Developing Improved Strategies for Management of Replant Problems

Project No.: **14-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:

This project focuses mainly on etiology and integrated control of replant disease (RD), a soilborne disease complex that widely suppresses growth and yield of replanted almond orchards even in the absence of plant parasitic nematodes. The project also contributes to development of almond rootstocks that tolerate the RD complex and crown and root rots caused by *Phytophthora* species.

To approach the etiology of RD in greater depth and open new horizons for integrated management of the complex, we began collaborations with the J. Eisen lab, which specializes in metagenomic technologies and their applications. The Eisen lab has helped the Browne lab refine metagenomic examination strategies for RD and gain experience using bioinformatics software. The Browne lab has begun constructing "DNA libraries" for high-throughput sequencing using the Illumina "Hi-Seq" system. After DNA is extracted and purified from samples collected in previous replant trials, libraries are created by fragmenting of the DNA (to appropriate lengths for sequencing) and indexing each sample's fragments with an adapter and a unique "bar code" that is used as a traceable label for all DNA in the sample. The bar codes are used to link the DNA fragments to their source— particular trees in particular plots. Although our previous experiments and data implicated several species of *Pythium*, *Phytopythium*, and *Cylindrocarpon* as contributors to RD, we hypothesize that metagenomic sequencing and associated approaches will: i) lead to stronger insights on the roles of the implicated organisms, ii) provide new insights on involvement of other soilborne microorganisms in the disease, and iii) support development of RD diagnostics that can help to predict the need for preplant fumigation or non-fumigant soil remediation.

For rootstock work under objective 2, we received 54 rootstock clones from M. Aradhya and C. Ledbetter for testing resistance to *Phytophthora* and RD. We transplanted them into standardized pots and grew them in a lath house in preparation for Phytophthora resistance testing in a field setting at UC Davis (Armstrong Field, Department of Plant Pathology). Before transplanting, the clones varied widely in size, number, methods used in propagation, etc. Additional rootstocks (Nemaguard, Hansen 536, Marianna 2624, Krymsk 86) of similar size were needed to serve as standards, which are essential for meaningful resistance interpretations. The most reliable assessments of resistance and tolerance will occur under field conditions, once plants have been "synchronized" by a cycle of growth and dormancy. Plant numbers to date are only sufficient for a Phytophthora trial; future trials will address tolerance to RD as well as resistance to *Phytophthora*.

Our bioassay work under objective 2 was completed with support from the California Department of Pesticide Regulation (Cal DPR). We continued an approach with a greenhouse-based peach seedling bioassay to learn more about the degree of need to preplant fumigate among the Central Valley's diverse soils. With additional help from the Almond Board of California (ABC), our goal is to develop predictive tests to aid preplant soil remediation decisions. In spring 2015, soils were collected from 26 locations throughout the Central Valley, representing mainly almond orchard soils but including various cropping histories and biological, chemical, and physical soil properties. Subsamples of each soil were mixed with course sand (2:1 soil:sand, v:v) to facilitate soil water drainage in pots; given different preplant treatments (fumigation, pasteurization, and a control); and planted with Nemaguard peach seedlings in a greenhouse (12 replicate seedlings per soil-treatment combination, one seedling per pot). The 2015 bioassay will be fully assessed at the end of September 2015, but by 20 August, growth in shoot length exhibited highly significant interaction between soils and preplant soil treatments ($P<0.0001$). The mean increases in shoot length growth resulting from fumigation and pasteurization ranged from -4 to 131% of the control. Percentages of increase resulting from fumigation were positively correlated with those resulting from pasteurization ($r=0.84$, $P<0.0001$). Also, shoot length increases resulting from fumigation or pasteurization were positively correlated with soil pH, cation exchange

capacity, and exchangeable potassium values (r=0.44 to 0.64; *P*=0.03 to 0.0006). There were no significant correlations between shoot length increases and populations of plant pathogenic nematodes (none were detected). In addition to the bioassay, seven orchard replant trials have been established for the purpose of i) validating greenhouse bioassay results among some of the collected soils and ii) demonstrating utility of GPS-controlled spot fumigation in commercial orchards. Collectively, results of the bioassay and coordinated field trials suggest that soil testing (i.e., bioassays and derived diagnostics) and spot fumigation technologies can ultimately lead to reduction in fumigant use for orchards. To date, the field results provide qualitative but not quantitative validation of bioassay results. For example, although positive growth responses to fumigation were observed in orchard trees in the Crows Landing, Kerman, and Parlier trials, the relative magnitudes of the growth responses were not highly correlated with the magnitudes of summer 2014 bioassay plant growth responses.

We established two new trials of anaerobic soil disinfestation (ASD) in 2014-15 and continued two ASD trials started in 2013-14. All four ASD trials are located Parlier at the Kearney Agricultural Center (KAC) in Hanford sandy loam soil impacted by RD but not plant pathogenic nematodes. Preplant ASD treatments were compared with Sudan grass crop rotation and shank fumigation for management of RD. In the trial established in 2014-15, multiple strip treatment widths and substrate application rates were tested for ASD. Efficacy of the preplant treatments was assessed according to effects on: i) survival bioassay inoculum of *Pythium ultimum* (a contributor to the RD complex, buried in nylon bags at 15 and 46 cm soil depths); ii) growth in stem circumference of the replanted almond trees, and iii) the percentage of photosynthetically active radiation (PAR) intercepted by the almond canopies in their second growing season after planting (i.e., in June 2015 for experiments 1 and 2). Data were subjected to analyses of variance and means were separated according to 95% confidence intervals.

All ASD treatments in 2014-15 trials quickly generated and maintained anaerobic conditions and elevated soil temperatures in the 6-wk treatment period (end of September to mid November 2014). All of the ASD and fumigation treatments in the trial reduced bioassay populations of *P. ultimum* to near or below detection limits, while the inoculum survived at relatively high populations in control treatments. After planting the plots in January 2015, tree circumference increases measured in mid-July were increased similarly by ASD-high-rate-wide-strip; ASD high-rate-narrow-strip; and fumigation-wide-strip treatments (by 85 to 174% of the control). The ASD low-rate-narrow strip treatment also stimulated tree growth significantly, but less so than the other ASD treatments (by 60% of control).

In second-year (July 2015) assessments of the trials established in 2013-14, ASD treatment benefits were still highly significant and were equivalent to those of preplant fumigation. Our results indicate that commercial adoption of ASD may be feasible but will require further optimization and testing. Total cost of the ASD treatments used in this study ranged from similar to that of the Telone C35 treatment to roughly double that of the fumigation treatment. Rice bran is a relatively expensive component of the tested ASD treatments. Research is planned to examine the effectiveness of alternative, less expensive ASD substrates and application methods for orchards.

Materials and Methods:

Objective 1. Determine causes of RD.

Initiating metagenomic examinations. To approach the etiology of RD in greater depth and open new horizons for integrated management of the complex, we began collaborations with the J. Eisen lab, which specializes in metagenomic technologies and their applications. The Eisen lab has helped the Browne lab refine metagenomic examination strategies for RD and gain experience using bioinformatics software. The Browne lab has begun construction "DNA libraries" for high-throughput sequencing using the Illumina "Hi-Seq" system. After DNA is extracted and purified from samples collected in previous replant trials, libraries are created by partial fragmenting of the DNA for sequencing and then indexing each sample's fragments with an adapter and a unique "bar code" that is used as a traceable label for all DNA in the sample. The bar codes are used to link the DNA fragments to their source—in these cases particular trees in particular plots. Although our previous experiments and data implicated several species of *Pythium*, *Phytopythium*, and *Cylindrocarpon* as contributors to RD, we hypothesize that metagenomic sequencing and associated approaches will: i) lead to stronger insights on the roles of the implicated organisms, ii) provide new insights on involvement of other soilborne microorganisms in the disease, and iii) support development of RD diagnostics that can help to predict the need for preplant fumigation or non-fumigant soil remediation.

Initially we will focus the metagenomic examinations on "shotgun sequencing", which is not dependent upon amplification of the DNA in a sample as is the case for "amplicon sequencing". Amplicon sequencing, although useful for many applications, has drawbacks for applications such as ours, including: i) PCR primers that are used for general amplification of diagnostic rDNA fragments from broad microorganism groupings such as bacteria, archaea, fungi, and stramenopiles typically fail to amplify from some key organisms in each grouping, both for known and unknown reasons; and ii) PCR amplification introduces many quantitative biases, skewing investigators' views of the relative abundance of different microorganisms. Shotgun sequencing, although requiring more intensive bioinformatic analysis, avoids these problems. As clues of interest are revealed from exploratory shotgun sequencing and associated bioinformatics, we will progress to qPCR to quantify specific organisms of interest and likely pursue supplementary amplicon sequencing of DNA and shotgun sequencing of rRNA.

Objective 2. Support the development of strategic approaches for management of RD and other soilborne diseases.

Rootstock resistance to RD and Phytophthora. In summer 2015, we received 54 rootstock clones from M. Aradhya and C. Ledbetter for testing resistance to *Phytophthora* and RD (**Table 1**). We transplanted them into standardized pots and grew them in a lath house in preparation for Phytophthora resistance testing in a field setting at UC Davis (Armstrong Field, Department of Plant Pathology). Before transplanting, the clones varied widely in size, number, methods used in propagation, etc. Additional rootstocks (Nemaguard, Hansen 536, Marianna 2624, and Krymsk 86) of similar size were needed to serve as standards, which are essential for meaningful resistance interpretations. The most reliable assessments of resistance and tolerance will occur under field conditions, once plants have been "synchronized" by a cycle of growth and dormancy. Plant numbers to date are only sufficient

for a Phytophthora trial, future trials will address tolerance to RD as well as resistance to *Phytophthora*.

Greenhouse bioassay and validation work. With support from the California Department of Pesticide Regulation (Cal DPR), soil samples were collected in spring 2015 from 26 orchards and vineyards in northern, central, and southern portions of the Central Valley (**Tables 2, 3;** soils 1-26). The samples were collected from soil depths of 0.3 to 2.0 ft [10 to 61 cm] at four random spots in each orchard using hand augers (hole diameter 3 inches [8 cm]). The 26 locations were chosen to represent i) diversity among soils used for almond and stone fruit production; ii) soils commonly being transitioned from vineyard to almond production; and iii) soils from orchards that had been or are being used for preplant fumigation / ASD replant research trials. All bioassay soils were collected from standing or cleared almond or stone fruit orchards, except for soils 11 and 12, which were collected from standing vineyards. Soils 1, 2, 6, 8-10, and 13-15 were from almond or stone fruit orchards that were hosting or had hosted replant trials with preplant fumigated and non-fumigated plots. The plan was to use incoming and previous data from replant trial locations to help assess validity of bioassay results. The four subsamples collected for each soil generally were pooled and mixed before being used for nematode assays, soil chemical and physical property tests, and greenhouse bioassays. Exceptions to the pooling were as follows: subsamples for soils 13-26 were used for nematode assays without pooling (i.e., the four subsamples were processed separately for soils 13-26), and four additional subsamples from all soils (1-26) were kept separate and frozen on dry ice immediately after collection in the field for subsequent metagenomic DNA sequencing.

The 2015 bioassay experiment was established as follows: the pooled subsamples of each soil were mixed with sterile sand (2:1, soil:sand, v:v). The sand-amended soils were subdivided into three portions; one for a non-treated control, one for preplant fumigation with chloropicrin (CP), and one for preplant pasteurization. The soil to receive CP was bagged doubly in polyethylene and placed inside a 5-gal [18.9-liter] bucket that was lined with a sheet of TIF (totally impermeable film; Vaporsafe, www.ravenag.com). The TIF was sealed shut around the bagged soil, and CP was injected into the soil (0.1 fl. Oz. [3 ml] CP per 15 quarts [14 liters] soil mixture). Soil pasteurization was achieved in a 5-gallon [19-liter] steaming apparatus that brought soil temperature to $\geq$176 °F [80 °C] for 30 min. On 15 July 2015, soil from each of the orchard locations and soil treatments was distributed to twelve 32-oz [0.9-liter] pots and planted with recently sprouted Nemaguard peach seedlings in a greenhouse that typically maintained air temperatures between 60 and 85 °F [16 and 30 °C]. The treatments were arranged in a randomized complete block design with six blocks. Each block had two potted Nemaguard seedlings (on plant per pot) per combination of soil number and soil treatment. The plants were watered daily/as-needed and fertilized with complete liquid fertilizer one to four times per week. Shoot growth length (height above pot rim) was measured 20 August. The experiment will be completed in late September, when plant top and root fresh weights will be determined and the roots will be visually evaluated to estimate the percentage of root cortex length that is necrotic (brown or black in color, compared to white, healthy root cortex tissue). The bioassay was used to predict field incidence and severity of RD based on the degree to which fumigation and pasteurization treatments stimulate seedling growth and improve root cortex health. Preliminary assessments of RD potential were made using the 20 August shoot growth data. The shoot growth length was subjected to analysis of variance using PROC

MIXED of SAS Version 9.4 software. PROC CORR of the software was used to examine correlations between shoot growth and soil variables.

Also with support from Cal DPR, we continued four orchard replant trials that were planted in 2014 after receiving treatments in 2013 and planted three new replant trials that had received preplant treatments in 2014 (**Table 4**). The trials were designed to afford comparisons between plant responses to soil remediation treatments in greenhouse vs. orchard settings. In the continuation trials, trunk circumferences were measured just after planting (winter 2014) and again after the trees entered dormancy (winter 2015). The net increases in trunk circumference from 2014 to 2015 (first growing season) were averaged within plots, subjected to analysis of variance, and used as the criterion by which to assess efficacy of soil treatments in the orchard. In the new trials, orchard fumigation treatments were applied as described previously, using shanks that were spaced 20 inches [51 cm] apart and injected fumigant at a soil depth of 18 to 22 inches [46 to 56 cm]; no tarp was used. The trials near Parlier (KAC) received fumigation treatments in October 2014, whereas the Delhi trial was fumigated in December 2014. Each trial included fumigated and non-fumigated plots that were at least 90 ft [27 m] (9 tree spaces) long and 20 ft [6.1 m] (1 tree space = 1 row) wide. The plots were arranged in three to six randomized complete blocks, depending on the experiment. The plots were planted with almond trees in January 2015. Initial stem diameters were measured in March 2015, and final stem diameters for the year will be measured in winter 2015/16, after trees enter dormancy.

Spot fumigation treatments were included in the Delhi trial along with strip fumigation treatments and the control (**Table 4**). The trial was designed to demonstrate efficacy of spot fumigation, which uses roughly 10 to 50% as much fumigant as conventional strip and broadcast fumigation. The treatments were administered within a trial managed by David Doll as part of his larger orchard replant experiment at the same location. Improved GPS hardware and software, adapted from previously used systems (Browne et al., 2013; Udompetaikul et al., 2013) were used to create a virtual “grid map” of all tree sites in the treated orchard. The grid provided digital guidance to the fumigation rig, turning off and on shanks as the rig traveled down the axes of future tree rows so that rectangular areas measuring 8.3 ft [2.5 m] wide × 8.0 ft [2.4 m] long, centered over tree planting sites, were treated with fumigant.

Anaerobic soil disinfestation. From 2013 to 2014, four orchard replant trials were initiated to test ASD where stone fruits (nectarines, peaches) had grown on Nemaguard rootstock for >12years (**Table 5**; experiments 1-4). ASD was compared with Sudan grass crop rotation and to preplant to soil fumigation for prevention of RD. Treatments were applied to 27.4 x 6.1-m plots, except that treatments 4 and 5 of experiment 3 were applied to 13.7 x 6.1 m plots; a randomized complete block design was used. There were five plots per treatment in experiments 1 and 3 and three plots per treatment in experiments 2 and 4. Depending on the experiment, differential preplant treatment programs were begun in the old stone fruit orchards as early as May, approximately 8 months before the orchards were to be replanted in January (see preplant treatment details in **Table 5**). ASD treatments were applied as described previously (Browne comprehensive report to ABC, 2013-14). Efficacy of the preplant treatments was assessed according to their effects on: i) survival bioassay inoculum of *Pythium ultimum* (a contributor to the RD complex, buried in nylon bags at 15 and 46 cm soil depths); ii) growth in stem circumference of the replanted almond trees; and iii) the percentage

of photosynthetically active radiation (PAR) intercepted by the almond canopies in their second growing season after planting (i.e., in June 2015 for experiments 1 and 2). Data were subjected to analyses of variance and means were separated according to 95% confidence intervals.

Results and Discussion:

Objective 1. Determine causes of RD.

A current working priority list for our metagenomics sequencing projects includes:

- Samples of roots from RD-affected and healthy trees from replicated orchard replant trials.
- Samples of bulk soil from non-fumigated and preplant fumigated plots in replicated orchard replant trials.
- Samples of bulk soil collected throughout the Central Valley for use in bioassay trials.
- Samples of roots from bioassay plants in fumigated and non-fumigated replant soils.
- Samples of soil and roots from orchard trials with anaerobic soil disinfestation (ASD) and fumigation treatments.

All of the samples listed above have already been preserved from our previous trials. We anticipate additions and alterations to this list as sequencing results accumulate and field experiments evolve to address soil management issues faced by almond growers.

Objective 2. Support the development of strategic approaches for management of RD and other soilborne diseases.

Rootstock resistance to RD and Phytophthora. We will report on this sub objective when field data become available. First data are anticipated in 2016. It was judged as important to equalize tree size and condition and secure sufficient numbers of rootstock standards before screening begins.

Greenhouse bioassay and associated field trials. By 20 August, seedling shoot growth in the 2015 greenhouse bioassay exhibited highly significant soil treatment × soil interaction ($P<0.0001$). Among the soils, mean increases in shoot growth resulting from fumigation and pasteurization ranged from -4 to 131% of the control (**Figure 1**). The percentages of increase resulting from fumigation were positively correlated with those resulting from pasteurization ($r=0.84$, $P<0.0001$). Shoot growth benefited relatively little from fumigation and pasteurization (i.e., ≤18% increase, compared to the control) in: two soils from standing almond orchards near Arbuckle (soils 6, 7); one soil from the fumigated plots near Delhi (soil 9); two soils from standing vineyards near Parlier at KAC (soils 11, 12), and one soil from fumigated plots of a cleared peach orchard near Parlier at KAC (soil 14) (**Figure 1**). Shoot growth benefited moderately to greatly from fumigation and pasteurization (i.e., >26 to 114% increases, compared to the control) in soils from almond or stone fruit orchards that had not been fumigated or otherwise remediated in the field before soil was collected (soils 1-5, 8, 10, 13, and 16-26) (**Figure 1**). The magnitude of shoot growth increases resulting from fumigation or pasteurization (**Figure 1**) were positively correlated with soil pH, cation exchange capacity, and exchangeable potassium values (**Table 3**) ($r=0.44$ to 0.64; $P=0.03$ to 0.0006). There were no significant correlations between populations of plant pathogenic nematodes (**Table 2**) and percentages of shoot length growth increases from preplant treatments (**Figure 1**).

Further assessments of plant growth and health, and biological, chemical, and physical parameters will occur at the completion of the 2015 bioassay experiment. As in 2014 bioassays, we will quantify levels of root infection with *Cylindrocarpon* and *Pythium* species (implicated as contributors to PRD) in all bioassay treatments and soils. Also, with support from the ABC, we will use "next-generation" sequencing of DNA to characterize soil microbial communities associated with growth suppression and stimulation.

In the first year after planting the Crows Landing trial, trunk circumference growth was increased by 44% in strip-fumigated plots and by 33% in spot-fumigated plots, compared to the control treatment (**Table 4**). Similarly, first-year trunk circumference growth in the Kerman replant trial was increased by 25% in strip-fumigated plots and 22% in spot fumigated plots, compared to the control (**Table 4**). In the two Parlier trials treated in 2013, strip fumigation increased trunk circumference growth by 89 to 115% in the first growing season, compared to the control (**Table 4**).

Comparison of the first-year growth responses in Crows Landing, Kerman, and Parlier-KAC field trials (**Table 4**) with corresponding responses in 2014 greenhouse bioassay experiments (2013-14 annual report to ABC, Browne et al) suggests that the greenhouse test can provide qualitative but not highly quantitative indications of the need to fumigate an orchard soil for management of RD. For example, trunk growth increases due to strip fumigation, compared to controls, observed in Crows Landing, Kerman, and Parlier-KAC trials (i.e., 44, 25, and 89 to 115%, respectively) were all positive but did not correspond closely in magnitude to the respective plant fresh weight increases in greenhouse bioassay tests in soil from Crows Landing, Kerman, and Parlier-KAC trials (81, 208, and 40%, respectively; 2013-14 annual report to ABC, Browne et al.). Informative comparisons were also possible in soils where field fumigation trials preceded greenhouse bioassay trials. For example, significant growth responses to field fumigation were not observed in an orchard preplant fumigation trial conducted 2007-2010 near Arbuckle at the Nickels Soils Lab, nor were significant responses to fumigation or pasteurization observed in greenhouse bioassays conducted with soil from the same and nearby locations (soil "4.ArbuckleNiT" in 2014 greenhouse bioassay [2013-14 annual report to ABC, Browne et al.] and soils 6 and 7 in 2015 greenhouse bioassay [**Figure 1**]). Conversely, positive orchard growth responses to fumigation were observed in preplant fumigation trials conducted 2003-2013 near Durham and Firebaugh (i.e., at same orchard locations that supplied soils 2, 3, and 8 for the summer 2014 greenhouse bioassay [2013-14 annual report to ABC, Browne et al.] and soils 1, 2, and 10 for the summer 2015 bioassay [**Figure 1**]). A possible explanation for the qualitative but incompletely quantitative correspondence of the orchard and greenhouse responses is that pathogens contributing to the RD complex, as well as their host plants, may be affected differentially under orchard vs. greenhouse conditions. Further evaluation of the field trial-greenhouse bioassay correspondence will be possible as data from additional field trials become available.

Anaerobic soil disinfestation. In 2013 and 2014 experiments, ASD quickly generated and maintained anaerobic conditions and elevated soil temperatures in the 6-wk treatment period (**Figure 2**). As in experiments 1 and 2 at Parlier-KAC, all 2014 ASD and fumigation treatments in experiments 3 and 4 reduced bioassay populations of *P. ultimum* to near or below detection limits, while the inoculum survived at relatively high populations in control treatments (**Table 6**). As indicated by canopy interception of PAR and trunk circumference measurements, ASD in

experiments 1 and 2 provided strong, persistent control of RD, equivalent to that of the fumigation treatments (**Table 7**). Although beneficial compared to the control, the low-rate, narrow-strip ASD treatment of experiment 3 stimulated tree growth less than the high-rate ASD treatments applied to either narrow or wide strips (**Figure 3A**). By midsummer, the high-rate ASD treatments, both in narrow and wide strips and both in experiments 3 and 4, were similar in effectiveness to soil fumigation in preventing PRD growth suppression (**Figure 3 A,B**). Sudan rotation alone provided a small but significant benefit compared to the control (**Fig. 3A**). Repeated soil sampling has indicated that plant parasitic nematodes are not contributing significantly to the PRD complex in the ASD experiments at Parlier, whereas sampling and pathogenicity tests have suggested that *Pythium* and *Cylindrocarpon* species present in the soil play a partial role in the growth suppression.

Our results indicate that commercial adoption of ASD may be feasible but will require further optimization and testing. Total cost of the ASD treatments used in this study ranged from similar to that of the Telone C35 treatment to roughly double that of the fumigation treatment. Rice bran is a relatively expensive component of the tested ASD treatments.
Research is planned to examine the effectiveness of alternative, less expensive ASD substrates and application methods for orchards. It is unknown whether ASD will be effective for management of nematode populations.

Research Effort Recent Publications:

In press:

Browne, G.T.*, and Schmidt, L.S. 2015. First report of *Phytophthora niederhauserii* causing almond tree losses in California. Plant Dis. published online as http://dx.doi.org/10.1094/PDIS-09-14-0995-PDN.

Submitted:

Browne, G.T.*, and Schmidt, L.S. 2015. Pathogenicity of *Pythium* and *Phytopythium* species associated with almond replant disease. Plant Dis.

Extension Activities:

Extension activities of this project in the last year have included:

"*Soilborne disease update for nut growers: a focus on replanting*"; 18 November 2014, Grape, Nut, and Tree Fruit Expo; Fresno, CA; Oral presentation by G.T. Browne.

"*Prune replant issues: insights from almond and peach experience*"; 20 February 2015, Prune Day; Red Bluff, CA; Oral presentation by G.T. Browne

"*An update on almond replant problems and orchard replant development discussion*"; 22 June 2015, Wonderful Orchards (formerly Paramount Farming Company); Shafter, CA, Belridge location; Oral presentation and interdisciplinary discussion including: Dr. A. Westphal, UC Specialist in Nematology; Dr. B. Holtz, UC Farm Advisor in Pomology, Stockton CA; and approximately 25 managers and pest control advisors of Wonderful Orchards. Oral presentation by G.T. Browne featured this project and a focus on planning research for non-fumigant management of RD.

"*Almond Replant Field Day*". The meeting will include multiple talks related to the subject, including "*Prunus replant disease and its implications for almonds*" by G.T. Browne, which

will feature results of this project. Also included will be a visit to KAC field plots that are part of this project.

References Cited:

Browne, G.T., Lampinen, B.D., Holtz, B.A., Doll, D.A., Upadhyaya, S.K., Schmidt, L.S. et al. 2013. Managing the almond and stone fruit replant disease complex with less soil fumigant. *California Agriculture* 67: 128-138.

Udompetaikul, V., Coates, R.W., Upadhyaya, S.K., Browne, G.T., Shafii, M., and Gillis, M. 2013. Tractor-mounted, GPS-based spot fumigation system manages Prunus replant disease. *California Agriculture* 67: 222-227.

Table 1. Rootstock germplasm destined for field trial

Source	Genotype	Genetic background
Aradhya	197-5	peach x P. argentia
	197-6	peach x P. argentia
	197-11	peach x P. argentia
	197-59	peach x P. tangutica
	197-95	peach x P. tangutica
	197-112	peach x P. tangutica
	197-113	peach x P. tangutica
	197-133	peach x P. tangutica
	197-137	peach x P. tangutica
	197-162	peach x P. tangutica
	197-176	peach x P. tangutica
	197-190	peach x P. dulcis
	197-198	peach x P. davidiana
	197-199	peach x P. davidiana
	197-200	peach x P. davidiana
	197-204	peach x P. kensuensis
	197-205	peach x P. kensuensis
	197-206	peach x P. kensuensis
	197-207	peach x P. kensuensis
	197-209	peach x P. kuramica
	197-214	peach x P. bucharica
	197-217	peach x P. kuramica
	198-3	DPRU0194 (P. argentea) OP
	198-13	DPRU0198 (P. webbii) OP
	198-18	Nemaguard x DPRU0582 (P. kansuensis)
	198-19	Tardy Nonpareil almond x P. argentea
	L-1-2	P. cerasifera OP
	P-2-1	Nemared x DPRU0194 (argentea)
	P-2-2	Nemared x DPRU0194 (argentea)
	P-2-4	Nemared x DPRU0194 (argentea)
	P-2-10	Nemared x DPRU0194 (argentea)
	P-2-11	Nemared x DPRU0194 (argentea)
	P-4-1	Nemared x fenzliana
	P-4-10	Nemared x fenzliana
	P-4-25	Nemared x fenzliana
Ledbetter	Nemaguard	peach x P. davidiana
	M2624	Marianna 2624
	Y115-175	*P. kansuensis* x Tskuba No. 4
	SunPEAL	Flordaguard x unknown almond
	P248-100	95-17 peach x GF557
	P58-25	*P. japonica* x Marianna 2624
	7-1	(P. kansuensis x peach)X P. davidiana 'Potanni'
	Y119-199	((peach x almond) x (peach x almond)) x peach
	Y119-246	((peach x almond) x (peach x almond)) x peach
	Ts X FG	Tsukuba No.4 x flordagard
	CA-A1	(P. kansuensis x peach) x peach
	CA-A10	(P. fenzliana x P. dulcis) x (P. davidiana x peach)
	CA-A12	(peach x peach-almond) x peach
	CA-A13	(peach x peach-almond) x peach
	CA-A14	(peach x peach-almond) x (P. davidiana x peach)
	CA-A3	(peach x peach-almond) x peach
	CA-A4	(peach x peach-almond) x peach
	CA-A5	(peach x peach-almond) x (P. davidiana x peach)
	CA-A7	(P. fenzliana x P. dulcis) x (P. davidiana x peach)

Table 2. Site histories and nematode populations of soils used in 2015 greenhouse bioassay

2015 soil number and code[a]	Crop history[b]	Nematode count (per 250 cc)[c]					
		Ring	Lesion	RKN	Dagger	Pin	Free living
1.Durham-Mea.Tri.CK.St	Almond/Lovell, 11 yr	0	0	0	0	62	92
2.Durham-Mtz.Tri.CK.St	Almond/Lovell, 11 yr	0	0	0	2	112	134
3.Durham-Mtz.S.St	Almond/Lovell, >20 yr	0	0	0	0	360	54
4.Durham-Gilb.N.St	Almond/Lovell, >20 yr	0	0	0	0	104	8
5.Durham-Gil.S.St	Almond/Lovell, >20 yr	0	0	0	0	26	22
6.Arbuckle-Nic.Tri.CK.St	Almond/Nemaguard, 6 yr	0	0	0	0	646	64
7.Arbuckle-Hen.St	Almond/Lovell, >20 yr	0	0	0	36	318	6
8.Delhi-Lit.Tri.CK.Cl	Almond/Nemaguard, >20 yr	30	0	0	0	0	54
9.Delhi-Lit.Tri.C35.Cl	Almond/Nemaguard, >20 yr	14	0	0	0	0	132
10.Firebaugh-WO.Tri.CK.St	Almond/Nemaguard, 8 yr	0	0	0	0	883	29
11.Parlier-KAC.Vin.S.St	Vineyard, >20 yr	808	0	15	7	317	149
12.Parlier-KAC.Vin.N.St	Vineyard, >20 yr	56	0	0	22	544	336
13.Parlier-KAC2014.Tri.CK.Cl	Peach/Nemaguard, ca. 12 yr	0	0	0	0	4	248
14.Parlier-KAC2014.Tri.C35.Cl	Peach/Nemaguard, ca. 12 yr	0	0	0	0	0	178
15.Parlier-KAC2014.Tri.ASD.Cl	Peach/Nemaguard, ca. 12 yr	0	0	0	0	0	586
16.Reedley-Klas.N.St	Nectarine/Nemaguard, ca. 12 yr	37	4	0	0	900	35
17.Reedley-Klas.S.St	Peach/Nemaguard, ca 15 yr	0	13	0	0	538	134
18.Sanger-MG.Rep.St	Plum/Nemaguard, 1 yr	0	38	0	0	45	70
19.Sanger-LTB.Hc.Cl	Almond/Nemaguard, >20 yr	0	0	0	0	186	146
20.Sanger-LTB.Rc.Cl	Almond/Nemaguard, >20yr	29	0	0	1	941	80
21.Traver-Famt.St	Nectarine/Nemaguard, ca. 15 yr	0	0	0	27	662	92
22.Shafter-3901.K&B.St	Almond/Nemaguard, >20 yr	892	184	3	38	179	42
23.Shafter-WO.3010.S.St	Almond/Nemaguard, >20 yr	0	0	0	0	268	34
24.Shafter-WO.3010.N.Stb	Almond/Nemaguard, >20 yr	0	0	0	0	184	33
25.Belridge-WO.3540.196.St	Almond/Nemaguard, >20 yr	0	0	0	0	824	58
26.Belridge-WO.3580.211.St	Almond/Nemaguard, >20 yr	0	4	0	45	500	89

[a] Soil location number is followed by nearest city or landmark and additional coded information. In code text, “Vin” indicates soil was from vineyard (all other soils were from almond or stone fruit orchards) “Tri” indicates that location had hosted or is hosting fumigation trial; “C35” indicates that soil was treated with Telone C35 before collection from the field; “ASD” indicates that soil was treated with anaerobic soil disinfestation before collection from the field; “CK” indicates soil was from control plots that did not receive C35 or ASD; “St” indicates standing orchard or vineyard; “Cl” indicates cleared orchard.

[b] Years are estimates

[c] Based on sugar flotation method. “RKN” indicates root knot nematode.

Table 3. Selected physical and chemical parameters of soils used in 2015 greenhouse bioassay

2015 soil number and code[a]	Soil series according to online soil survey	Measured parameters[b]														
		Sand (%)	Silt (%)	Clay (%)	Texture by analysis	pH	EC (dS/m)	Ca (meq/L)	Mg (meq/L)	Na (meq/L)	SAR	ESP (%)	CEC (meq/100g)	N (Total) (%)	exch. K (ppm)	exch. K (meq/100g)
1.Durham-Mea.Tri.CK.St	Edjobe silty clay	36	37	27	clay loam	7.81	0.77	3.56	3.19	0.77	<1	<1	27.2	0.058	179	0.46
2.Durham-Mtz.Tri.CK.St	Conejo clay loam	55	30	15	sandy loam	7.91	0.82	3.81	2.68	0.74	<1	<1	23.7	0.056	276	0.71
3.Durham-Mtz.S.St	Conejo/Busacca clay loam	63	28	9	sandy loam	7.95	0.96	4.66	3.02	1.06	<1	<1	23.6	0.054	119	0.30
4.Durham-Gilb.N.St	Almendra loam	41	37	22	loam	7.08	0.70	2.90	2.70	0.85	<1	<1	31.8	0.094	153	0.39
5.Durham-Gil.S.St	Conejo clay loam	41	32	27	clay loam	6.95	0.55	2.24	1.99	0.73	<1	<1	37.1	0.090	134	0.34
6.Arbuckle-Nic.Tri.CK.St	Arbuckle sandy loam	65	22	13	sandy loam	5.75	0.81	2.89	1.81	2.71	2	1	9.8	0.037	76	0.19
7.Arbuckle-Hen.St	Arbuckle-Hillgate complex	61	27	12	sandy loam	5.61	1.44	5.45	4.65	3.61	2	1	11.0	0.041	93	0.24
8.Delhi-Lit.Tri.CK.Cl	Delhi sand	91	9	<1	sand	6.34	1.07	5.50	2.38	1.65	<1	<1	3.2	<0.020	32	0.08
9.Delhi-Lit.Tri.C35.Cl	Delhi sand	92	8	<1	sand	6.80	0.50	2.63	1.00	0.83	<1	<1	2.8	<0.020	24	0.06
10.Firebaugh-WO.Tri.CK.St	Dinuba/El Peco fine sandy loam	77	16	7	sandy loam	7.85	2.98	14.39	2.39	16.00	6	6	6.0	0.038	254	0.65
11.Parlier-KAC.Vin.S.St	Hanford fine sandy loam	62	32	6	sandy loam	7.34	0.59	2.87	1.41	1.31	<1	<1	4.1	0.024	52	0.13
12.Parlier-KAC.Vin.N.St	Hesperia fine sandy loam	57	35	8	sandy loam	7.57	0.60	2.74	1.21	1.75	1	<1	6.5	0.029	63	0.16
13.Parlier-KAC2014.Tri.CK.Cl	Hanford fine sandy loam	66	28	6	sandy loam	7.55	1.81	7.54	3.73	5.80	2	2	6.0	0.024	50	0.13
14.Parlier-KAC2014.Tri.C35.Cl	Hanford fine sandy loam	66	29	5	sandy loam	7.12	1.69	7.72	3.93	4.15	2	1	5.8	0.026	51	0.13
15.Parlier-KAC2014.Tri.ASD.Cl	Hanford fine sandy loam	68	26	6	sandy loam	6.43	1.26	6.47	3.46	1.33	<1	<1	6.5	0.030	64	0.16
16.Reedley-Klas.N.St	Hanford course sandy loam	78	15	7	sandy loam	6.80	1.04	5.48	2.76	1.56	<1	<1	6.7	0.021	77	0.20
17.Reedley-Klas.S.St	Greenfield sandy loam	73	19	8	sandy loam	7.28	2.94	21.32	10.17	3.84	<1	<1	8.0	0.033	65	0.17
18.Sanger-MG.Rep.St	Hanford sandy loam	55	42	3	sandy loam	6.79	1.62	6.66	7.13	2.08	<1	<1	7.1	0.027	58	0.15
19.Sanger-LTB.Hc.Cl	Hanford sandy loam	71	24	5	sandy loam	6.18	1.02	4.70	3.12	1.55	<1	<1	4.5	0.028	51	0.13
20.Sanger-LTB.Rc.Cl	Ramona loam	70	22	8	sandy loam	6.68	0.78	2.48	3.09	1.58	<1	<1	9.3	0.026	92	0.24
21.Traver-Famt.St	Calgro complex	68	23	9	sandy loam	7.60	1.29	5.94	1.92	4.47	2	2	7.5	0.032	79	0.20
22.Shafter-3901.K&B.St	Wasco sandy loam	86	10	4	loamy sand	6.07	1.78	8.72	1.53	7.19	3	3	4.3	<0.020	45	0.11
23.Shafter-WO.3010.S.St	Wasco sandy loam	72	17	11	sandy loam	7.57	1.99	7.16	1.08	12.24	6	7	6.3	0.020	117	0.30
24.Shafter-WO.3010.N.Stb	Driver coarse sandy loam	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
25.Belridge-WO.3540.196.St	Milham sandy loam	66	18	16	sandy loam	7.68	3.30	19.34	5.98	11.38	3	3	12.0	0.039	99	0.25
26.Belridge-WO.3580.211.St	Panoche clay loam	45	31	24	loam	7.79	3.02	16.13	4.34	12.46	4	4	18.1	0.062	132	0.34

[a] Soil location number is followed by nearest city or landmark and additional coded information. In code text, "Vin" indicates soil was from vineyard (all other soils were from almond or stone fruit orchards); "Tri" indicates that location had hosted or is hosting fumigation trial; "C35" indicates that soil was treated with Telone C35 before collection from the field; "ASD" indicates that soil was treated with anaerobic soil disinfestation before collection from the field; "CK" indicates soil was from control plots that did not receive C35 or ASD; "St" indicates standing orchard or vineyard; "Cl" indicates cleared orchard or vineyard;

[b] Texture analysis by suspension settling / hydrometer method. "--" indicates no data available. pH measured in 1:10 dilution of soil with water. "EC" (electrical conductivity) and concentrations of cations (Ca, Mg, and Na) measured quantitatively in saturated paste extract by atomic emission spectometry. "SAR" (sodium absorption ratio) and "ESP" (exchangeable sodium percentage) based on concentrations of Ca, Mg, and Na. "CEC" (cation exchange capacity) based on barium displacement method. "N total" measured by Kjeldahl method. Exchangeable K measured semi-quantitatively based on displacement with ammonium acetate solution.

Table 4. Status of orchard replant trials conducted for purposes of bioassay validation and spot fumigation demonstration

Year field plots treated	"Location" of field plots	Designation of soil in greenhouse bioassays, year(s) tested in greenhouse bioassays[a]	Field treatment	Field treatment details			Increase in circumference (cm)	
				Fumigant rate, lb per treated acre (& kg/ha)	Proportion of treated area	Fumigant per orchard acre, lb (& kg/orchard ha)	First growing season (95 % CI)[b]	Second growing season (95 % CI)[c]
2013	Crows Landing	5. GoT soil, 2013-14	Control	0	0.00	0	7.8 (6.0-9.6)	--
			Telone C35, strip	520 (582)	0.53	276 (309)	11.2 (9.5-12.9)	--
			Telone C35, spot	520 (582)	0.21	109 (122)	10.4 (8.6-12.1)	--
	Kerman	9. AvT soil, 2013-14	Control	0	0.00	0	9.7 (8.9-10.4)	--
			Chloropicrin, strip	350 (392)	0.38	133 (149)	12.2 (11.5-12.9)	--
			Chloropicrin, spot	350 (392)	0.19	67 (75)	11.8 (11.0-12.5)	--
	Parlier, trial A	12. Parlier KAC, 2013-14	Control	0	0.00	0	3.7 (3.2-4.2)	16.4 (15.1-17.7)
			Telone C35, strip	540 (605)	0.58	313 (350)	7.0 (6.6-7.5)	25.6 (24.3-26.9)
	Parlier, trial B	12. Parlier KAC, 2013-14	Control	0	0.00	0	3.3 (2.6-4.0)	17.1 (16.0-18.1)
			Telone C35, strip	540 (605)	0.58	313 (350)	7.1 (6.7-8.1)	27.3 (26.2-28.3)
2014	Delhi	8. Delhi-Lit.Tri.CK.Cl, 2015	Control	0	0.00	0	--	--
			Telone C35, strip	540 (605)	0.50	270 (303)	--	--
			Telone C35, spot	540 (605)	0.19	103 (115)	--	--
	Parlier, trial C	13.Parlier-KAC2014.Tri.CK.Cl, 2015	Control	0	0.00	0	2.2 (1.8-2.6)	--
			Telone C35, strip	540 (605)	0.58	313 (350)	5.1 (4.8-5.5)	--
	Parlier, trial D	13.Parlier-KAC2014.Tri.CK.Cl, 2015	Control	0	0.00	0	2.6 (2.1-3.1)	--
			Telone C35, strip	540 (605)	0.58	313 (350)	4.9 (4.4-5.4)	

[a] Soils tested in 2013-14 bioassays described in 2014 annual report to CalDPR, Browne et al., 2014; soils tested in 2015 bioassay described in Tables 2,3 in this report.

[b] Measured from winter 2014 to winter 2015.

[c] Measured from winter 2015 to July 2015

Table 5. Overview of trials testing anaerobic soil disinfestation and other preplant treatments near Parlier at Kearney Agricultural Center

Year	Expt.	Trt. no.	Treatment name	Month of old orchard tree removal	Month of sudan rotation	Fall/winter soil disinfestation treatment
2013	1	1	Control, no sudan	Sep	None	None
		2	Control, with sudan	May	May-Oct	None
		3	ASD, high bran rate, wide strip, with sudan	May	May-Oct	ASD, 20 metric tons /treated ha, 3.0-m-wide strips
		4	Fumigation in Oct, no sudan	Sep	No	Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips
		5	Fumigation in Oct, with sudan	May	May-Oct	Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips
		6	Fumigation in Dec, no sudan	Sep	None	Telone C35, 600 kg/treated ha in Dec, 3.4-m-wide strips
	2	1	Control, no sudan	May	None	None
		2	ASD, high bran rate, wide strip, no sudan	May	None	ASD, 20 metric tons /treated ha, 3.0-m-wide strips
		3	Fumigation in Oct, no sudan	May	None	Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips
2014	3	1	Control, no sudan	Sep	None	None
		2	Control, with sudan	May	May-Oct	None
		3	ASD, high bran rate, wide strip, with sudan	May	May-Oct	ASD, 20 metric tons /treated ha, 3.0-m-wide strips
		4	ASD, high bran rate, narrow strip, no sudan	Sep	None	ASD, 20 metric tons /treated ha, 1.8-m-wide strips
		5	ASD, low bran rate, narrow strip, no sudan	Sep	None	ASD, 12 metric tons /treated ha, 1.8-m-wide strips
		6	Fumigation in Oct, no sudan	Sep	None	Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips
		7	Fumigation in Oct, with sudan	May	May-Oct	Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips
	4	1	Control, no sudan	May	None	None
		2	ASD, high bran rate, wide strip, no sudan	May	None	ASD, 20 metric tons /treated ha, 3.0-m-wide strips
		3	Fumigation in Oct, no sudan	May	None	Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips

Table 6. Effects of preplant treatments on survival of *Pythium ultimum* in buried bags of ASD trials near Parlier at Kearney Agricultural Center

Expt.	Trt. no.	Treatment	Depth of bioassay inoculum in soil	Survival of bioassay inoculum	
				Mean	(S.E. of Mean)
3	1	Control, no sudan	15	2315	(70)
			46	1998	(471)
	2	Control, with sudan	15	2330	(556)
			46	2030	(363)
	3	ASD, high bran rate, wide strip, with sudan	15	0	(0)
			46	0	(0)
	4	Fumigation in Oct, no sudan	15	0	(0)
			46	190	(190)
	5	Fumigation in Oct, with sudan	15	0	(0)
			46	0	(0)
	6	ASD, high bran rate, narrow strip, no sudan	15	0	(0)
			46	0	(0)
	7	ASD, low bran rate, narrow strip, no sudan	15	0	(0)
			46	5	(5)
4	1	Control, no sudan	15	3663	(354)
			46	2008	(284)
	2	Fumigation in Oct, no sudan	15	0	(0)
			46	8	(8)
	3	ASD, high bran rate, wide strip, no sudan	15	0	(0)
			46	0	(0)

Table 7. Effects of preplant treatments on second-year growth of trees in experiments 1 and 2 near Parlier at the Kearney Agricultural Center (treatment details in **Table 6**)

Expt.	Treatment	Increase in trunk circumference by Nov 2014 (cm)	Increase in trunk circumference by July 2015 (cm)	% PAR June 2015
1	Control, no sudan	3.7	16.4	11.9
	Control, with sudan	4.9	19.1	14.2
	ASD, hi bran rate, wide strip, with sudan	7.4	27.0	24.0
	Fumigation in Oct, no sudan	7.0	25.6	20.8
	Fumigation in Oct, with sudan	7.5	26.6	22.3
	Fumigation in Dec, no sudan	6.7	24.7	20.0
	(95% confidence limits):	*(+/- 0.5)*	*(+/-1.3)*	*(+/- 1.9)*
2	Control, no sudan	3.3	27.1	12.8
	ASD, hi bran rate, wide strip, no sudan	7.1	27.3	26.6
	Fumigation in Oct, no sudan	7.4	17.1	24.8
	(95% confidence limits):	*(+/-0.7)*	*(+/- 1.0)*	*(+/- 2.1)*

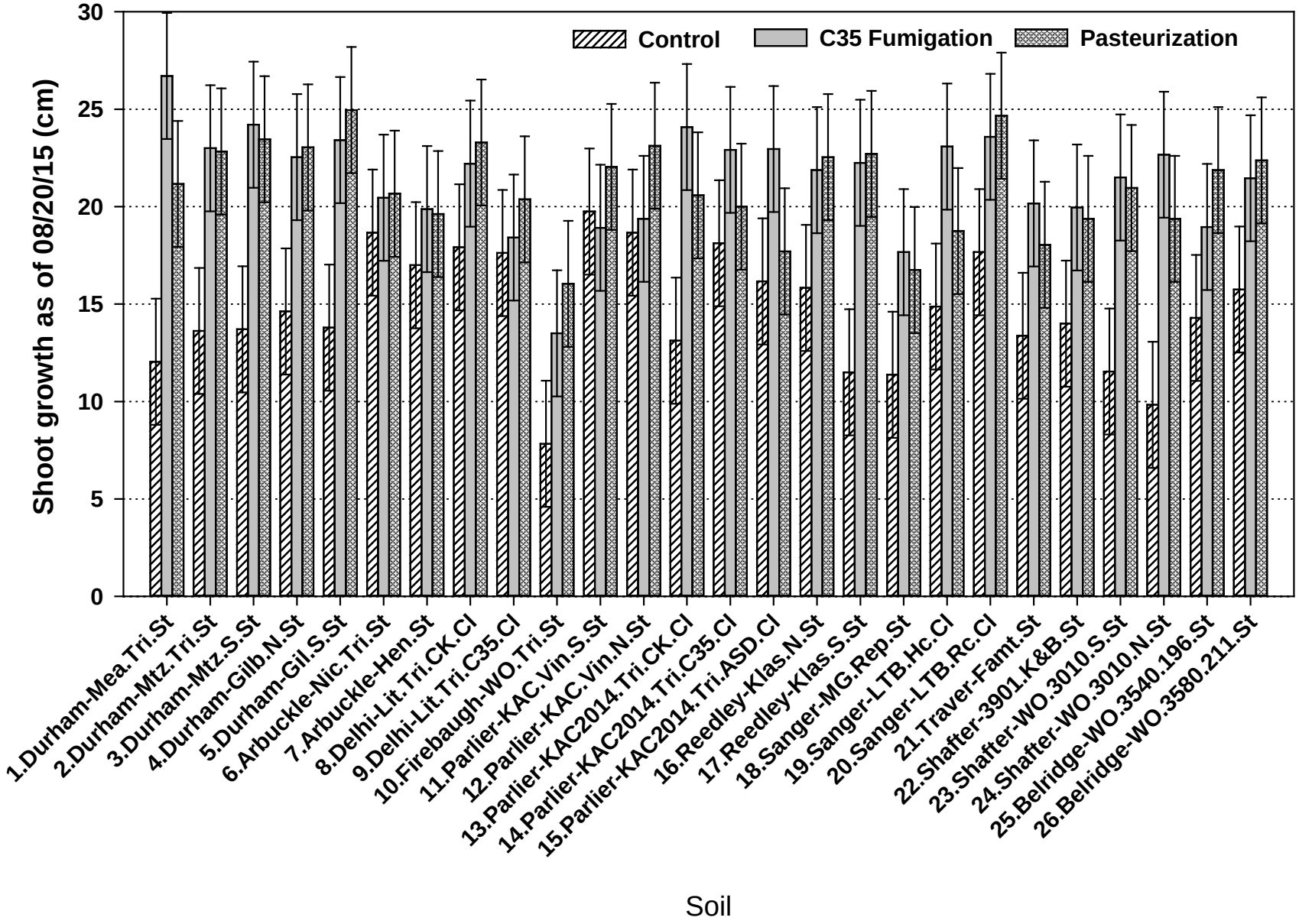

Figure 1. Response of 'Nemaguard' rootstock seedlings to preplant soil treatments in 2015 greenhouse bioassay of 26 soils as of 20 Aug 2015.

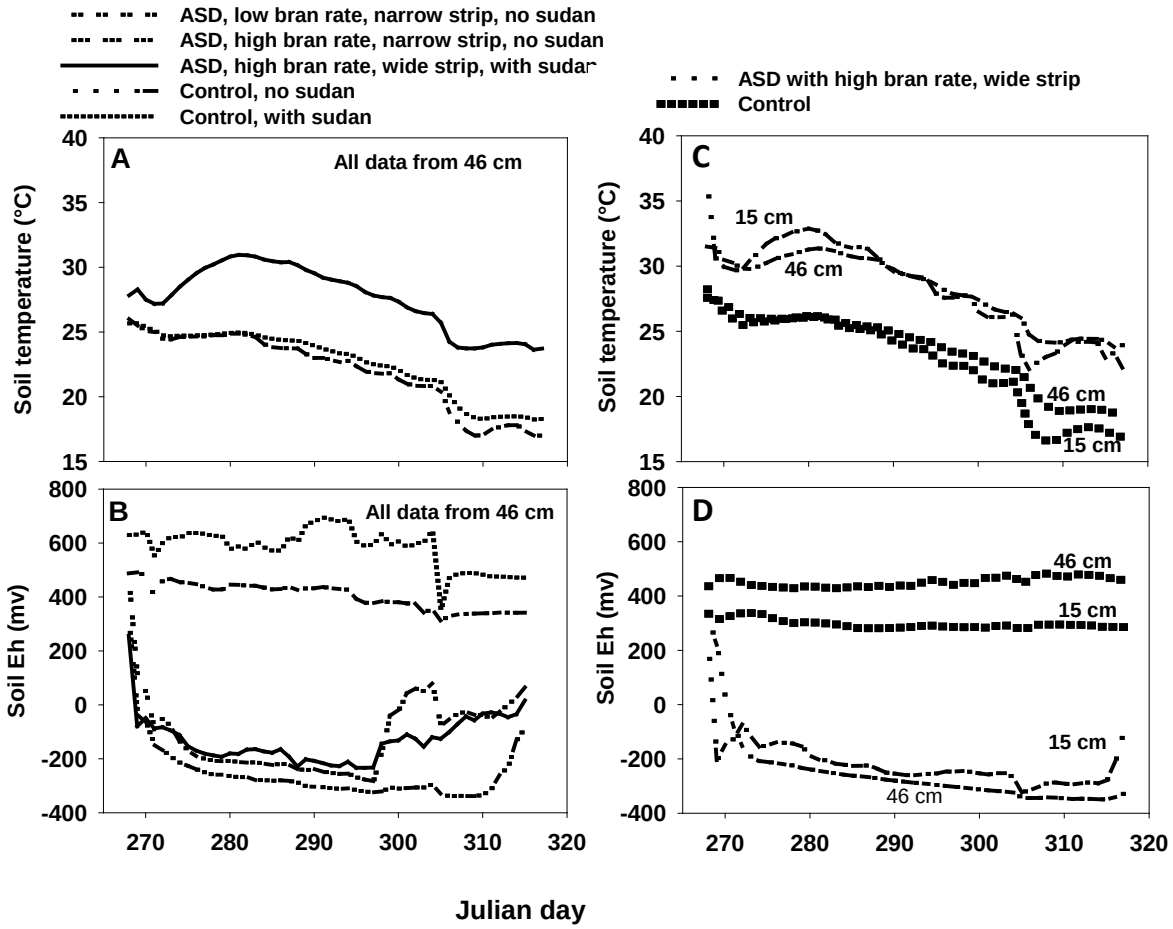

Figure 2. A and **B,** Effects of ASD and control treatments on temperature and reduction potential in soil, experiment 3; **C** and **D**, effects of ASD and control treatments on temperature and reduction potential in soil, experiment 4.

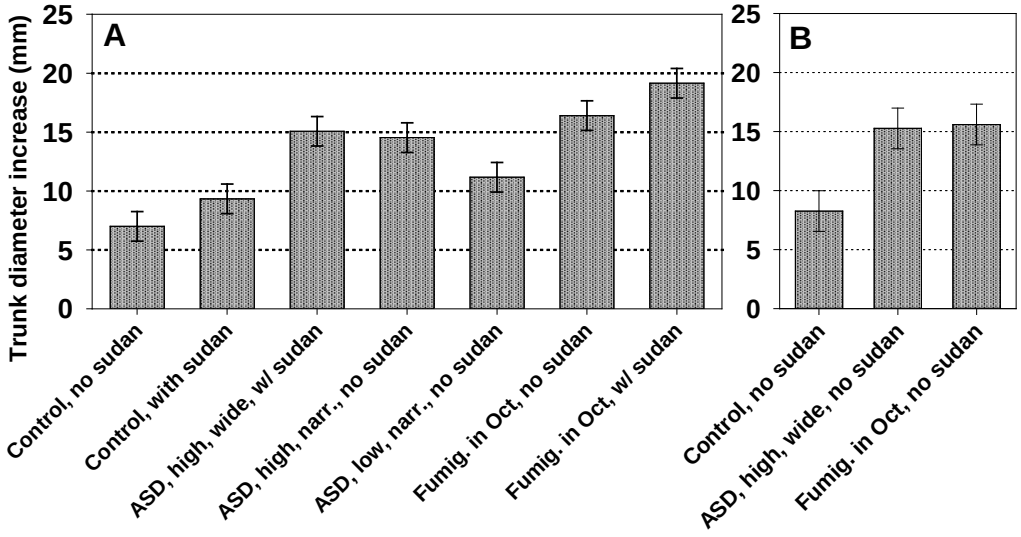

Figure 3. A, and **B**, effects of preplant treatments on growth of trees in 2014 experiments 3 and 4, respectively. Shown are increases in trunk diameter from the time of planting (Jan 2015) to Jul 2015.