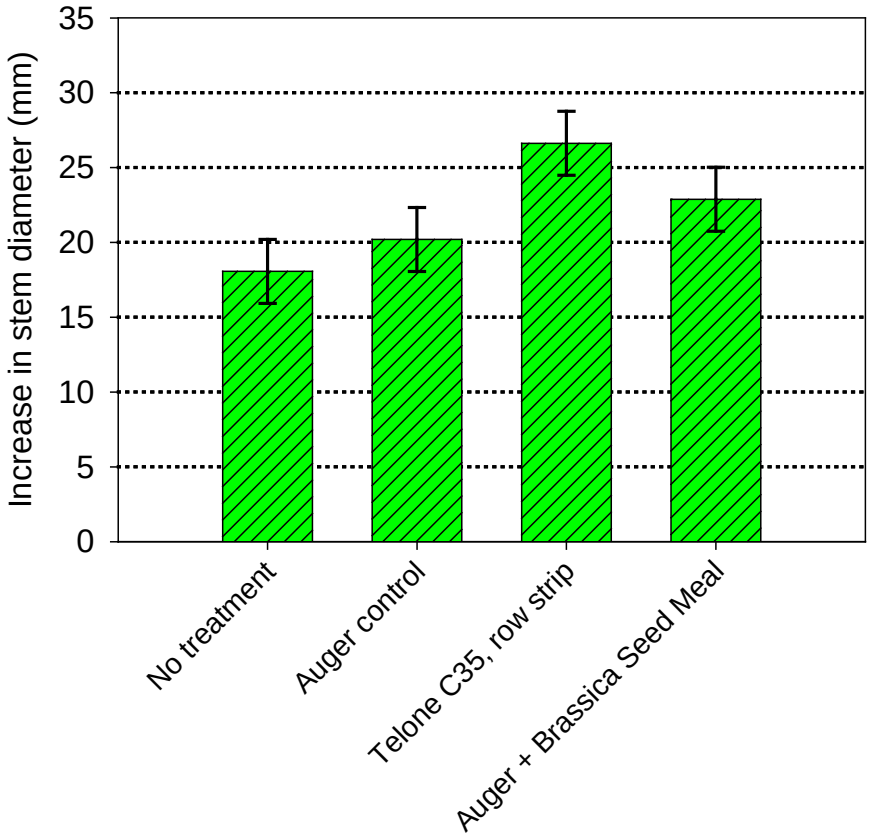

Figure 6. Response of almond trees in a replanted orchard to pre-plant spot treatment with Brassica seed meal (6 tons/treated acre) applied with a 2.5-ft. diameter auger to tree planting sites, in comparison to non-treated and auger controls and conventional row-strip fumigation with Telone C35. For each treatment there were seven replicate plots of 2 to 6 trees). Vertical bars are 95% confidence intervals.