Diagnostics and Non-Fumigant Management Approaches for Prunus Replant Disease

Project No.: **17-PATH1-Browne**

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Objectives:

1. Develop molecular diagnostics for characterization and prediction and of PRD.
2. Optimize ASD for affordability and ease of commercial implementation.

Interpretive Summary:

We continued investigating causes, prediction approaches, and control measures for Prunus replant disease (PRD). PRD is a soilborne complex that, in some soils, suppresses growth and productivity of successive plantings of stone fruit and nut orchards. Towards completing objective 1, we: (i) completed a study of root microbial

communities from a previous greenhouse peach seedling bioassay, (ii) completed "draft" examinations of root and soil microbial communities from almond replant trials planted in early 2017, and (iii) collected and initiated microbial analyses of root and soil samples from plots in two almond replant experiments planted in January 2018 at CSUF. The greenhouse bioassay (i) assessed PRD potential among different by soils by determining whether peach seedling growth was suppressed in non-treated soil portions, compared to growth in fumigated and pasteurized soil portions. Our analyses of bioassay root communities resolved that *Streptomyces scabiei*, *S. lincolnensis*, *Steroidobacter denitrificans*, *Steroidobacter* sp., and *Povalibacter uvarum* were enriched in roots from PRD-inducing soils, and their relative abundances correlated negatively with peach seedling biomass. Many fungi and oomycetes also were enriched in PRD-inducing soils, but their relative abundances did not correlate significantly with peach seedling biomass. To determine whether the bacterial associations were causal or just coincidental, we will focus on culturing the bacterial suspects and testing their pathogenicity. It is still possible that fungi and oomycetes that we have found to be pathogenic on peach play a role in PRD, but our recent findings suggest important contributions from bacteria. Among field trials, we collected and processed soil and root samples collected from trials planted in 2017 (ii), including: Shafter 1 (WO3371) and Shafter 2 (WO3381), and the Parlier-KARE trial; we extracted total DNA, and, as described above, amplified rRNA gene fragments from bacteria, fungi, and oomycetes. We have completed draft bioinformatics and statistical analyses for most of these samples. In the CSUF field trials (iii), we sampled soil from all treatments before planting, then, each month from April through July, we collected 96 root samples and 72 soil samples representing 18 preplant treatments (784 samples total). Total DNA was extracted from the samples, and multiple sets of PCR primers are being used to amplify diagnostic rRNA gene fragments from the DNA of bacteria, fungi, and oomycetes. As for the bioassay, bioinformatics and statistical approaches will be used to assess treatment effects on microbial community structure and to relate abundances of specific microorganisms to tree growth.

For objective 2, optimizing ASD, we measured tree responses (trunk cross sectional growth increases) to ASD treatments in three trials planted in 2017 and established two new ASD trials planted at CSUF in 2018. The 2017 trials were described previously (Browne et al., Annual Report to Almond Board of California [ABC], 2017). The CSUF trials are testing alternative ASD carbon sources and ASD application methods. The carbon sources included ground rice bran, almond hull + shell mixture, and tomato pomace. We also tested whole-orchard-recycling (WOR) treatments in various combinations with ground almond hull and shell mixture. Normally, for ASD, application of the carbon source(s) is followed by covering the soil with tarp and irrigating it to saturate the soil profile with water and maintain the soil moisture at or above field capacity for 4 to 6 weeks. However, we tested rice bran and almond hull + shell substrates in three ways: (i) solely as amendments (i.e., without added water or tarp), (ii) with water only and (iii) with water and tarp. Based on tree responses to date in the CSUF trial, it appears that ground almond hull + shell has good potential as an alternative, low-cost ASD carbon source. Both rice bran and almond hull + shell mixture

improved tree growth significantly, compared to the controls, and they both performed best, nearly as well as preplant fumigation, with water and tarp.

Materials and Methods:

Objective 1. To resolve PRD etiology and targets for its prediction, we continued examining the soil and root microbial communities that induce the disease. We (i) completed characterizations root microbial communities from a peach seedling bioassay involving multiple replant soils tested in a greenhouse (for bioassay background, see Browne et al., Annual Reports to the Almond Board of California [ABC] from 2015-2017); (ii) completed "draft" examinations of root and soil microbial communities from almond replant trials planted in early 2017; and (iii) processed samples for microbial analyses in roots and soil from plots replanted in January 2018 at CSUF.

For examinations of root microbial communities in the greenhouse bioassay (i), we included 10 of 26 bioassayed soils. Among the 10 included soils were eight that had recently hosted *Prunus* sp. and two that had been used for *Vitis* sp. (**Table 1**). Three of the soils, numbered 7, 8, and 9 (**Table 1**) were from different plots in a single field trial at KARE, Parlier and had received field treatments of: a control, fumigation (1,3-D 340 lb/a + chloropicrin 200 lb/a; shank applied), or ASD with rice bran (9 t/a), respectively. After collection, each soil was apportioned to three treatments: a control, fumigation with chloropicrin, and pasteurization with steam. After treatment, the soils were potted and planted to Nemaguard peach seedlings in a greenhouse bioassay for PRD. Ten weeks after planting, we used peach seedling fresh weight suppression in the control treatment, compared to fresh weights in fumigated and pasteurized treatments, to assess PRD-inducing capacity of soil. Also, we immediately froze the peach root systems after washing them free from soil to preserve them for subsequent examinations of their surface and internal microbial communities. Total DNA was extracted from the roots, and we used PCR to amplify rRNA gene region fragments from microbial communities; the primer sets targeted: 16S rRNA gene regions v4 and v5-v7 of bacteria, ITS1 and ITS2 of fungi, and ITS1 and ITS2 of oomycetes. The amplicons were tracked for sample (and treatment) identity by attaching sample-unique DNA barcodes to them. We filtered the sequences for quality and used the DADA2 algorithm (1) for resolving amplicon sequence variants (ASVs). Online databases of the National Center for Biotechnology Information and Barcode of Life Datasystem were queried to match the amplicon DNA sequences to their source microorganisms. We assessed bacterial, fungal, and oomycete libraries, in turn, for richness (number and diversity of taxa) and composition (relative abundance of individual taxa) as a function of soil source and bioassay treatment. We also examined root community richness and composition as a function of whether or not the control (i.e., non-treated) soil had induced PRD in the greenhouse bioassay. For individual taxa (ASVs) that represented $\geq$ 1% of the population, we tested for correlations between relative abundance of the ASV and final biomass in peach seedlings. Of special interest were ASVs that had high relative abundance in PRD-inducing soils and had relative abundances that correlated positively with peach seedling growth suppression.

For soil and root samples collected from trials planted in 2017 (ii), including: Shafter 1 (WO3371) and Shafter 2 (WO3381), and the Parlier-KARE trial, we extracted total DNA, and, as described above, amplified rRNA gene fragments from bacteria, fungi, and oomycetes. We have completed draft bioinformatics and statistical analyses for most of these samples and are revising them as needed.

In both CSUF trials (iii), we sampled soil from all treatments before planting. After planting, each month from April through July, we collected 96 root samples and 72 soil samples representing 18 preplant treatments (784 samples total) (**Tables 2, 3**). Total DNA was extracted from the samples, and multiple sets of PCR primers are being used to amplify diagnostic rRNA gene fragments from the DNA of bacteria, fungi, and oomycetes. The amplicons will be sequenced and subjected to bioinformatics and statistical analyses as described for bioassay samples and samples from trials planted in 2017.

Objective 2**.** To assess treatments designed to help optimize ASD treatments for efficacy and affordability in almond replant settings, we monitored first-year tree performance in three previously established ASD trials (one planted in 2017 near Parlier at KARE that is testing nine different ASD carbon sources; two planted in 2017 near Shafter, testing ASD treatment components of rice bran substrate, irrigation, and tarp). Also, we established two new experiments at CSUF. The CSUF trials include treatments with different ASD application methods and carbon sources (**Tables 2, 3**). Normally, for ASD, soil incorporation of the carbon source(s) is followed by covering the soil with tarp and irrigating it to water saturate the soil profile and maintaining soil moisture at or above field capacity for 4 to 6 weeks. Experiment 1 at CSUF (**Table 2**) tested rice bran and almond hull + shell substrates in three ways: solely as amendments (i.e., without added water or tarp), with water only, and with water and tarp (**Table 2**). Experiment 2 (**Table 3**) tested carbon sources of ground rice bran, almond hull + shell mixture, tomato pomace, and whole-orchard-recycling (WOR) chips, and some of the treatments with almond hull + shell mixture involved combinations with ammonium sulfate.

Results and Discussion:

Objective 1. In the bioassay, PRD was induced in soils 1, 2, 4, 7, 9, and 10, all of which had hosted almond or peach trees on a peach rootstock (**Figure 1, Table 1**), but not in soils 3, which had also hosted almond on peach, or soils 5 and 6, which were planted to grape, or soil 8, which had hosted peach on Nemaguard, but was fumigated before collection. Fumigation or pasteurization after soil collection but before the bioassay afforded relatively good growth of peach seedlings in all soils (**Figure 1**).

The fumigation and pasteurization treatments of the bioassay significantly reduced richness (i.e., the number of taxa observed) of bacterial and fungal root communities (data not shown, $P<0.05$). Also, PCR amplification of oomycete sequences from roots in the fumigated and pasteurized treatments was challenging, and resulting low numbers of oomycete amplicons in these treatments precluded routine characterizations of their

richness and composition; this suggested that fumigation and pasteurization had nearly eliminated oomycetes from the soils.

When we compared bacterial populations at the class level in non-PRD-inducing soils vs. PRD-inducing soils, with two different sets of primers, we observed that Actinobacteria were enriched in PRD-inducing soils, compared to the non-inducing soils (**Figure 2 A, B**). DESeq2 analysis at ASV level revealed taxa that were differentially abundant in PRD-inducing soils vs. non-inducing soils. Among the differentially abundant ASVs that that represented ≥1% of total amplicons, we found that *Streptomyces scabiei*, *S. lincolnensis*, *Steroidobacter denitrificans*, *Steroidobacter* sp., and *Povalibacter uvarum* were enriched in PRD-inducing soils, and their relative abundances correlated positively with peach seedling biomass reduction (**Table 4**). Interestingly, *S. scabiei* (also known as *Streptomyces scabies*) is the well-known cause of scab disease in vegetables (i.e., potato, beet, carrot, parsnip, radish, rutabaga, and turnip) and can inhibit growth of monocot and dicot seedlings (4-7). Other species of *Streptomyces* are known to suppress plant diseases and can be used as biocontrol agents due to the ability to produce multiple antibiotics (2, 9, 11). *Steroidobacter* species and *Povalibacter uvarum* are closely related and were described relatively recently, but little is known about their potential for causing disease in plants (3, 8, 10). Further studies involving enrichment of these bacterial taxa from peach seedling roots followed by pathogenicity testing in isolation or as consortia are needed to confirm the role of these microbes in PRD.

Among fungi at the class level in non-inducing vs. PRD-inducing soils, Pezizomycetes were more enriched in the latter (**Figure 3 A, B**). At genus level among oomycete root communities, little difference was apparent between non-PRD-inducing soils and PRD-inducing soils (**Figure 4 A, B**). When DESeq2 analyses were used at ASV level, there were several fungal and oomycete taxa that were differentially abundant in roots from PRD-inducing vs. non-inducing soils and that represented ≥1% of the amplicons, but the relative abundances of these taxa did not correlate significantly with plant biomass (data not shown).

The look into the root microbial communities that developed in PRD-inducing vs. non-inducing soils provided new evidence for involvement of several bacterial taxa in PRD, but little evidence for contributions of fungi or oomycetes. Nevertheless, these findings must be viewed as qualitative, because relative abundances do not adequately reflect absolute abundances (12). Quantitative determinations are possible by qPCR (limited to specific organisms) and by a recently developed approach developed for simultaneous quantifications of cross-domain tax in complex communities (12). We will proceed to quantifications in our studies. To determine whether the bacterial associations with PRD were causal or just coincidental, we will focus on culturing the bacterial suspects and testing their pathogenicity. It is still possible that fungi and oomycetes that we have found to be pathogenic on peach play a role in PRD, and quantitative examinations will help to resolve possible contributions.

Objective 2**.** In Parlier KARE experiment 1 planted in 2017, year-one trunk-cross sectional growth was affected significantly by preplant soil treatment (P<0.0001). ASD with any of the nine carbon sources (rice bran, almond hull, almond shell, almond hull + shell, grape pomace, olive pomace, tomato pomace, pistachio hull, all at 9 t/treated acre; and mustard seed meal, at 3 t/treated acre), conducted with full irrigation and TIF tarp, significantly improved tree growth, compared to the non-treated control (**Figure 5**). Most carbon sources produced similar results, but ASD based on rice bran improved tree growth more than that with almond shell or olive pomace. Preplant fumigation stimulated tree growth more than all ASD treatments, except that with rice bran, which was equivalent to fumigation. Details of the ASD treatments and application methods were given previously (Browne et al., 2017 Annual Report to ABC).
In Parlier KARE experiment 2 planted in 2017, which included the rice bran and almond hull ASD treatments of experiment 1, except with no tarp, the preplant soil treatments also significantly affected first-year TCSA increase (P<0.0001) (**Figure 6**). Though means are not strictly comparable between experiment 1 and 2, tree growth was similar between the controls of each experiment and the fumigated treatments of each experiment, suggesting comparable conditions between the experiments, which were adjacent and had the same crop history. Interestingly, rice bran ASD without tarp in experiment 2 performed as well as fumigation and similar to rice bran with tarp in experiment 1. The almond hull ASD treatment without tarp only marginally improved tree growth, compared to the non-treated control. We discussed performance of the WOR in the accompanying Annual Report to ABC on WOR by Holtz et al.

Results of the Parlier experiments suggested that almond hull and shell mixture, at least when used with irrigation and tarp for ASD, affords nearly as good performance as rice bran. Also, the former carbon source, though fluctuating in price, may be strategically superior carbon source for the almond industry and has been less expensive than the other carbon sources tested. Further trials have been initiated at CSUF and Chowchilla to examine efficacy of rice bran and almond hull and shell mixture with and without tarp.

In the Shafter 1 experiment (location WO3371), which tested control, strip fumigation, and spot fumigation treatments against all possible combinations of rice bran ASD treatment components (i.e., components were rice bran substrate, irrigation, and tarp), preplant treatments significantly affected first-year increases in TCSA (P<0.0001), but effects were relatively small in magnitude (**Figure 7 A**). The rootstocks in the experiment were affected similarly by the treatments, but potted trees on Hansen 536 (TCSA 15.4) increased more than bare root trees on Nemaguard (TCSA 14.0) (P=0.007). Spot fumigation slightly improved tree growth, compared to the control, but strip fumigation did not. Treatments that had received rice bran substrate generally grew better than treatments that had not, suggesting that in this trial, nutritional benefits of ASD had overshadowed other effects.

Tree growth responses to treatments that were in common among the trials planted in 2017 (Parlier-KARE experiments 1, 2 and Shafter 1 (WO3371) and Shafter 2 (3381)) were not highly consistent. The response to fumigation in the Shafter trials were less pronounced and consistent than in the Parlier trials. Also, the full rice bran ASD

treatment offered no benefit in Shafter 2, but did improve tree growth in Shafter 1 and in Parlier experiments 1,2. Tree growth in all trials exhibited benefit from rice bran without tarp. Site factors, including soils, management factors, and timing of planting probably affected treatment responses.

By midsummer of the first growing season after planting, preplant soil treatments in CSUF experiment 1 had significant effects on tree growth (P<0.0001), but only the preplant fumigation treatment and the full ASD treatment, i.e., ground almond hull + shell, water, and TIF tarp, had increased tree growth significantly above that of the nontreated control (**Figure 8**). Increasing the rate of almond hull + shell mixture from 9 to 12 to 16 t/a did not improve tree growth, nor did application of ammonium sulfate before ASD. In CSUF experiment 2 by mid-summer, soil treatments also significantly affected TCSA growth (P<0.0001). The almond hull + shell and rice bran ASD treatments, each administered with 9 t/a with water and tarp, more than doubled TCSA growth compared to control treatments, and statistically matched growth in fumigated plots. Addition of WOR chips to almond hull and shell ASD treatments, with or without ammonium sulfate, reduced the benefit of almond hull and shell with water and tarp. Tomato pomace was not as effective as almond hull and shell or rice bran. It will be important to reassess the treatment effects in CSUF experiments 1 and 2 after the growing season is completed.

In retrospect, almond hull and shell mixture appears to offer a less-expensive alternative to rice bran for ASD used to prevent PRD. More time will be required to assess effects of these treatments on plant parasitic nematodes, which can take years to build to damaging populations. The CSUF trials and Shafter trial 2 had measurable populations of plant parasitic nematodes at the beginning of their treatments and may over time afford assessments of effects on nematode populations. At this time, data are insufficient to conclude how much benefit TIF tarp offers to the ASD process with rice bran or other substrates. Nevertheless, our data to date indicate that at least partial benefit, and sometimes equivalent benefit may result from applying the substrate alone or with water, compared to using tarp and water with the substrate. Timing of application may factor into tarp benefit, but more research will be required to determine this.

Research Effort Recent Publications:

Wicaksono, W., Ott., N.J., Poret-Peterson, A.T., Browne, G.T. Amplicon-based sequencing examines associations of bacteria, fungi, and oomycetes with Prunus replant disease. Applied and Environmental Microbiology (submitted).

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Tkacz, A., Hortala, M., and Poole, P. S. 2018. Absolute quantitation of microbiota abundance in environmental samples. Microbiome 6 (1):110.

Table 1. Properties of soils used for greenhouse bioassay for Prunus replant disease and associated root microbial community characterizations

Soil number & code[a]	Source County	Crop history[b]	Texture classification	pH	Nematode count (per 250 cc)[c]			
					Ring	Lesion	RKN	Dagger
1.Durham_Mea_St	Butte	Al/Lov 11 yr	clay loam	7.81	0	0	0	0
2.Durham_Mtz_St	Butte	Al/Lov >20 yr	sandy loam	7.95	0	0	0	0
3.Arbuckle_Nic_St	Colusa	Al/Nem 6 yr	sandy loam	5.75	0	0	0	0
4.Firebaugh_Wo_St	Madera	Al/Nem 8 yr	sandy loam	7.85	0	0	0	0
5.Parlier_KViS_St	Fresno	Vin >20 yr	sandy loam	7.34	808	0	15	7
6.Parlier_KViN_St	Fresno	Vin >20 yr	sandy loam	7.57	56	0	0	22
7.Parlier_KaldCon_Cl	Fresno	Pe/Nem 12 yr	sandy loam	7.55	0	0	0	0
8.Parlier_KaldFum_Cl	Fresno	Pe/Nem 12 yr	sandy loam	7.12	0	0	0	0
9.Parlier_KaldASD_Cl	Fresno	Pe/Nem 12 yr	sandy loam	6.43	0	0	0	0
10.Shafter_Wo_St	Kern	Al/Nem >20 yr	sandy loam	7.57	0	0	0	0

[a] In code, first portion of text indicates nearest city, “St” indicates that orchard or vineyard was standing when soil was collected, and “Cl” indicates that orchard had been cleared before soil was collected.
[b] “Al/Lov” indicates orchard was almond trees on Lovell peach rootstock; “Al/Nem” indicates orchard was almond trees on Nemaguard peach rootstock’ “Vin” indicates grape vineyard; and “Pe/Nem” indicates orchard was peach trees on Nemaguard rootstock.
[c] “Ring” = *Mesocricinema xenoplax*; “Lesion” = *Pratylechus* sp.; “RKN” = *Meloidogyne incognita*; “Pin” = *Paratylenchus* sp. All nematodes extracted by centrifugal flotation and identified by morphological examination.

Table 2. Experiment 1, CSU Fresno, and samples collected for microbial community analyses

Mainplot treatment	Subplot Treatment	Samples collected		
		Preplant soil	Postplant soil	Postplant roots
Control	Control	+1	+4	+4
	Strip fumigation 1,3-D + Pic 330 + 200 lb/trt. ac	+1	+4	+4
	Almond hull:shell, 16 tons/trt. ac.	+1		
	Rice bran, 9 tons/trt. ac.	+1	+4	+4
	Almond hull:shell, 9 tons/trt. ac.	+1	+4	+4
Water	Control	+1	+4	+4
	Almond hull:shell, 16 tons/trt. ac.	+1		
	Rice bran, 9 tons/trt. ac.	+1	+4	+4
	Almond hull:shell, 9 tons/trt. ac.	+1	+4	+4
	Almond hull:shell, 12 tons/trt. ac.	+1		
	Almond hull:shell, 12 tons/trt. acre + AMS 360 lb./trt. ac.	+1		
Water+tarp	Control	+1		
	Almond hull:shell, 16 tons/trt. ac.	+1	+4	+4
	Rice bran, 9 tons/trt. ac.	+1	+4	+4
	Almond hull:shell, 9 tons/trt. ac.	+1	+4	+4
	Almond hull:shell, 12 tons/trt. ac.	+1		
	Almond hull:shell, 12 tons/trt. acre + AMS 360 lb./trt. ac.	+1		

[a]Mainplot treatments indicate whether or not ASD treatment components of (i) 5 weeks of irrigation ("water") or (ii) water plus cover with TIF tarp ("water + tarp") were included with the subplot soil treatments. "AMS" = ammonium sulfate. All treatment combinations included four replicate plots of four trees per plot, plus additional bordering guard trees.

Table 3. Experiment 2, CSU Fresno, and samples collected for microbial community analyses[a]

Preplant treatment combination			Samples collected		
Soil amendment / disinfestation	ASD irrigation	TIF tarp	Preplant soil	Postplant soil	Postplant roots
Control	No	No	+1	+4	+4
Control	Yes	No	+1		
Control	Yes	Yes	+1	+4	+4
Telone + chloropicrin	No	No	+1	+4	+4
Ald hull+shell, 9 t/trt. ac.	Yes	Yes	+1	+4	+4
WOR chips, 60 t/trt. ac.	Yes	Yes	+1	+4	+4
Ald hull+shell, 9 t/trt. ac. + WOR chips 60 t/trt. ac.	Yes	Yes	+1	+4	+4
Ald hull:shell, 9 t/trt. ac. + WOR chips 60 t/trt. ac. + AMS 360 lb/trt. ac.	Yes	Yes	+1	+4	+4
Rice bran 9 t/trt. ac.	Yes	Yes	+1	+4	+4
Tomato pomace 9 t/trt.ac.	Yes	Yes	+1		
Ald hull+shell, 9 t/trt. ac. + WOR chips 60 t/trt. ac. + AMS 360 lb/trt. ac.	Yes	No	+1		

[a]All treatment combinations included four replicate plots of four trees per plot, plus additional bordering guard trees.

Table 4. DESeq2 analysis of relative abundances of bacterial amplicon ASVs in PRD inducing vs. non-inducing soil sources and correlation of ASV relative abundances with peach seedling biomass in greenhouse bioassay[a]

			DESeq2 non-inducing vs PRD inducing soils		Correlation analysis between taxa relative abundance and plant growth reduction[c]			
					% Reduction: Top fresh weight		% Reduction: Root fresh weight	
(Primer set, rRNA gene target region) and ASV	Rel. abund. (%)	Identity (NCBI accession no.)	log_2 fold change[b]	P value	r value	P value	r value	P value
(515F-806R, v4 region)								
51121c8f8cc8a7a03d9d7f1dd5142aa5	28.18	*Streptomyces scabiei* (LBNJ01000196)	-2.77	0.019	0.42	0.021	0.47	0.009
ec6470d2512662ae27e4dbd6b11bf38d	5.27	*Steroidobacter denitrificans* (CP011971)	-2.15	0.041	0.38	0.039	0.36	n.s.d
120eba657e42a11a5c29f97b90f02035	3.03	*Streptomyces galbus* (X79852)	6.70	<0.001	-0.31	n.s.d	-0.4	0.027
cb640ecfa90d3aff38869257557b1348	2.47	*Steroidobacter denitrificans* (CP011971)	-2.93	0.012	0.41	0.024	0.3	n.s.d
bf321c05d79f766c0099afb9ecc42f52	1.61	*Streptomyces turgidiscabies* (AB026221)	-2.83	0.014	0.096	n.s.d	0.022	n.s.d
(799F-1193R, v5-v7 region)								
37a9d0d51cdc404da88875705bb63b05	17.25	*Streptomyces scabiei* (LBNJ01000196)	-2.77	0.005	0.39	0.031	0.43	0.019
40b59f5b98e2fc10445174e4aa94cba6	13.48	*Streptomyces scabiei* (LBNJ01000196)	-2.58	0.012	0.49	0.006	0.5	0.005
375be3b5d189bdc5d584c59d1943719d	4.18	*Povalibacter uvarum* (AB548216)	-2.14	0.049	0.36	n.s.d	0.33	n.s.d
c185a514f0bae05e6ee15b1440d977f5	2.00	*Povalibacter uvarum* (AB548216)	-2.22	0.045	0.43	0.019	0.33	n.s.d
a6dae0ea1b001760e9ff2aaf9b233158	1.92	*Streptomyces canus* (KQ948708)	2.30	0.011	-0.45	0.013	-0.51	0.004
bf6c0f77cf10ab096fafb786889f4505	1.41	*Streptomyces lincolnensis* (CP016438)	-3.58	0.001	0.45	0.014	0.33	n.s.d
fb69da1479dd7147a1e8c6ad399f7277	1.26	*Streptomyces griseorubiginosus* (KQ948757)	5.59	<0.001	-0.42	0.023	-0.44	0.014
dcc323752ebda91f52c005254f1f8bd3	1.14	*Steroidobacter* sp. (HQ119093)	-6.45	<0.001	0.41	0.024	0.39	0.035

[a]Only ASVs of overall relative abundance ≥1% and with significant log_2-fold changes in DESeq2 included.
[b]Positive log_2 fold change value indicates taxa enriched in non-inducing soil and negative value indicates taxa enriched in PRD inducing soil.
[c]Top and root fresh weight reductions were percentages for plant growth in control (untreated) soil compared to pasteurized soil; "n.s.d" indicates $P \geq 0.05$.

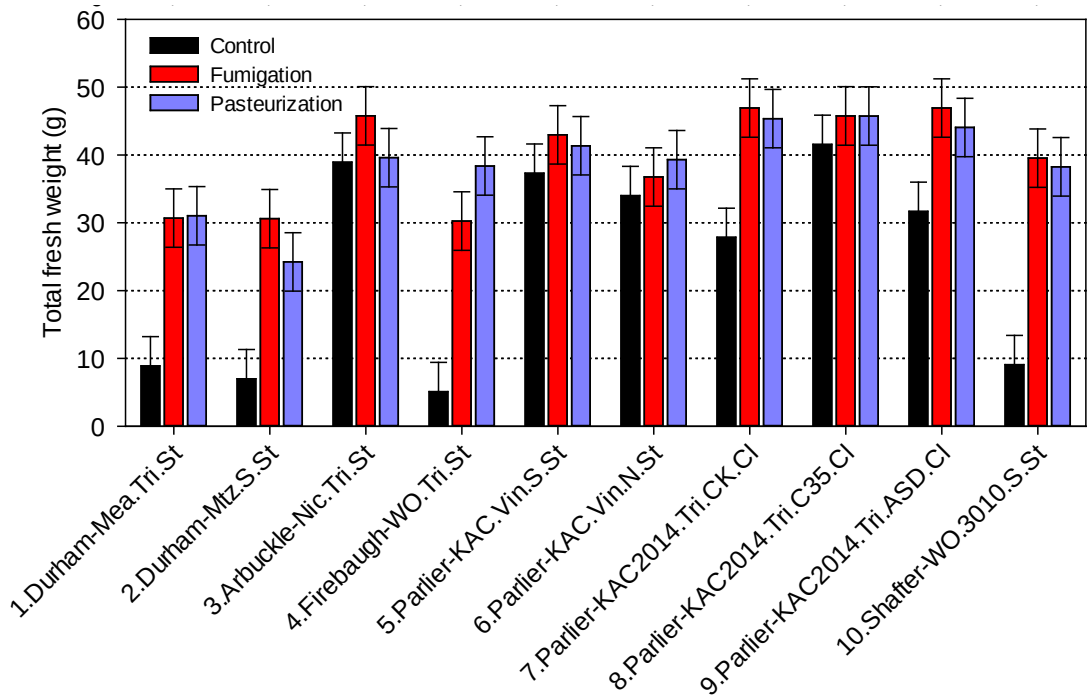

Figure 1. Growth of peach seedlings in 10 soils in greenhouse bioassay. In x-axis labels, soil number is followed by name of nearest city to soil collection site, and code in which: "St" indicates that orchard or vineyard was standing when soil was collected, and "Cl" indicates that orchard had been cleared before soil was collected. Soils 1-4 and 7-10 were from plantings of almond or peach, and soils 5 and 6 were from vineyards.

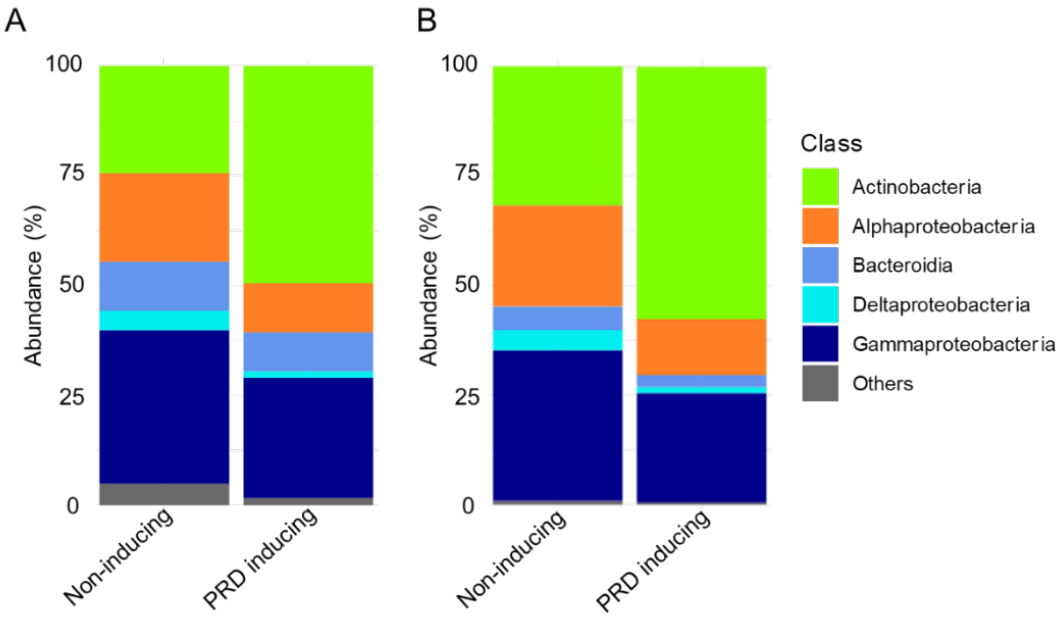

Figure 2. Class-level bacterial community composition in rRNA gene amplicons from roots of greenhouse bioassay as a function of the PRD-inducing capacity of soil source. **A**, community determined with primers 515F-806R (v4 region) and **B**, community determined with 799F-1193R (v5-v7 region).

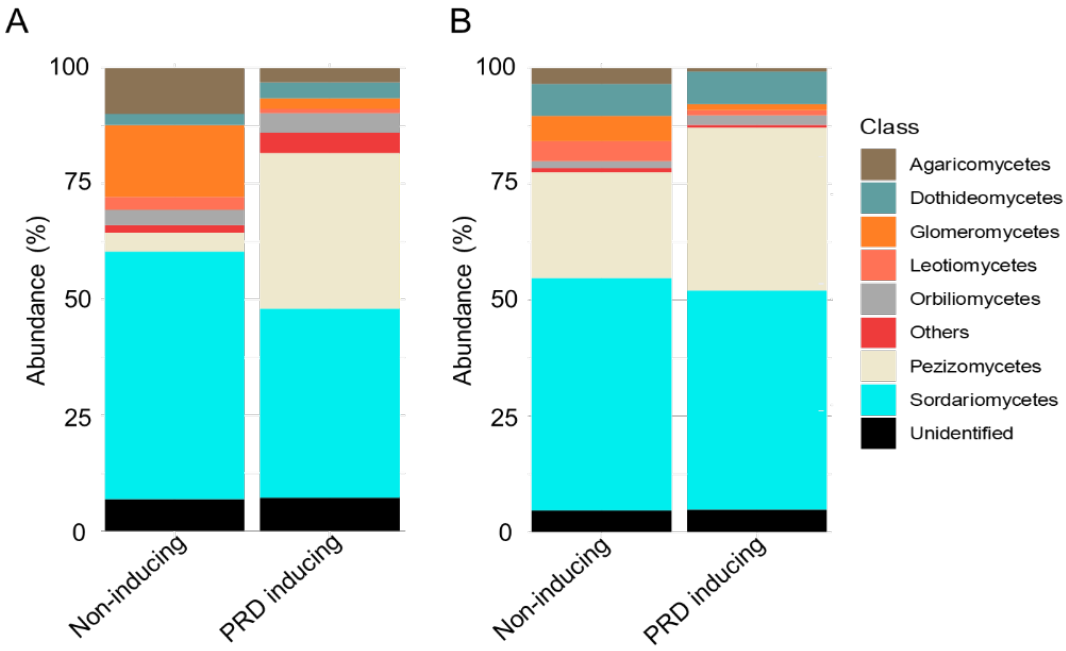

Figure 3. Class-level fungal community composition in rRNA gene amplicons from roots of greenhouse bioassay as a function of the PRD-inducing capacity of soil source. **A**, community determined with ITS1f-ITS2 (ITS1 region) and **B**, community determined with fITS7-ITS4 (ITS2 region).

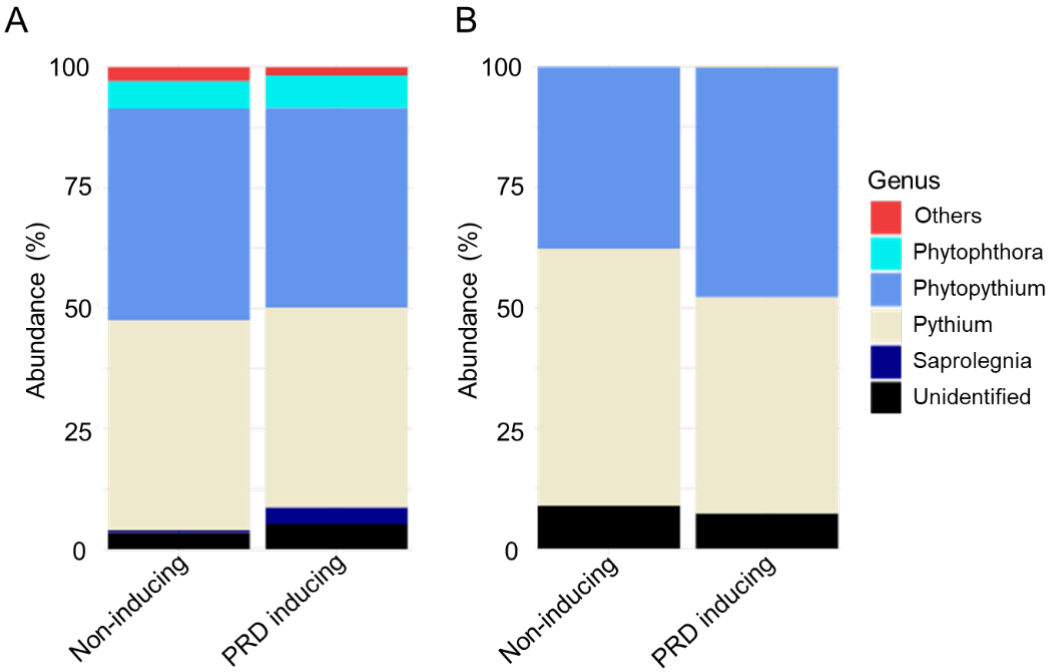

Figure 4. Genus-level oomycete community composition in rRNA gene amplicons from roots of greenhouse bioassay as a function of the PRD-inducing capacity of soil source. **A**, community determined with ITS1oo-ITS7 (ITS1 region) and **B**, community determined with ITS3ooITS4 (ITS2 region).

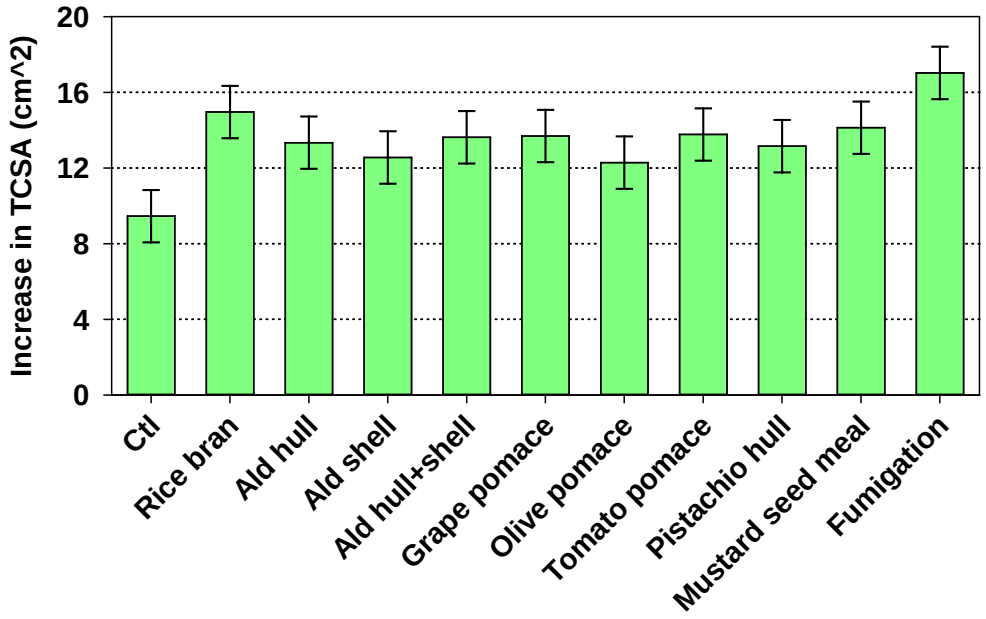

Figure 5. First-year increases in trunk cross sectional area (TCSA), Parlier-KARE, Experiment 1, planted in 2017. "Ctl" = non-treated control, "Fumigation" was 1.3-D 340 lb/a + chloropicrin 200 lb/a, shank applied without tarp. All other treatments were applied as ASD treatments with TIF tarp and supplemental irrigation; mustard seed meal applied at 3 t/treated acre, all other ASD carbon sources applied at 9 t/treated acre. Error bars are 95% confidence intervals.

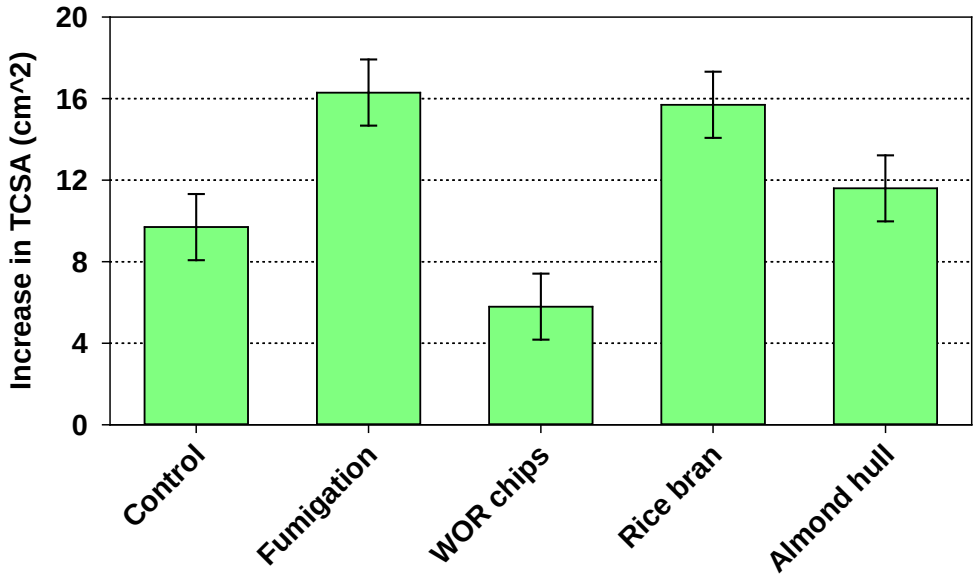

Figure 6. First-year increases in trunk cross sectional area (TCSA), Parlier-KARE, Experiment 2, planted in 2017. "Control" = non-treated, "Fumigation" was 1.3-D 340 lb/a + chloropicrin 200 lb/a, shank applied without tarp. "WOR" = recycled almond orchard chips at 80 t/a. Rice bran and almond hull (both ground) were applied as ASD treatments with supplemental irrigation, but without tarp. Error bars are 95% confidence intervals.

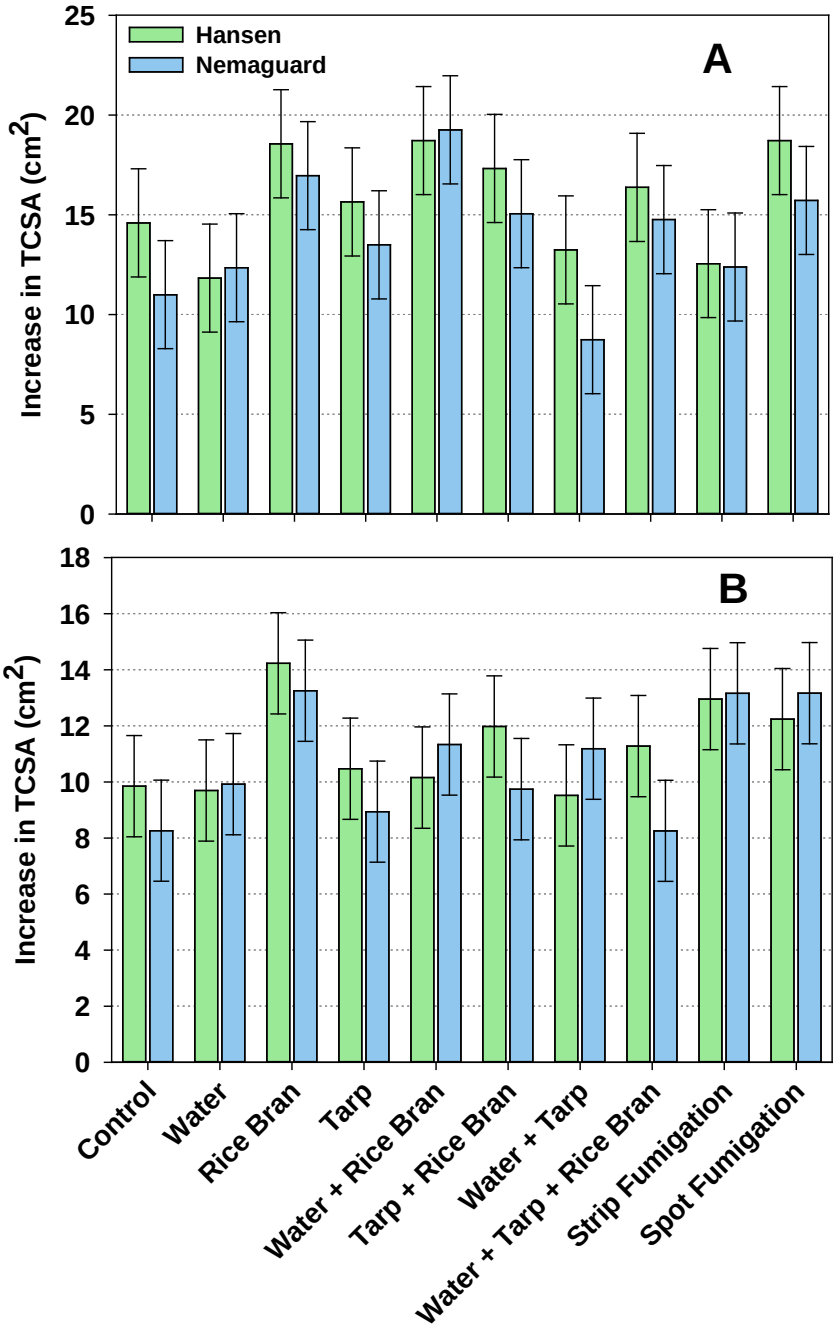

Figure 7. First-year increases in trunk cross sectional area (TCSA), **A**, Shafter experiment 1 (WO3371) and **B**, Shafter experiment 2 (WO3381), both planted in 2017. “Control” = non-treated, “Strip Fumigation” was 1.3-D 340 lb/a + chloropicrin 200 lb/a applied in 8-ft x 8-ft squared centered over tree planting sites, and ‘Strip Fumigation” was same fumigant mixture applied in 11.6-ft-wide strips over tree rows before planting, both treatments shank-applied without tarp. Treatment components of anaerobic soil disinfestation: “Water”= preirrigation to saturate soil profile and maintain soil moisture at or above field capacity for 5 weeks; “Rice bran” = incorporation of rice bran into soil at 9 t/a in 10-ft-wide strips before water; “Tarp” = covering treated strips with TIF tarp after rice bran incorporation and before water application. See text for details of treatment effects. Error bars are 95% confidence intervals.

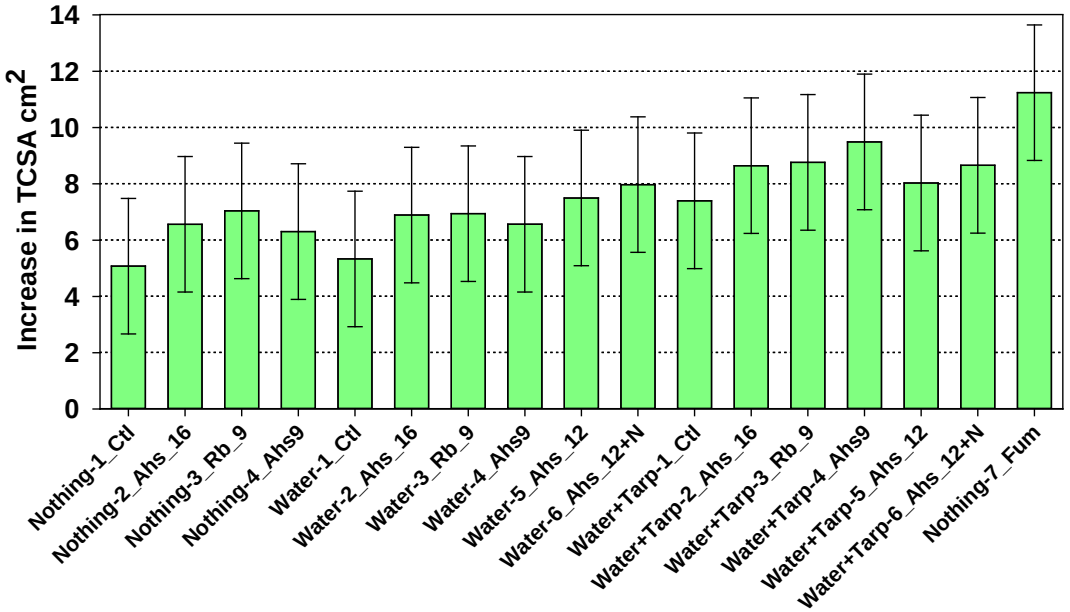

Figure 8. Increases in trunk cross sectional area (TCSA) by mid August, CSUF experiment 1, planted in January 2018. Treatment abbreviations on x axis: "Nothing" indicates no irrigation or TIF tarp applied; "Water" = preirrigation to saturate soil profile and maintain soil moisture at or above field capacity for 5 weeks; "Water+Tarp" = the preirrigation applied under TIF tarp. "Ahs" = ground almond hull + shell applied, followed by 9, 12, or 16 (rates in t/treated acre); "N' = ammonium sulfate applied at 360 lb/treated acre, with Ahs; "Rb" indicates rice bran applied at indicated rate of 9 t/treated acre; "Fum" indicates strip fumigation with 1,3-D (340 lb/treated acre) and chloropicrin (200 lb/treated acre) applied in 11.6-ft-wide strips over tree rows before planting. See text for details of treatment effects. Error bars are 95% confidence intervals.

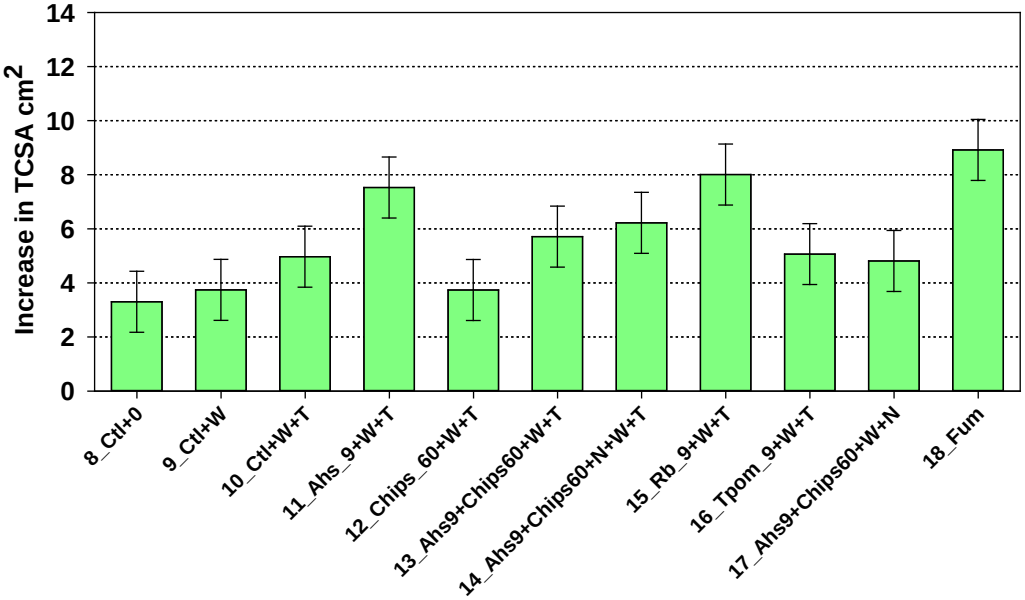

Figure 9. Increases in trunk cross sectional area (TCSA) by mid August, CSUF experiment 2, planted in January 2018. Treatment abbreviations on x axis: "Ctl" = no substrate added; "Ahs" = almond hull + shell added at 9 t/treated acre; "Chips60" = whole orchard recycling ships added at 60 t/treated acre; "Rb" indicates rice bran added at 9 t/treated acre; "Tpom" = tomato pomace added at 9 t/treated acre; and "Fum" indicates strip fumigation with 1,3-D (340 lb/treated acre) and chloropicrin (200 lb/treated acre) applied in 11.6-ft-wide strips over tree rows before planting. See text for details of treatment effects. Error bars are 95% confidence intervals.