Developing Improved Strategies for Management of Replant Problems

Project No.: **12-PATH1-Browne**

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Objectives:

1) Determine the causes of replant disease (RD).
2) Support the development of strategic approaches for management of RD and other soilborne diseases, by:
   a) Identifying rootstocks with genetic resistance or tolerance to:
      i) RD
      ii) *Phytophthora* (previous funding is being used to complete 2013 activities)
   b) Developing greenhouse bioassays to:
      i) Predict risk of RD in commercial orchards.
      ii) Facilitate broad examination of RD causes.
   c) Quantifying impacts of orchard replacement scheduling, intensive pre-plant soil ripping, and pre-plant soil fumigation on RD expression.

Interpretive Summary:

The overall project goal is to improve almond orchard replacement strategies by maximizing their economy and minimizing their dependence on soil fumigation. Specific project objectives are to: 1) determine causes of almond replant disease (RD) (a widespread yet poorly understood soilborne complex that suppresses growth and cumulative yields in successive plantings of *Prunus species*, even in the absence of plant parasitic nematodes), and 2) support strategic approaches to orchard replacement by: a) identifying rootstocks with resistance or tolerance to RD and *Phytophthora* species; b) developing a predictive bioassay to identify and assess RD risk factors in orchard soils; and c) examining the value of pre-plant cultural practices that may reduce dependence on soil fumigation for control of RD.

Under **objective 1**, our 2013 progress includes: 1) determining pathogenicity of several RD-associated *Pythium* species on Nemaguard rootstock (in three repeated trials, *P. ultimum*, *P. intermedium*, *P. irregulare,* and *P. spinosum*, which all were associated with RD in our earlier work, repeatedly caused shoot and root system stunting on the rootstock, whereas *P. pareocandrum*, *P. vexans* and two unidentified *Pythium* species relatively little or no disease); 2) establishing a trial to assess whether RD severity is affected by interactions among *Pythium* species, *Cylindrocarpon* species (both often associated with RD), and *Trichoderma* species (often associated with roots of healthy almond trees replanted in fumigated soils); and 3) completing culture-independent identification of microorganisms associated with RD in a field rootstock trial. Under **objective 2**, our 2013 progress includes: 1) near completion of a repeat field trial evaluating resistance of 22 commercially available almond and stone fruit rootstocks to RD (no rootstocks were RD-immune, but several [e.g., Empyrean 1, Hansen 536, Bright's Hybrids 5 and 106, Garnem] were tolerant); 2) completion of a repeat greenhouse trial evaluating the same 22 rootstocks for resistance to *Phytophthora niederhauseri* (a "new" pathogen we find killing almond in CA; rootstock genotypes including plum parentage were resistant, while others were susceptible); 3) establishment of a trial evaluating resistance 13 novel rootstock hybrids (genotypes contributed by the National Clonal Germplasm Repository; USDA-ARS, Davis) to *P. niederhauseri*; and 4) initiating a replant trial at Kearney Ag Center (KAC) which will be used for continued development of our RD bioassay and to evaluate remediation of RD via short-term fallowing, pre-plant soil ripping, and anaerobic soil disinfestation (ASD).

The demonstrations of pathogenicity among several RD-associated *Pythium* species on Nemaguard rootstock indicate that several of them contribute to the disease complex and give us a "target" for new RD remediation strategies. For example, mefenoxam and phosphonates, both systemic in plants, have controlled some diseases caused by *Phytophthora* and *Pythium* species and therefore may help to control *Pythium*-induced RD.

The findings of RD tolerance in Empyrean 1 rootstock (peach) and Hansen 536, Bright's Hybrid 5 and Bright's Hybrid 106, Garnem (all peach x almond hybrids) suggest these

rootstocks will be suppressed less than Nemaguard or Lovell rootstocks by RD in non-fumigated replant situations. Nevertheless, all rootstock traits, not just tolerance to RD, should be considered carefully by growers selecting rootstock(s). For example, our evaluations and observations indicate that growers will need to use care with peach x almond hybrids where *Phytophthora* is present, and at least some peach almond hybrids are susceptible to the ring nematode/bacterial canker complex in sand.

We will report on the impacts of non-fumigant and fumigant treatments being tested for management of RD in the KAC replant trial as the results become available, starting in 2014.

Growers considering replanting almonds in 2014 or 2015 and interested in having their soil tested with an experimental bioassay for RD prediction are encouraged to email the principal investigator (email address: gtbrowne@ucdavis.edu). About 15 gallons of soil are used for the assay.

Materials and Methods:

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

Testing pathogenicity of microorganisms associated with RD. In 2012 and earlier years, we had detected elevated incidence of *Pythium* spp. in roots from RD-affected trees in non-fumigated plots of orchard replant trials, compared to their incidence in roots from healthy trees in adjacent pre-plant fumigated plots. Similarly, in greenhouse trials with Prunus replant soil, we found higher incidence of *Pythium* spp. in roots from PRD-affected peach seedlings grown in non-treated portions of the soil, compared to the incidence in roots from healthy plants in fumigated or pasteurized portions of the soil. However, to resolve the causal role of these *Pythium* species in RD, we needed to specifically identify them and conclusively assess their pathogenicity. These latter steps were a main focus in 2013.

We identified the *Pythium* species by rDNA sequencing (of diagnostic ITS1 and 2 regions) and BLAST (basic local alignment search tool) matching (i.e., conducting computer searches in an online database to match our isolate's sequences with those of known standards). We identified *P. intermedium* (1 isolate), *P. irregulare* (5), *P. pareocandrum* (7), *P. spinosum* (5), *P. ultimum* (9), and *P. vexans* (4), and two unknown but distinctive ribotypes of *Pythium* sp. (4 and 8 isolates).

Pathogenicity of 41 of the isolates identified by sequencing was tested in three greenhouse trials; 2 to 3 week-old Nemaguard peach rootstock seedlings were transplanted into UC mix potting soil artificially infested with isolates of *Pythium* on V8 juice-vermiculite-oat substrate (5 to 10% soil vol.). Controls received sterile substrate. Treatments were randomized in complete blocks, with 5 to 10 seedlings per treatment. Three months after transplanting, pathogenicity was assessed according to top and root fresh weights and root cortex necrosis. Culture based isolations and DNA-based

identifications were used to confirm that inoculants were associated with disease and controls remained free of the inoculants.

Culture-independent identification of organisms associated with RD in rootstock trial. Culture-independent methods can provide critical insights into causes of a disease by revealing the presence of microorganisms that are not easily detected in culture media. In 2013 we used culture-independent cloning and sequencing of rDNA fragments to identify microorganisms associated with RD in Nemaguard and Lovell trees in one of our rootstock trials. The trial was planted in April 2011 to test resistance to RD in 22 rootstocks for almond and stone fruits; each rootstock was planted in replicate fumigated and non-fumigated plots (see details in Comprehensive Report to the Almond Board of California [ABC], Browne et al., 2011). By late summer, severe RD developed in Nemaguard, Lovell, and several other rootstocks in the non-fumigated plots, while all rootstocks in the fumigated plots remained healthy. Roots (<1 mm diameter) were sampled from four replicate trees of Nemaguard and Lovell in each soil treatment in late summer 2011 and preserved at -80 °C for subsequent culture-independent characterizations. DNA was purified from 150 mg of each root sample using a modified CTAB method, and PCR primers were used to amplify diagnostic rDNA fragments from bacteria, stramenopiles, and fungi as described previously (details in the Comprehensive Report to ABC, Browne et al., 2010). The amplicons from each primer set were cloned (Promega P-Gem T Easy Vector System; Promega Corp, Madison, WI) and sequenced (UC DNA Sequencing Facility). The source organism for each clone was identified to the extent possible using BLAST searches in NCBI (National Center for Biotechnology Information) sequence databases. Within each microorganism grouping (i.e., bacteria, fungi, stramenopiles), identified sequences appearing at least four times were tabulated according to their pre-plant soil treatment (i.e., non-fumigated control or pre-plant fumigated with Telone C35). Microorganisms with counts proportionally shifted towards the control (i.e., with significantly more than 0.5 [i.e., 50%] of the counts occurring in preplant control treatment [i.e., the RD-affected treatment]) were considered to be positively associated with RD, while microorganisms with counts proportionally shifted away from the control (i.e., with less than 0.5 of counts occurring in the control) were considered to be negatively associated with RD (and positively associated with soil fumigation/root and tree health).

Objective 2. Support development of new management strategies for RD and other soilborne diseases.

Field evaluations of genetic resistance to RD. A repeat of the rootstock trial planted in 2011 (report to ABC, Browne et al., 2012) was planted in May 2012 at the USDA station near Parlier, CA. The rootstocks included in the 2012 planting (**Table 1**) were the same as used in 2011. The test site had been cleared from almonds on Nemaguard rootstock in summer 2011. In Oct 2011, 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 May 2012. Resistance was assessed in Nov 2012 by determining NF/F (non-fumigated/fumigated) 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 accumulated in fumigated plots.

Evaluations of genetic resistance to *Phytophthora*. The rootstock genotypes tested for resistance to RD at Parlier were also tested for resistance to *Phytophthora niederhauseri* in our greenhouse at UC Davis. In spring, actively growing plants of each rootstock were transplanted into fresh 600-ml pots filled with UC potting mix and maintained in a greenhouse. Three repeated evaluations of resistance are being completed this summer and fall. In each trial, 30 ml of V8 juice-oat substrate colonized by isolates of *P. niederhauseri* is added to each inoculated pot, and 30 ml of sterile substrate is added to each control pot. In each experiment, there are six replicate inoculated plants and six non-inoculated controls per rootstock. All plants are subjected to biweekly 48-hour periods of flooding and watered and fertilized normally otherwise. Two months after inoculation all plants are evaluated for susceptibility to the pathogen by washing their root systems free from the potting soil, determining shoot and root system fresh weights, and visually rating severity of root and crown rot. Isolations were used to confirm that inoculated plants have been exposed to the pathogen and that control plants remain free from the pathogen. Below we present results from one of the trials. Additional trials are underway and include evaluations of resistance of novel rootstock genotypes from USDA-ARS; results from the latter trials will be reported in the future.

Field evaluation of cultural approaches for managing RD. A nectarine orchard on Nemaguard rootstock at the Kearney Agricultural Center (KAC) was removed and is being used for two almond replant experiments. The trials are designed to assess effects of various cultural and fumigant treatments on RD severity (see **Table 2** for treatment details). One of the cultural treatments, anaerobic soil disinfestation (ASD), is new to the almond industry but has shown promise in California strawberries; we are adapting the approach to orchard replant applications. All plots of both trials will be replanted by February 2014, and it is anticipated that early results of the trials will be available by fall 2014. The KAC trials also will provide soil for use in development of our bioassay for RD prediction.

Results and Discussion:

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

Testing pathogenicity of microorganisms associated with RD. Among 41 isolates tested for pathogenicity in the greenhouse, pathogenicity was repeatedly demonstrated on Nemaguard peach by *Pythium intermedium*, *P. irregulare*, *P. spinosum*, and *P. ultimum* (**Figure 1**). As a group, isolates of *P. ultimum* were the most aggressive. *Pythium pareocandrum*, *P. vexans*, and two unknown but distinctive ribotypes of *Pythium* sp. caused little or no disease (**Figure 1**). All pathogenic species were re-isolated from roots of their inoculated plants, while *Pythium* was undetectable in the controls.

The pathogenicity data, combined with previous data associating the pathogenic *Pythium* species with incidence of RD in field trials, implicate *P. intermedium*, *P. irregulare*, *P. spinosum*, and *P. ultimum* as contributors to almond RD in California. Our previous work (reports to ABC, Browne et al., 2010-2012) identified *Cylindrocarpon macrodidymum* as another contributor to the complex. We will continue to assess these and other microorganisms' roles and interactions in the complex. In August, we established a trial to assess interactions among *Pythium* species, *Cylindrocarpon* species (we have shown both genera contribute to RD), and *Trichoderma* species (we suspect it suppresses RD in fumigated soils). Data from this trial should be available in our next report.

The implication of several *Pythium* species as RD contributors suggests that mefenoxam and phosphonate treatments might be helpful in managing a portion of the RD complex. Mefenoxam and phosphonate treatments can become systemic in plants and suppress some diseases caused by *Phytophthora* and *Pythium* species. Additional field trials would be needed to test efficacy of the treatments against RD.

We are testing qPCR primers for the species of *Pythium* that were pathogenic in our trials. The primers would allow rapid quantification in roots (and perhaps in soil), with applications in etiology (and possibly RD prediction).

Culture-independent identification of organisms associated with RD in rootstock trial. Among the counts of fungal rDNA sequences amplified from Nemaguard and Lovell root samples in the rootstock trial, several were skewed towards occurrence in RD-affected plants, including a sequence with similarity to *Phaeonectriella lignicola* and several uncultured fungi with affinity to diverse fungal groups (**Table 3**). We have detected the rDNA sequence with similarity to *P. lignicola* in association with RD in several previous culture independent assays (report to ABC, Browne et al., 2011). Conversely, amplified rDNA sequences of several other fungal groups were skewed towards occurrence in root samples from the healthy rootstocks (e.g., this was true for "Agaricaceae.sp.", "Sordariales sp.", and "Uncultured.Auriculariales", **Table 3**).

In comparison to fungal rDNA sequences, less of the bacterial rDNA sequence groups were skewed in occurrence towards or away from RD in the control treatment (**Table 4**). Only a "Psuedomonas.sp" group was skewed towards RD incidence, and only a small "Pseudoxanthomonas.sp" of three sequences was skewed towards healthy roots from the fumigated plots. Similarly, RD-associated shifts in stramenopile sequences occurred only in a sequence identified from *Pythium vexans*, while a sequence from a *Pythium* sp. was only found in healthy roots (**Table 5**).

In the coming months we will work to refine the identities of the rDNA sequences that are positively or negatively associated with RD and look for their occurrence in previous culture-independent data sets. The fungal sequence with affinity to *P. lignicola* is of particular interest. Our future culture-independent work will focus on deep sequencing, which has become more economical than before and may provide more in-depth views into RD-mediating and RD-suppressing microbial communities.

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

Field evaluations of genetic resistance to RD. Overall, RD severity in the repeat rootstock trial (2012/13) has been less than in the first trial (2011/12), but NF/F stem diameter increase rankings were similar between the experiments. As in our previous rootstock trial planted in 2011, all of the 22 tested rootstocks grew less in non-fumigated soil than in fumigated soil, but the severity of the growth reductions varied by rootstock (**Figure 2, A and B**). The rootstock–soil treatment interaction was highly significant ($P < 0.0001$). Most rootstocks with only peach parentage were relatively susceptible to the PRD complex in non-fumigated soil (**Figure 2B**). 'Empyrean 1,' also a peach, was the least susceptible of rootstocks with this parentage. The hybrid rootstocks that combined peach and almond parentage were less susceptible than most peaches. Rootstocks with plum parentage, including 'Controller 5,' 'Krymsk' clones 1, 2, 9 and 86, 'Marianna 2624,' and 'Myrobalan,' varied in susceptibility to the complex in non-fumigated soil.

Evaluations of genetic resistance to *Phytophthora*. 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 3**). Some of the peach x almond selections, as well as the Empyrean 1 and PAC 9908-02 rootstocks were relatively susceptible to crown rot. All control plants remained free from the inoculant, and *P. niederhauseri* was recovered from plants of the inoculated treatment.

The relatively low susceptibility of Krymsk 86 to *P. niederhauseri* is fortunate considering its recent popularity in the upper Sacramento Valley. We will report results from further repeat tests of these 22 rootstocks and from tests of new USDA rootstock genotypes as the results become available. 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 almond orchards.

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.

Research Effort Recent Publications:

Browne, GT., Lampinen, B.D., Holtz, B.A., Doll, D.A., Upadhyaya, S.K., Schmidt, L.S., Bhat, R.G., Udompetaikul, V., Coates, R.W., Hanson, B.D., Klonsky, K.M., Gao, S., Wang, D. , Gillis, M., Gerik J., Johnson, R.S. 2013. Managing the almond and stone fruit replant disease complex with less soil fumigant. *California Agriculture* 67 (3) 128-138. Online:http://californiaagriculture.ucanr.edu/landingpage.cfm?article=ca.v067n03p128&fulltext=yes doi: 10.3733/ca.v067n03p128

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. 2012. Integrated pre-plant alternatives to methyl bromide for almonds and other stone fruits. pp. 19-1 to 19-2, Proceedings, Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, Orlando. available online: http://mbao.org/2012/Proceedings/19BrowneG.pdf

Browne, G.T., Beede, R.H., and Schmidt, L.S. 2012. Irrigation Water Relation to the Health of Deciduous Fruit and Nut Crops. 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 Prunus replant disease complex.

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

[a]Al=almond, Ap=apricot, Pe=peach and nectarine, Pl=plum, Pr=prune, and parentheses indicate that not all varieties of the crop are compatible with the rootstock.

Table 2. Status of almond replant trial at Kearney Agricultural Center.

Experiment	Treatment	Previous orchard removal	Sudan hybrid June-Aug 2013	Soil ripping depth (ft) Sept. 2013	Additional pre-plant treatment
1	1	Sept 2013	No	1.5-2.0	None
1	2	Sept 2013	No	1.5-2.0	Fumigation, Sept/Oct
1	3	Sept 2013	No	1.5-2.0	Fumigation, Nov/Dec
1	4	April 2013	Yes	1.5-2.0	None
1	5	April 2013	Yes	1.5-2.0	Fumigation, Sept/Oct
1	6	April 2013	Yes	1.5-2.0	Anaerobic soil disinfestation
2	1	April 2013	No	1.5-2.0	None
2	2	April 2013	No	4.0-4.5	None
2	3	April 2013	No	1.5-2.0	Fumigation, Sept/Oct
2	4	April 2013	No	4.0-4.5	Fumigation, Sept/Oct
2	5	April 2013	No	1.5-2.0	Anaerobic soil disinfestation
2	6	April 2013	No	4.0-4.5	Anaerobic soil disinfestation

Table 3. Identities and counts of microorganism rDNA sequences amplified by fungal PCR primers as a function of pre-plant soil treatment in 2011 rootstock trial.

	Sequence count[b]			Proportion in control treatment		
Organism sequence identity[a]	Telone C35 (healthy)	Non-fumigated Control (with RD)	Total	Mean	Lower 95% confidence limit	Upper 95% confidence limit
Agaricaceae.sp.	26	7	33	0.21	0.09	0.39
Ceratobasidium.sp.	3	7	10	0.70	0.35	0.93
Cylindrocarpon.sp.	7	2	9	0.22	0.03	0.60
Daedaleopsis.rubescens	6	2	8	0.25	0.03	0.65
Exophiala.sp.	2	3	5	0.60	0.15	0.95
Fungal.endophyte.sp.	5	1	6	0.17	0.00	0.64
Fungal.sp.	4	5	9	0.56	0.21	0.86
Fusarium.solani	2	2	4	0.50	0.07	0.93
Glomus.intraradices	13	11	24	0.46	0.26	0.67
Glomus.sp.	2	4	6	0.67	0.22	0.96
Magnaporthe.poae	3	0	3	0.00	0.00	0.63
Phaeonectriella.lignicola	5	28	33	0.85	0.68	0.95
Pleosporales.sp.	3	0	3	0.00	0.00	0.63
Podospora.communis	4	3	7	0.43	0.10	0.82
Rhizophagus.intraradices	0	3	3	1.00	0.37	1.00
Rhizophagus.irregularis	7	4	11	0.36	0.11	0.69
Sordariales.sp.	12	0	12	0.00	0.00	0.22
Stemphylium.sp.	0	3	3	1.00	0.37	1.00
Uncultured.Ascomycota	4	6	10	0.60	0.26	0.88
Uncultured.Auriculariales	35	5	40	0.13	0.04	0.27
Uncultured.Basidiomycota	64	62	126	0.49	0.40	0.58
Uncultured.Diaporthales	1	2	3	0.67	0.09	0.99
Uncultured.endophytic.fungus	10	6	16	0.38	0.15	0.65
Uncultured.fungus	12	38	50	0.76	0.62	0.87
Uncultured.Glomeraceae	1	14	15	0.93	0.68	1.00
Uncultured.Glomeromycota	5	6	11	0.55	0.23	0.83
Uncultured.Glomus	19	39	58	0.67	0.54	0.79
Uncultured.Halosphaeriales	3	12	15	0.80	0.52	0.96
Uncultured.Rhizophagus	3	5	8	0.63	0.25	0.92
Uncultured.fungus	17	13	30	0.43	0.26	0.63
Uncultured.Sebacinaceae	3	4	7	0.57	0.18	0.90
Uncultured.soil.basidiomycete	1	12	13	0.92	0.64	1.00
Uncultured.soil.fungus	13	20	33	0.61	0.42	0.77
Uncultured.Sordariales	7	9	16	0.56	0.30	0.80

[a]These identities are subject to revision.
[b]Counts were combined for Nemaguard and Lovell rootstocks.

Table 4. Identities and counts of organism rDNA sequences amplified by bacterial PCR primers as a function of pre-plant soil treatment in 2011 rootstock trial.

	Sequence count[b]			Proportion in control treatment		
Organism sequence identity[a]	Telone C35 (healthy)	Non-fumigated Control (with RD)	Total	Mean	Lower 95% confidence limit	Upper 95% confidence limit
Actinoplanes.globisporus	5	4	9	0.44	0.14	0.79
Agrobacterium.tumefaciens	3	2	5	0.40	0.05	0.85
Caulobacter.sp.	0	4	4	1.00	0.47	1.00
Cryptosporangium.japonicum	1	3	4	0.75	0.19	0.99
Devosia.sp.	1	2	3	0.67	0.09	0.99
Duganella.nigrescens	0	3	3	1.00	0.37	1.00
Enterobacter.ludwigii	2	2	4	0.50	0.07	0.93
Flavobacterium.sp.	2	7	9	0.78	0.40	0.97
Methylibium.sp.	2	1	3	0.33	0.01	0.91
Pseudomonas.sp.	11	2	13	0.82	0.57	0.96
Pseudoxanthomonas.sp.	3	0	3	0.15	0.02	0.45
Rhizobium.sp.	7	8	15	0.00	0.00	0.63
Rhizobium.sullae	3	1	4	0.53	0.27	0.79
Streptomyces.diastatochromogenes	30	23	53	0.25	0.01	0.81
Streptomyces.scabies	2	5	7	0.43	0.30	0.58
Streptomyces.sp.	18	15	33	0.71	0.29	0.96
Uncultured.alpha	1	2	3	0.46	0.28	0.64
Uncultured.bacterium	179	230	409	0.67	0.09	0.99
Uncultured.Chloroflexi.bacterium	1	2	3	0.56	0.51	0.61
Uncultured.gamma.proteobacterium	2	4	6	0.67	0.09	0.99
Uncultured.soil.bacterium	5	8	13	0.67	0.22	0.96
Uncultured.Variovorax	4	0	4	0.62	0.32	0.86
Variovorax.paradoxus	5	0	5	0.00	0.00	0.53
Variovorax.sp.	3	3	6	0.00	0.00	0.45

[a] These identities are subject to revision

[b] Counts were combined for Nemaguard and Lovell rootstocks.

Table 5. Identities and counts of organism rDNA sequences amplified by stramenopile PCR primers as a function of pre-plant soil treatment in 2011 rootstock trial.

	Sequence count[b]			Proportion in control treatment		
Organism sequence identity[a]	Telone C35 (healthy)	Non-fumigated Control (with RD)	Total	Mean	Lower 95% confidence limit	Upper 95% confidence limit
Aplanochytrium.sp.	9	34	43	0.79	0.64	0.90
Eimeriidae	2	3	5	0.60	0.15	0.95
Glomus.macrocarpum	0	7	7	1.00	0.65	1.00
Glomus.sp.	9	5	14	0.36	0.13	0.65
Labyrinthula.sp.	9	11	20	0.55	0.32	0.77
Nais.inornata	0	4	4	1.00	0.47	1.00
Pythium.sp.	5	0	5	0.00	0.00	0.45
Pythium.splendens	2	3	5	0.60	0.15	0.95
Pythium.vexans	8	16	24	0.67	0.45	0.84
Sorangium.cellulosum	3	0	3	0.00	0.00	0.63
Thraustochytriidae.sp.	46	1	47	0.02	0.00	0.11
Uncultured.chrysophyte	2	1	3	0.33	0.01	0.91
Uncultured.eukaryote	16	11	27	0.41	0.22	0.61
Uncultured.stramenopile	10	5	15	0.33	0.12	0.62

[a] These identities are subject to revision.
[b] Counts were combined for Nemaguard and Lovell rootstocks.

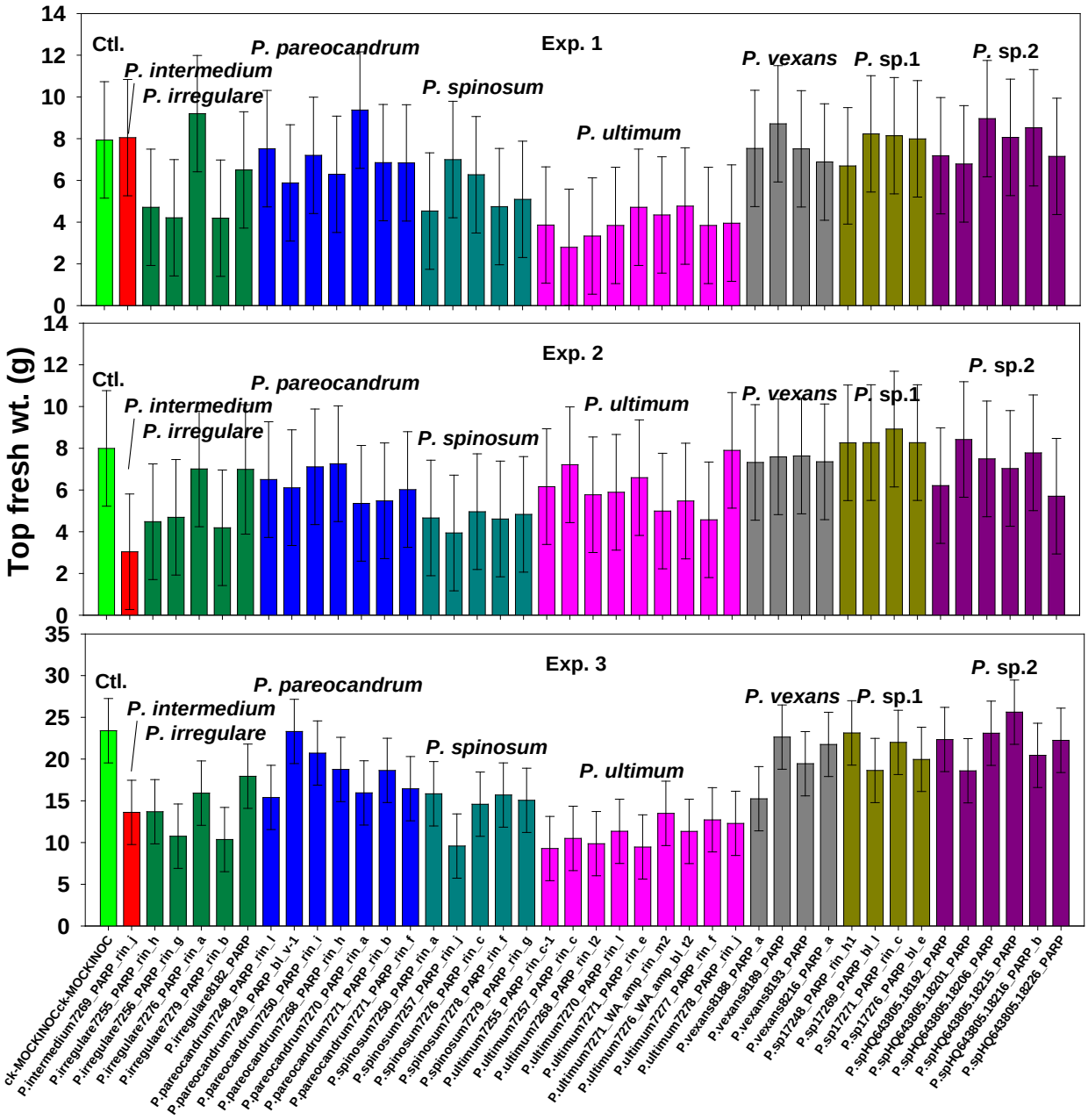

Figure 1. Pathogenicity of 41 isolates of *Pythium* spp. in repeated greenhouse trials. Vertical bars are 95% confidence intervals.

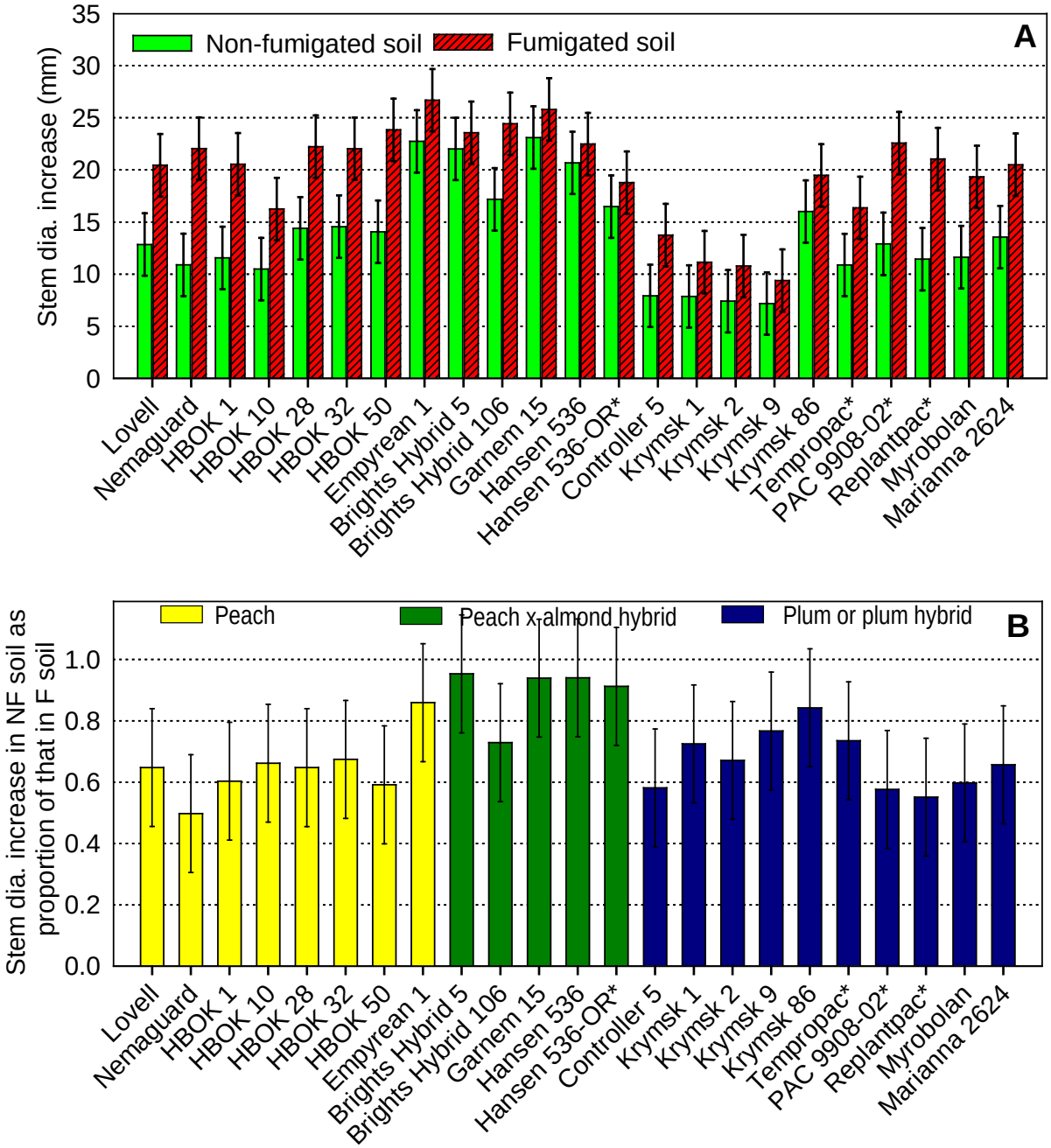

Figure 2. Relative resistance of 22 rootstocks for almond and stone fruits to the Prunus replant disease (RD) complex. The resistance was assessed according to the degree to which rootstocks grew as well in non-fumigated soil as in pre-plant fumigated soil. The trial was conducted at a site subject to RD but not plant parasitic nematode infestation. Stem diameter growth increases measured from May 2012-Nov 2012. **A**) actual stem diameter increases; **B**) stem diameter increases in non-fumigated soil divided by stem diameter increases in fumigated soil. Vertical bars are 95% confidence intervals.

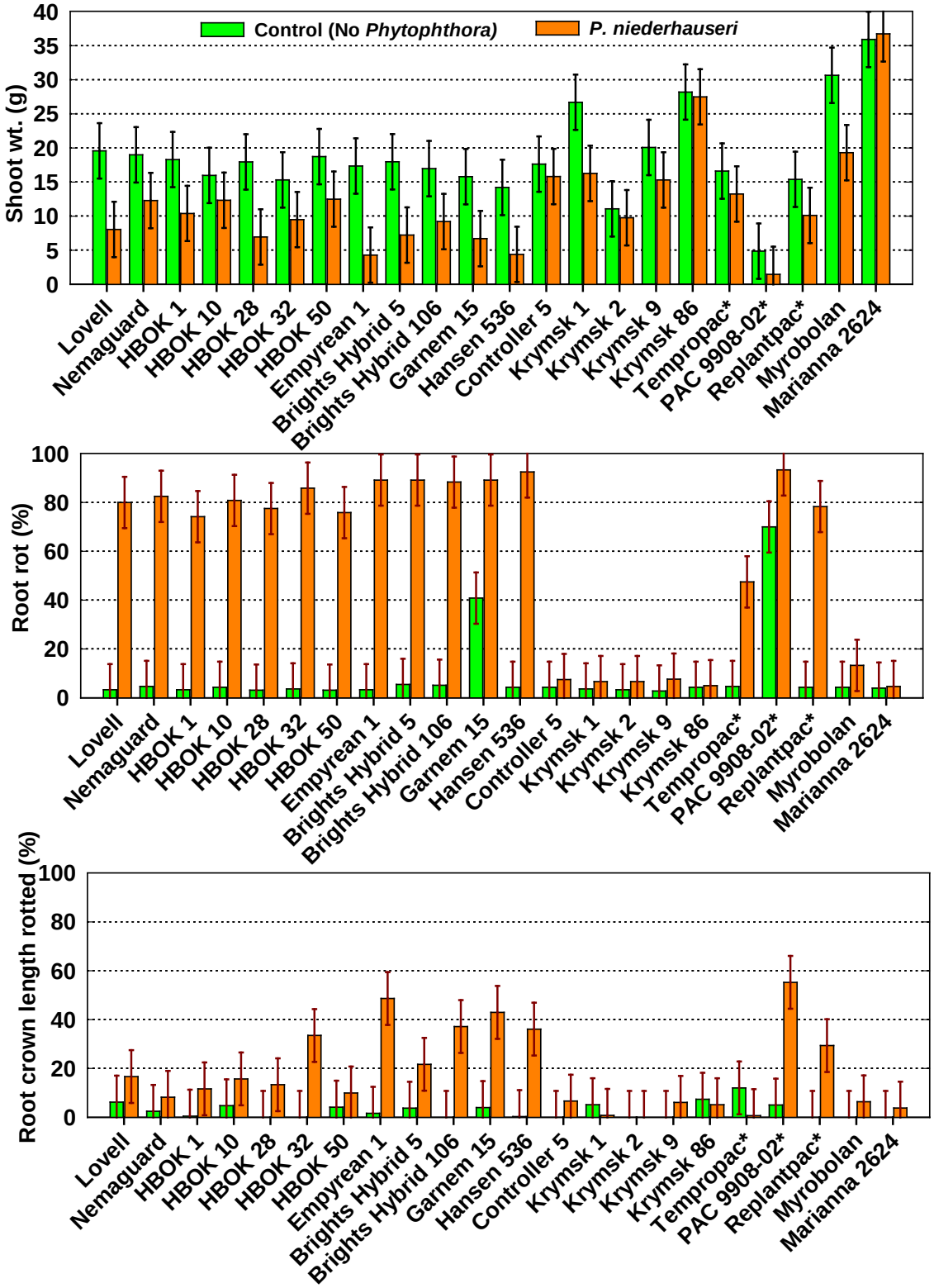

Figure 3. Relative resistance of 22 rootstocks for almond and stone fruits to *Phytophthora niederhauseri*, an important pathogen of almond in California.