Developing Improved Strategies for Management of Replant Problems

Project No.: **10-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 work is to improve almond orchard replacement strategies, maximizing their economy while reducing their dependence on soil fumigation. Central project concerns are: 1) determining causes of almond replant disease (RD), which is 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 2010-11, we focused on the following: 1) determining the specific identity of *Cylindrocarpon* species associated with RD, 2) developing and applying a qPCR (quantitative PCR) assay to quantify the amount of *Cylindrocarpon* in root samples from RD-affected orchards, 3) establishing new almond replant trials to support further study of RD causes and test fumigant and non-fumigant alternatives for RD control, and 4) conducting field and greenhouse evaluations of rootstock resistance to RD and *Phytophthora*.

We reported previously that *Cylindrocarpon* sp., *Pythium* sp., and a fungus with DNA sequence similarity to *Phaeonectriella* sp. each had exhibited positive associations with RD occurrence in multiple orchard replant trials, although no single organism's presence was highly correlated with RD at all orchards. The site-associated variation in these results is not too surprising, given that the disease may be induced by a microbial complex that interacts with soil and other environmental factors. Important steps in sorting out the roles of organisms suspected as RD causes include: identifying the organisms specifically; examining, quantitatively, the degree and consistency of each organism's association with the disease; and testing isolates for pathogenicity (i.e., the ability to induce disease, in this case RD). To specifically identify *Cylindrocarpon* sp. associated with RD on almond, we completed multi-locus gene sequencing for 79 isolates putatively identified and *Cylindrocarpon* sp. from six of our previous RD trials; each isolate was from the roots of a different tree. The sequencing revealed that the vast majority (77) of these isolates were *C. macrodidymum*. Pathogenicity of these isolates is being tested repeatedly. To quantitatively examine to association between *Cylindrocarpon* and RD, we developed and applied a quantitative PCR (qPCR) assay specific for *C. macrodidymum* and used it on roots from healthy and RD-affected trees in five RD trials. Based on the qPCR results, in three of the five orchards *Cylindrocarpon* was present in higher quantities in roots from RD-affected trees, compared to healthy trees. We are presently investigating ingression of *Cylindrocarpon* into vascular tissues and will continue development of qPCR assays for it and other suspected RD pathogens. The assays will help in our determinations of RD etiology and may have value in disease diagnosis and prediction (i.e., to determine a need for soil fumigation).

Two new almond replant trials were established with commercial growers under the Pacific Area-Wide Program for Integrated Methyl Bromide Alternatives (PAW-MBA) (Browne et. al) in 2010-11. One of them is in Merced County on a sand soil texture with the ring nematode present, and the other is in Kern County on a sandy loam soil texture without known significant parasitic nematode populations. Both sites were expected to express RD, based on their recent history of almond production and relatively coarse soil texture. The trials include treatments known to prevent RD (e.g., strip fumigation with chloropicrin, Telone C35, or methyl bromide + chloropicrin); alternative non-fumigant spot treatments of uncertain efficacy (e.g., spot treatment of tree planting sites with steam, Brassica seed meal, and various fungicides); and non-treated controls. Based on the effectiveness of spot fumigation in controlling RD in previous trials, we had hypothesized that spot treatments with non-fumigants also may be effective. To investigate causes of RD in these replant trials, we sampled the microbial populations from roots of trees in chloropicrin-fumigated and non-fumigated plots. The microorganisms are being identified and tested for pathogenicity in Nemaguard rootstock. Efficacy of all of the field treatments is being assessed according to growth and health of the trees. Completion of the microbial assessments and early tree growth assessments will require additional years for these trials.

A field trial was established in 2010-11 at USDA-ARS Parlier to evaluate resistance to the RD complex in 22 rootstocks (including peach, peach × almond, plum, and plum

hybrid backgrounds). Most of these rootstocks are of interest for almond production, although some are suited only for peaches or other fresh fruits. The rootstocks were planted into replicate fumigated and non-fumigated plots in April 2011. To date, all of the rootstocks, regardless of parentage, have expressed growth suppression in the non-fumigated plots (i.e., indicating a degree of susceptibility to RD), but some rootstocks have performed marginally better than others. A "companion" greenhouse trial was established with soil collected from non-fumigated plots in the Parlier rootstock trial. The soil was mixed thoroughly, and then half of it was pasteurized with steam. The pasteurized and non-pasteurized soil portions each were mixed with sterile sand (2:1, soil:sand, v:v), placed in pots, and planted with the 22 rootstocks. Our aim in the greenhouse experiment is to determine whether plant growth and root health in a greenhouse test will reflect RD resistance expressed by the same rootstocks in the field. The field rootstock trial will continue for another year, while the greenhouse experiment is to be completed this month.

The same rootstocks tested for their response to RD were evaluated in a greenhouse trial for resistance to *Phytophthora niederhauserii*, a species we have found killing almond trees in Fresno and Kern Counties and that previously was reported to do so in Spain. In this experiment, some of the rootstocks with plum parentage were more resistant to the pathogen than peach and peach × almond backgrounds. We will repeat this experiment and conduct screens with additional species of *Phytophthora* from almond. Growers are encouraged to report severe suspected Phytophthora problems to their UC Farm Advisor; this will permit us to keep our *Phytophthora* collection current and ensure that our rootstock evaluations will adequately represent field performance. Results from the evaluations of rootstock resistance to RD and *Phytophthora* may suggest beneficial directions for rootstock breeding efforts and will help growers to select rootstocks and planting densities appropriate for their orchard sites.

Materials and Methods:

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

Sampling of new replant trials. Two new replant trials were established with commercial growers under the Pacific Area-Wide Program for Integrated Methyl Bromide Alternatives (PAW-MBA) (1) for microbial sampling (described here, objective 1) and to test efficacy of new RD control treatments (described below, objective 2). One of the trials is in Merced County on a sand soil texture with the ring nematode present, and the other is in Kern County on a sandy loam where significant populations of plant parasitic nematodes have not been detected. Both sites were expected to express RD, based on their recent history of almond production and relatively coarse soil texture. To investigate causes of RD in these trials, we sampled the microbial populations from roots of trees in chloropicrin-fumigated and non-fumigated plots in the first few months after orchard replanting.

Characterizing RD-associated *Cylindrocarpon* populations. In-depth analyses of *Cylindrocarpon* populations associated with RD were continued. We previously

reported that *Cylindrocarpon* sp., *Pythium* sp., and a fungus with DNA sequence similarity to *Phaeonectriella* sp. exhibited positive associations with RD occurrence, although no single organism's presence was highly correlated with RD at all orchards (Browne et al., Annual Report to the Almond Board of California, 2010). In 2010-11, we used multi-locus gene sequencing (i.e., DNA sequencing in multiple gene regions) to speciate representative cultured *Cylindrocarpon* isolates from the previous RD trials (2003-2010). Seventy-nine isolates of putative *Cylindrocarpon* sp. were included, each from the roots of a different tree among six CA almond and peach orchards affected by RD. DNA was extracted from each isolate, and the gene regions of interest (i.e., rDNA ITS1-5.8S-ITS2; partial beta-tubulin gene, and partial mtSSU rDNA) were amplified and sequenced (i.e., the order of the nucleotides making up the DNA backbone, which can serve as an organism-specific genetic "fingerprint", was determined). The *Cylindrocarpon* DNA sequences were used in BLAST (Basic Local Alignment Search Tool) search queries in the NCBI (National Center for Biotechnology Information) sequence database to locate named species with matching DNA sequences. The *Cylindrocarpon* DNA sequences also were subjected to phylogenetic cluster analyses (i.e., analyses that cluster DNA sequences according to genetic relationships inferred from the sequences). Well-documented sequences from known species of *Cylindrocarpon* in the NCBI database were included in our cluster analyses as standards.

In addition to the species identifications completed for cultured isolates of *Cylindrocarpon* as described above, we attempted to identify *Cylindrocarpon* species detected by culture-independent amplification of ITS2 rDNA fragments directly from roots. As was done for the gene region sequences from cultured isolates, ITS2 rDNA sequences from culture-independent amplifications were used for BLAST searches and cluster analyses.

Using qPCR to quantify *Cylindrocarpon* levels in healthy and RD-affected roots. We developed and applied a quantitative PCR (qPCR) assay for *Cylindrocarpon*. The ITS1-5.8S-ITS2 region of the rRNA gene was sequenced from many target and non-target organisms present in roots of RD-affected and healthy roots. These sequences and additional ones from public DNA sequence databases were used to develop primers specific for *C. macrodidymum*. After the primers were tested to confirm intended specificity, they were used for qPCR assays in root samples from healthy and RD-affected trees in replicate pre-plant fumigated and non-fumigated plots, respectively. The samples had been collected within the first 15 months after planting in five replant trials located in the Sacramento and San Joaquin Valleys between 2003 and 2010. DNA was extracted from the samples using a modified CTAB method after overnight incubation of the root tissues in 5% alconox solution; a total of 118 root samples, each collected from a particular tree at a particular time after planting, were processed.

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

New replant trials. The two replant trials established in 2010-11 (site characteristics detailed above, Objective 1, "Sampling of new replant trials") include treatments known to prevent RD (e.g., strip fumigation with chloropicrin, Telone C35 or methyl bromide + chloropicrin); alternative non-fumigant treatments of uncertain efficacy (e.g., spot treatment of the soil in tree planting sites with steam, Brassica seed meal, or various fungicides); and non-treated controls. The spot treatments were injected into the soil at tree planting sites before planting. Based on the effectiveness of spot fumigation in controlling RD in previous trials, we had hypothesized that spot treatments with non-fumigants also may be effective. Effectiveness of the treatments is being assessed according to tree growth and periodic disease severity ratings.

Previously established replant trials. Monitoring of tree growth responses was continued in two replant trials initiated in 2009 in Madera and Merced Counties. The Madera County trial included spot drip fumigation treatments, spot shank-fumigation treatments, and spot steam treatments in addition to conventional shank-applied strip fumigation treatments. The Merced County trial included spot fumigation treatments and spot treatments with Brassica seed meal and steam in addition to conventional shank-applied strip fumigation treatments.

Rootstock resistance to RD. A field trial was established in 2010-11 at USDA-ARS Parlier to evaluate resistance to the RD complex in 22 different rootstocks (including peach, peach × almond, and plum and plum hybrid backgrounds, **Table 1**). On 29 October 2010, a shank strip fumigation treatment with Telone C35 (540 lb/acre in 15-ft wide strips) and a non-fumigated control (shank ripped only) each were applied to eight replicate plots. In April 2011, potted trees of the rootstocks were planted into the plots. The experiment had a split-plot design, with fumigation treatments occurring in mainplots and rootstocks occurring in subplots. Three trees of each rootstock were planted 2 ft. apart in each fumigated and non-fumigated plot. It was necessary to compare the rootstocks in two sets ("set 1" and "set 2") to accommodate plant shipments from two different nurseries (plant and pot sizes differed between nurseries). Krymsk 86 rootstock was included in both set 1 and set 2 to serve as a common point of reference.

Non-fumigated soil was collected from the Parlier rootstock trial in spring 2011 to use in a complementary trial evaluating resistance to RD in a greenhouse at Davis. Half of this soil was pasteurized with steam (30 min at >95 °C); and the steamed and non-steamed portions were mixed with sterile sand (2:1, soil:sand, v:v), placed in pots, and planted with the 22 rootstocks (small ca. 2" potted plants were used).

Rootstock resistance to *Phytophthora*. The same 22 rootstocks tested for their response to RD in the field and greenhouse as described above were evaluated for

resistance to *Phytophthora niederhauserii* in a greenhouse trial. We isolated *P. niederhauserii* from dying almond trees in Fresno and Kern Counties and identified it based on multi-locus gene sequencing. This pathogen has been reported to cause serious almond tree losses in Spain. In the greenhouse trial, replicate plants of the rootstocks were transplanted into 0.7-liter pots of UC mix soil (a peat:sand 50:50 v:v mixture with added nutrients). The UC mix either received V8-vermiculite-oat substrate infested with one of the isolates of *P. niederhauserii* (40 ml of the infested substrate was added per liter of UC mix) or 40 ml of the substrate in sterile form. There were 10 replicate plants per combination of inoculum treatment and rootstock in a split plot design (inoculum treatments were added to mainplots, while rootstocks were randomized among subplots).

Results and Discussion:

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

Sampling new replant trials. The microorganisms sampled from the new replant trials are still being identified. Information on their incidence and pathogenicity will be summarized in future reports.

Characterizing RD-associated *Cylindrocarpon* populations. BLAST searches using the partial beta tubulin and mtSSU gene sequences from the cultured isolates of *Cylindrocarpon* each identified 77 of the isolates as *C. macrodidymum*, one isolate as *C. liriodendri*, and one isolate as *Fusarium* sp. In contrast, ITS1 and 2 rDNA gene sequences from the cultured isolates and ITS2 rDNA sequences from culture-independent clones did not permit unambiguous identification of *Cylindrocarpon* species. Results of the phylogenetic cluster analyses were consistent with the BLAST identifications; all clustering methods grouped the 77 cultured isolates identified as *C. macrodidymum* into one large cluster. The other cultured fungi (*C. liriodendri, Fusarium* sp.) were clustered separately (**Figure 1**). In a separate cluster analysis, the ITS2 sequences from cloned fragments, which could not be definitively identified using BLAST searches, clustered into one large clade of 118 sequences and a small clade of 4 sequences (data not shown).

These results indicate that *C. macrodidymum* has been the predominant species of *Cylindrocarpon* associated with RD of almond. In contrast with this result, *C. destructans* was reported as the most prevalent species associated with RD of apple in WA (2). Both *C. liriodendri* and *C. macrodidymum* were associated with black foot disease of grapevine (3). It is possible, though not investigated, that host specificity in *Cylindrocarpon* populations contributes to host specificity observed in replant diseases. For example, severe RD is not expected in almond planted after grapes nor vice versa (Browne, *unpublished*; although the ring nematode, a separate replant problem, can follow from grape to almond). The fact that ITS sequences alone were not sufficient to discriminate between *C. macrodidymum* and some other *Cylindrocarpon* species, whereas partial beta tubulin gene and mitochondrial sequences were sufficiently

discriminating indicates that for best specificity, qPCR assays should be developed and tested for the latter gene targets.

Using qPCR to quantify *Cylindrocarpon* levels in healthy and RD-affected roots. In three of the five orchards sampled, significantly more target DNA of *Cylindrocarpon* was detected by qPCR in roots from RD-affected trees than in roots from healthy trees (**Table 2**) (*P*=0.01 to 0.0001). Differences in target DNA concentration were not significantly different for the other two orchards (*P*=0.19-0.23). These results generally coincide with our previous experiences using culture-based isolations and semi-quantitative DNA based analyses, each of which have generally, but not always, found RD-*Cylindrocarpon* associations. We are investigating possible predictive uses of qPCR for managing RD problems (i.e., testing for *Cylindrocarpon* and other RD-associated organisms in soil and root samples collected before replanting).

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

New replant trials. Preliminary tree growth assessments in the new Merced County trial are reported in a companion project "*Development and Optimization of the Steam Auger for Management of Almond Replant Disease*" (Hanson et al., 2011 report to the Almond Board of California), which shared plots in the trial. Preliminary tree growth assessments for the new Kern County replant trial are reported below (**Tables 3, 4**). Since the Kern County trial was only planted in May 2011, continued growth monitoring will be required for meaningful assessment of the treatments.

Previously established replant trials. The Madera County replant trial initiated in fall 2009 and planted in winter 2010 had to be discontinued. A few months after planting the new trees, severe, spatially erratic glyphosate injury required the grower to replant approximately 25% of the trees, and many of the remaining trees still suffered from glyphosate exposure. The glyphosate injuries precluded meaningful assessment of the treatments.

The Merced County trial initiated in fall 2009 and planted in winter 2011 got off to a rough start due to irrigation irregularities and other horticultural factors, but treatment effects seem to be emerging in 2011. Early results from this trial are reported in the companion project (Hanson et al., 2010-11 report to the Almond Board of California).

Rootstock resistance to RD and *Phytophthora*. To date, in the field assessment of rootstock resistance to RD, all of the rootstocks, regardless of parentage, have expressed growth suppression in the non-fumigated plots (i.e., indicating a degree of susceptibility to RD), but some rootstocks have performed marginally better than others (**Figures 2-4**). The field rootstock trial will continue for another year, and it is important to realize that relative performance of the rootstocks may change as their root systems expand according to their genetic potential. The greenhouse trial is still running and will be completed later this month.

In the greenhouse assessment of resistance to *P. niederhauseri*, some of the rootstocks with plum parentage were more resistant than peach and peach × almond backgrounds (**Figure 5**). We will repeat this experiment and conduct screens with additional species of *Phytophthora* from almond. Growers are encouraged to report severe suspected Phytophthora problems to their UC Farm Advisors; this will permit us to keep our *Phytophthora* collection current and ensure that our evaluations of Phytophthora resistance will reflect field performance of rootstocks in the presence of *Phytophthora* species found in California almonds. Results from the evaluations of rootstock resistance to RD and *Phytophthora* may suggest beneficial directions for rootstock breeding efforts and will help growers to select rootstocks and planting densities appropriate for their orchard site histories.

Table 1. Rootstocks included in field and greenhouse evaluations of resistance to replant disease complex and *Phytophthora niederhauserii*

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.*
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*
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*
Replantpac	Pl hybrid	*P. ceracifera x P. dulcis*
Myrobalan	Pl hybrid	*P. ceracifera?*
Marianna 2624	Pl hybrid	*P.munsoniana x P. cerasifera*

Table 2. Quantitative PCR (qPCR) testing results using samples from previous replant trials

Replant trial	Number of times sampled	Number of trees sampled (RD-affected + healthy)	*Cylindrocarpon* DNA in roots (ng DNA / g root)		*P* value
			RD-affected trees	Healthy trees	
1	5	20+20	615	169	0.01
2	4	16+16	606	135	0.0001
3	2	12+12	277	2	0.0001
4	1	6+6	61	21	0.19
5	1	5+5	35	10	0.23

Table 3. Preliminary assessment of tree performance in experiment 1 of Kern County replant trial

Trt no.	Rootstock	Fumigant	Fumigant rate (lb/treated acre)	August disease severity rating[a]	
				Nonpareil	Monterey
1	Hansen 536	None-control	0	0.11	0.00
2	Hansen 536	MB:CP (57:43)	350	0.08	0.11
3	Hansen 536	Chloropicrin	200	0.03	0.06
4	Hansen 536	Chloropicrin	300	0.03	0.00
5	Hansen 536	Chloropicrin	400	0.03	0.06
6	Nemaguard	None-control	0	0.67	0.50
7	Nemaguard	Chloropicrin	400	0.22	0.50
P value for effect of treatment:				*0.009*	*0.0019*

[a]Disease severity ratings based on a scale of 0 to 5, with 0 indicating a healthy tree; 1, 2, 3, and 4; indicating increasing increments of decline, and 5 indicating a dead tree.

Table 4. Preliminary assessment of tree growth in experiment 2 of Kern County replant trial

Trt no.	Treatment details[a]	August disease severity rating[b]	
		Nonpareil	Monterey
1	Chloropicrin, 300 lb per treated acre (strip treatment)	0.16	0.59
2	Non-treated control	0.38	0.50
3	2.0-ft auger[b] control (spot trt.)	0.69	1.13
4	2.5-ft auger control (spot trt.)	0.25	0.63
5	3.0-ft auger control (spot trt.)	0.19	1.25
6	2-ft auger + steam (spot trt.)	0.63	0.88
7	3-ft auger + steam (spot trt.)	0.81	0.44
8	2.5-ft auger + Fludioxonil 50 WP (0.18 oz.) (spot trt.)	0.31	0.31
9	2.5-ft auger + Fludiox.50 WP (0.18 oz), Abound 2.08 SC (0.13 fl oz), and Ridomil Gold 4SL (0.14 fl oz) (spot trt.)	0.13	0.25
10	2.5-ft auger + Abound 2.08SC (0.13 fl oz) (spot trt.)	0.75	0.06
11	2.5-ft auger + mefenoxam (0.14 fl oz) (spot trt.)	0.50	1.31
12	Pre-plant root drench and postplant foliar spray with Fungiphite, (with 0.5% and 0.2% solutions, respectively)	0.31	0.88
13	2.5-ft auger + yeast formulation (5.3 oz) (spot treatment)	0.25	0.38
14	2.5-ft auger + pre-plant root drench with Actigard (0.03 oz)	0.75	0.88
15	2.5-ft auger + Brassica seed meal (13.9 oz) spt treatment)	0.31	0.25
P value for effect of treatment:		0.42	0.18

[a]For the spot treatments: 1) the auger dimension specified is diameter (depth of augering was approx. 2 ft for all augers), and 2) fungicide and amendment amounts were added per tree site.

[b]Disease severity ratings based on a scale of 0 to 5, with 0 indicating a healthy tree, 1, 2, 3, and 4 indicating increasing increments of decline; and 5 indicating a dead tree.

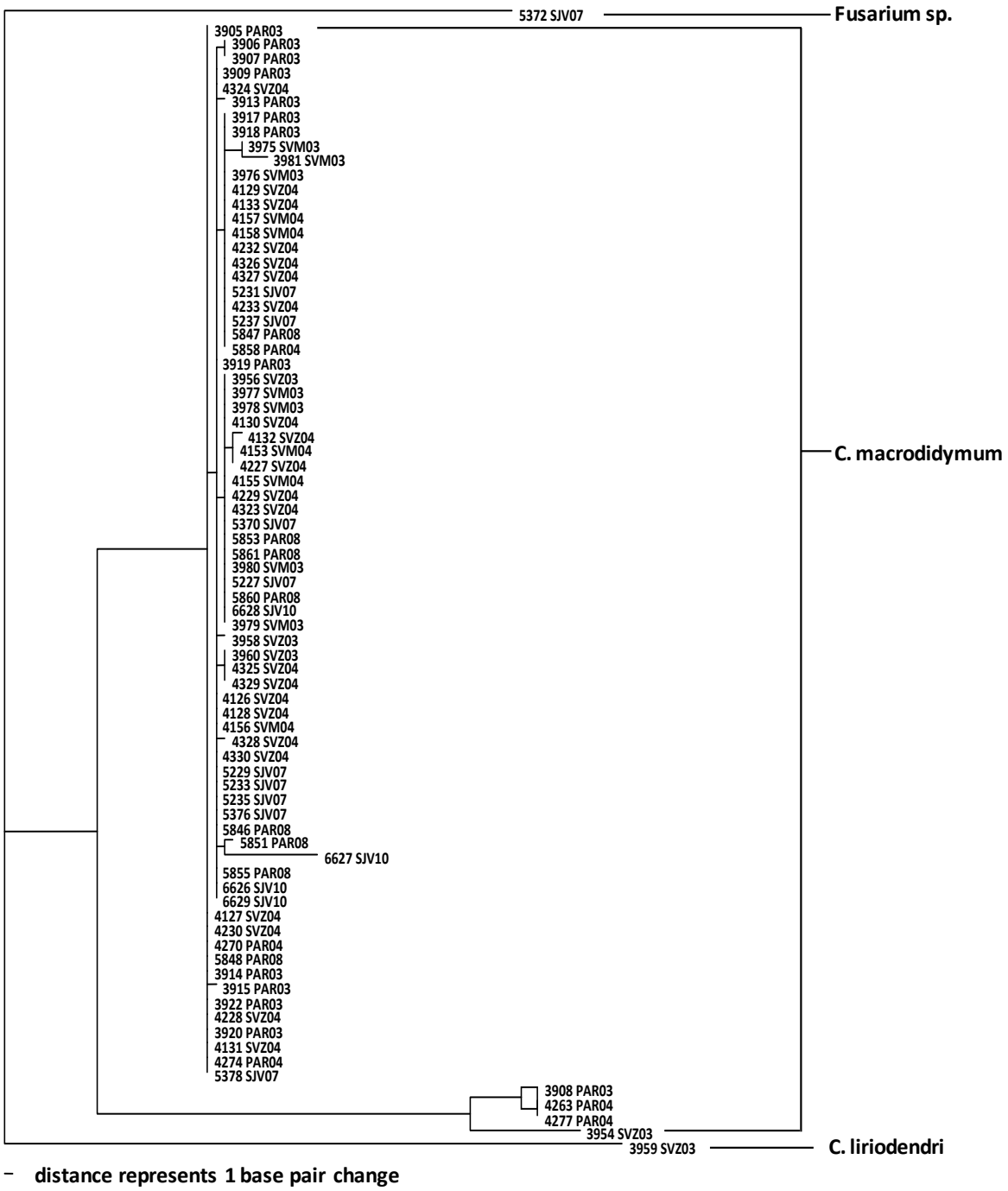

Figure 1. Phylogenetic cluster analysis of 79 isolates of *Cylindrocarpon* from roots of trees in RD-affected orchards. Clustering was based on partial DNA sequences from three genes (ITS regions of rDNA, partial beta tubulin, and partial mtSSU rDNA). Species names were assigned to the clusters according to BLAST searches on the

NCBI database. Note that *C. macrodidymum* predominates (i.e., there were: 1 isolate of *Fusarium* sp., 77 isolates of *C. macrodidymum*, and 1 isolate of *C. liriodendri*)

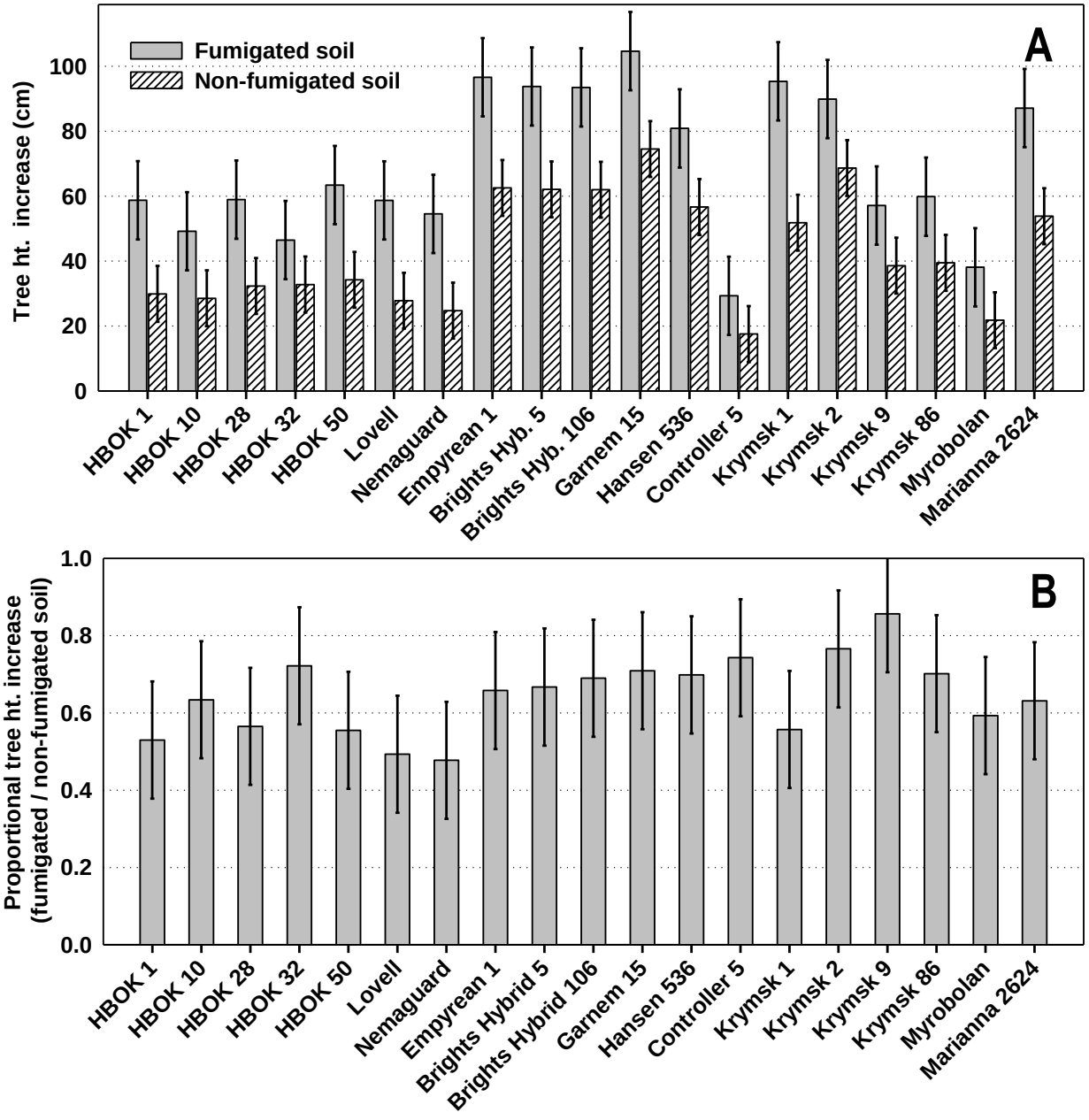

Figure 2. Growth of rootstocks (set 1) in fumigated and non-fumigated plots at USDA-ARS Parlier site previously planted to almonds on Nemaguard rootstock and affected by replant disease. The preceding orchard was removed in fall 2010, the fumigation and non-fumigated plots were established in October 2010, and the new rootstocks were planted in April 2011. **A**, tree height increase as of 25 July, 2011, and **B**, proportion of height increase in non-fumigated plots as compared to that in fumigated plots. Vertical bars indicate 95% confidence intervals.

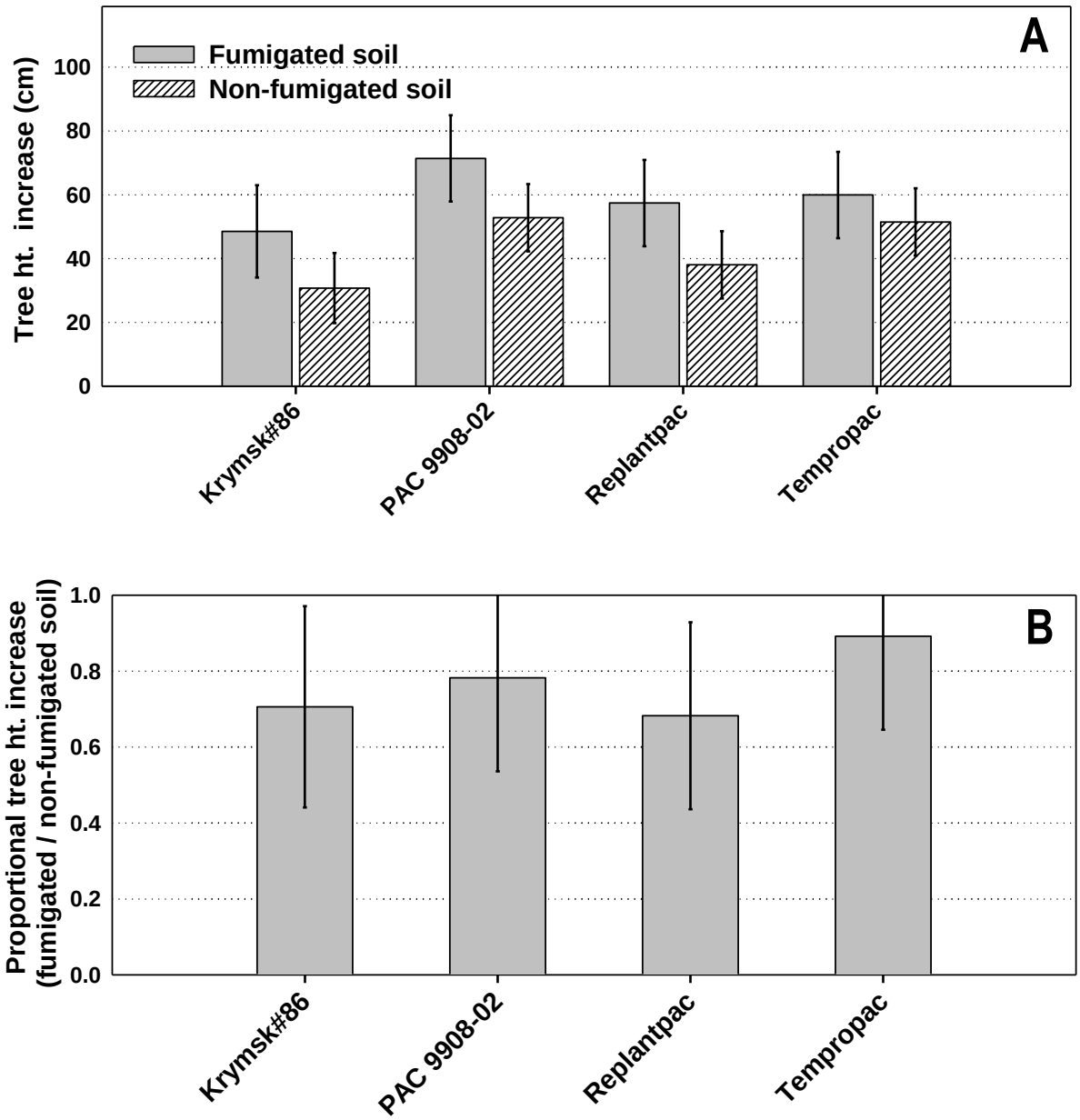

Figure 3. Growth of rootstocks (set 2) in fumigated and non-fumigated plots at USDA-ARS Parlier site previously planted to almonds on Nemaguard rootstock and affected by replant disease. The preceding orchard was removed in fall 2010, the fumigation and non-fumigated plots were established in October 2010, and the new rootstocks were planted in April 2011. **A**, tree height increase as of 25 July, 2011, and **B**, proportion of height increase in non-fumigated plots as compared to that in fumigated plots. Vertical bars indicate 95% confidence intervals.

Figure 4. Portion of trial testing resistance of rootstocks to replant disease. Row in foreground is planted in non-fumigated soil; row in background is planted in pre-plant fumigated soil. Rootstocks planted April 2011, photo taken August 2011.

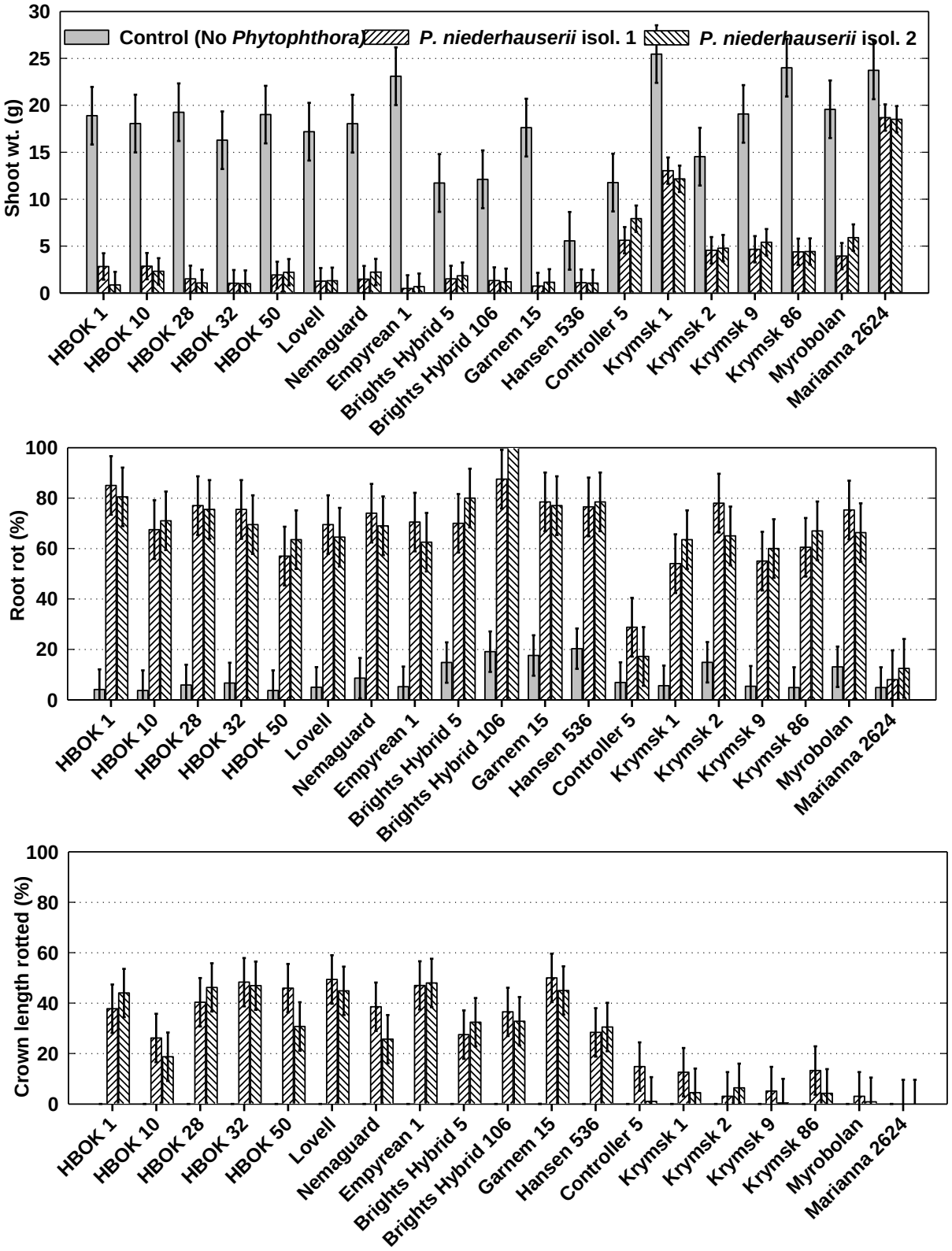

Figure 5. Relative resistance of rootstocks to two isolates of *Phytophthora niederhauserii* in greenhouse experiment.

Research Effort Recent Publications:

Browne, G., Lampinen, B., Doll, D., Holtz, B., Upadhyaya, S., Schmidt, L., Wang, D., Fennimore, S., Hanson, B., Gao, S., Klonsky, K., and Johnson, S. 2010. Integrated pre-plant alternatives to methyl bromide for almonds and other stone fruits. Pp. 28-1 to 28-4, Proceedings, Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, available online: http://mbao.org/2010/Proceedings/028BrowneG.pdf

Bhat, R. G., Schmidt, L. S., and Browne, G. T. 2011. Quantification of *Cylindrocarpon macrodidymum* in roots of almond and peach trees from orchards affected by Prunus replant disease. Phytopathology 101: S15

Schmidt, L. S., Bhat, R. G., and Browne, G. T. August 2011. Characterization of Cylindrocarpon populations associated with replant disease of almond and peach. Phytopathology 101: S15

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Mazzola, M. 1998. Elucidation of the microbial complex having a causal role in the development of apple replant disease in Washington. Phytopathology 88:930-938.

Petit, E., and Gubler, W. D. 2007. First Report of *Cylindrocarpon liriodendri c*ausing black foot disease of grapevine in California. Plant Disease 91 (8):1060-1060.