Developing Improved Strategies for Management of Replant Problems

Project No.: **13-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 goal of this project is to strengthen management strategies for almond replant problems, especially 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. Our approach to this goal is to investigate the complex biology underlying RD while improving practical control strategies for RD and other replant problems. In this report, we feature: 1) continuing work on the underlying biology of RD (under objective 1); 2)

use of a greenhouse bioassay to predict RD severity (and the need to fumigate) and examine RD causes among Central Valley orchards (under objectives 1 &2); and 3) efficacy of preplant soil ripping depths, fumigation dates, sudan grass rotation, and anaerobic soil disinfestation (ASD) for management of RD (under objective 2).

Our 2013-14 research on underlying RD biology focused on determining whether interactions among *Pythium*, *Cylindrocarpon* (fungal-like and fungal organisms, respectively), and *Trichoderma* (a fungus) have important impacts on RD severity. In past almond replant trials, we found that soil fumigation stimulated root populations of *Trichoderma* spp. and reduced root populations of *Pythium* spp. and *Cylindrocarpon* spp. A greenhouse trial was established to determine if *Trichoderma* may directly stimulate growth of almond rootstocks or if *Trichoderma* may protect the roots from infection by *Pythium* and *Cylindrocarpon*. Nemaguard rootstock seedlings were planted in soil infested with mixtures of *P. ultimum* isolates, mixtures of *C. macrodidymum* isolates, and mixtures of *Trichoderma* isolates. Additional treatments included co-inoculations of the soil with *Trichoderma* and *C. macrodidymum* and with *Trichoderma* and *P. ultimum*. Only *P. ultimum* caused significant plant weight reductions, and *Trichoderma* did not significantly increase or decrease plant growth, with or without either of the pathogens. These results did not support the hypothesis that *Trichoderma* impacts growth of replanted orchards, but they did emphasize important contributions of *P. ultimum* to the disease complex.

In additional trials on RD biology, *Pythium helicoides*, which we previously found associated with almond tree decline in recently replanted and mature almond orchards, was aggressive on Nemaguard rootstock, causing root and crown rot. Periods of soil flooding greatly increased disease severity but were not required for disease. These findings indicate that *P. helicoides* can contribute, along with other organsims and factors, to RD and decline of almond trees and that soil water saturation can favor the disease development. In future work, we plan to determine whether phosphonate treatments can reduce disease losses caused by *P. helicoides* and closely related *Pythium* species that contribute to RD.

With funding from the California Dept. of Pesticide Regulation, we expanded our research on use of a greenhouse-based Nemaguard peach bioassay to predict RD severity (and the need to fumigate) and examine microbial contributions to the disease among diverse “replant” soils. The soils were collected from 20 almond and stone fruit orchards in northern, central, and southern portions of the Central Valley in Fall 2013. Two bioassay experiments were completed with the soils. Each experiment included one or more preplant soil treatments expected to prevent RD (i.e., pasteurization, fumigation) and a control. Soils that supported greater bioassay plant growth after the treatments, compared to the control, were considered likely to benefit from preplant fumigation in orchard replant settings. Responses of Nemaguard peach seedlings to the preplant treatments varied greatly among the 20 soils. In experiment 1 (conducted in Fall/Winter 2013/14), preplant soil pasteurization increased total bioassay plant weights by 0 to 42%, compared to the non-treated controls. In experiment 2 (conducted in Spring/Summer 2014), preplant soil pasteurization increased total plant weights by 8 to 268%, and preplant fumigation increased total plant weights by 19 to 351%. Across the soils and experiments, growth suppression was associated with root incidence of *Pythium* spp. and *C. macrodidymum*, and in experiment 2 the percentage increase in plant weight resulting from pasteurization and fumigation (i.e., compared to the control) was positively correlated with soil

pH. Overall, the bioassay results indicate that there is potential for use of soil sampling to predict RD severity and that *Pythium* spp. and *C. macrodidymum* may play at least a partial role in RD etiology in many almond replant soils. The fact that plants in the Fall/Winter bioassay (2013/14) benefited less from pasteurization than those in the Spring/Summer bioassay (2014) suggests that time of year may affect development of RD in the bioassay. We will continue our bioassay experiments to learn more about RD and its prediction statewide.

We conducted two orchard replant trials at the Kearney Agricultural Center (KAC) to explore the potential for control of RD using non-fumigant replant remediation treatments, including deep soil ripping, sudan grass rotation, and anaerobic soil disinfestation (ASD). ASD was generated by incorporating a readily available carbon substrate into soil while maintaining soil moisture near field capacity and using an impermeable tarp to elevate soil temperature and reduce gas exchange.The KAC experiments also included preplant soil fumigation and non-treated control treatments, and they compared the efficacy of fall vs. winter fumigation. Both KAC trials were conducted where peaches had grown on Nemaguard rootstock for >12years in Hanford sandy loam soil. In Spring and Summer 2014, significant growth suppression of the replanted trees resulted without preplant remediation treatments in both experiments. The ASD treatments quickly generated and maintained anaerobic conditions in the 6-wk treatment period during which they were imposed. All preplant soil fumigation and ASD treatments markedly reduced inoculum survival in bioassays with *P. ultimum* and markedly stimulated tree growth in the replanted orchard. There was no significant impact of preplant soil ripping depth (i.e., 2 vs. 4 ft), and sudan grass rotation had little effect on replanted tree growth. Our results indicate ASD has excellent potential as a non-fumigant method to prevent RD; further optimization of ASD is justified and will be pursued. A key focus will be to reduce ASD costs while retaining the efficacy.

Materials and Methods:

Objective 1. Determine causes of RD.

Interactions among *Trichoderma*, *Pythium*, and *Cylindrocarpon* species. In past almond replant trials, we found that soil fumigation stimulated root populations of *Trichoderma* spp. and reduced root populations of *Pythium* spp. and *Cylindrocarpon* spp. We conducted a greenhouse trial to investigate whether the positive growth response to fumigation in replanted orchards may result from direct growth stimulation by *Trichoderma*, or whether the response may be due to *Trichoderma* protecting roots from infection by *Pythium* and *Cylindrocarpon*.

Nemaguard rootstock seedlings were planted in 20-oz pots of potting soil infested with mixtures of *P. ultimum* isolates, mixtures of *C. macrodidymum* isolates, and mixtures of *Trichoderma* isolates. Each organism was grown on a V8 juice oat medium substrate, then the colonized substrate (and sterile control substrate) was used to inoculate soil at 5% and 10% rates by volume. Additional treatments included co-inoculations of the soil at 5 and 10% rates with mixtures of *Trichoderma* and *C. macrodidymum* and with mixtures of *Trichoderma* and *P. ultimum*. Nemaguard peach seedlings were grown for 2 months in soil given each of the treatments to determine effects of each inoculant or inoculant mixture on RD severity. RD severity assessments were based on plant fresh weights and severity of root cortex necrosis at the end of the experiment.

Pathogenicity of *Pythium helicoides*. We tested *Pythium helicoides* for its ability to cause almond root disease. Although *P. helicoides* is not known as an almond pathogen we have it in young almond and stone fruit trees affected by RD and also in mature almond trees affected by decline. Nemaguard peach seedlings were transplanted into potting soil artificially infested with *P. helicoides* or sterile control inoculum substrate. Half of the plants were watered only as needed, without flooding, while the other plants were subjected to 48-h soil flooding periods once every 2 weeks and otherwise watered as needed. There were 8 to 10 plants per treatment combination, arranged in four or five complete blocks, depending on the experiment. Two months after transplanting, the seedlings were washed free from soil, weighed, and rated for severity of root and crown rot.

Objective 2. Support development of strategic approaches to management of RD.

Use of a greenhouse bioassay to predict RD severity and examine RD causes in different soils. For bioassays, soil samples were collected from 20 almond and stone fruit orchard locations in northern, central, and southern portions of the Central Valley from 23 October to 8 November 2013 (**Table 1**). The samples were collected from 0.3- to 2.0-ft depths from four random spots in each orchard using hand augers (3" diameter). Orchards 1-4, 6, 8, 11, 13, and 16-18 were standing when samples were collected, whereas trees had been uprooted or removed from the other sampled orchards. All of the sites had been used for almond or stone fruit production, but site 5 had an intervening rotation with alfalfa in 2013 after removal of its almond orchard in 2012. Samples from the same orchard were pooled and mixed, then used for pH measurement, nematode quantification, and bioassay experiments.

Bioassay experiment 1 was conducted in a fall/winter period as follows: Subsamples of each soil were mixed with sterile sand (2 vol. soil to 1 vol. sand). The mixed, sand-amended soils were subdivided into two portions; one for a non-treated control and the other for preplant pasteurization (i.e., pasteurization achieved in a 5-gal steaming apparatus that brought soil temperature to $\geq$80 C [176 F] for 30 min). On 25 November 2013, soil from each of the orchard locations and soil treatments was distributed to 10 1-liter (32-oz) pots and planted with recently sprouted Nemaguard peach seedlings in a greenhouse. The treatments were arranged in a randomized complete block design with five blocks. Each block had two potted Nemaguard seedlings (i.e., subplots) per soil location and soil treatment combination. The plants were watered daily/as-needed and fertilized with complete liquid fertilizer once per week. Final assessments of experiment 1 were made 5 Feb 2014. At this time the plant top and root fresh weights were determined, and the roots were washed free from soil and visually evaluated to estimate the percentage of root cortex length that was necrotic (brown or black in color, compared to white, healthy root cortex tissue). The potential for RD was assessed according to plant fresh weight suppression and root cortex necrosis in the non-treated control soil, as compared to values in the pasteurized soil.

Experiment 2 was conducted during spring/summer. It included, in addition to the control and pasteurization treatments, a preplant soil fumigation treatment with chloropicrin (CP). The soil to receive CP was bagged doubly in polyethylene, and then placed inside a 5-gal bucket that was lined with a sheet of TIF (totally impermeable film; Vaporsafe, www.ravenag.com). After inserting the bags of soil, the TIF was sealed shut around the soil, and CP was injected into

the soil (3 ml [0.1 fl. Oz.] CP per 14 liters [15 qts.] soil). On 23 April 2014, 3 weeks after fumigation was completed and the soil had vented thoroughly, all soil treatments were placed in pots and planted with Nemaguard peach seedlings. Results of experiment 2, which was otherwise conducted as described for experiment 1, were measured 25 June 2014.

Comparing fumigant and non-fumigant treatments for management of RD. We conducted two orchard replant trials at the Kearney Agricultural Center (KAC) to explore the potential for control of RD using non-fumigant replant remediation treatments, including deep soil ripping, sudan grass rotation, and anaerobic soil disinfestation (ASD). ASD is a preplant remediation treatment that produces pathogen-suppressing anaerobic conditions, organic acids, etc. “ASD conditions” are generated and maintained for several weeks by incorporating a readily available carbon substrate into soil while maintaining soil moisture near field capacity and using an impermeable tarp to elevate soil temperature and reduce gas exchange (Shennan et al., 2007). The KAC experiments also included preplant soil fumigation and non-treated control treatments, and they compared the efficacy of fall vs. winter fumigation. Both KAC trials were conducted where peaches had grown on Nemaguard rootstock for >12years in Hanford sandy loam soil.

Additional details of the KAC replant trials included: in the first trial, the six preplant treatments, administered after removal of the old peach trees and before replanting with almond on Nemaguard rootstock in January 2014, were: 1) the old peach trees were removed in September with no further preplant treatment before replanting (i.e., the “No-sudan control”), 2) the peach trees were removed in May, followed by a sudan grass rotation and no further treatment (i.e., the “sudan control”), 3) after peach tree removal in May, the sudan rotation was followed by anaerobic soil disinfestation (i.e., “Sudan + ASD”), 4) peach trees removed in September, followed by October soil fumigation (i.e., “No sudan + Oct. fumigation”), 5) peach trees removed in May, followed by sudan rotation and October soil fumigation(i.e., sudan + Oct. fumigation), and 6) the peach trees were removed in September, followed by December soil fumigation (i.e., “No sudan + Dec. fum.). In the first KAC trial, after sudan rotation, the ASD treatment was generated as follows: dry rice bran (9 tons/acre [20 metric tons ha^{-1}]) was incorporated into a 6” (15-cm) soil depth, covered with clear plastic film (TIF), and irrigated continuously for 50 h (10” water [25 cm] water, 1 drip emitter per sq. ft. [930 cm^2]). ASD was maintained for 6 wk by irrigating daily for 1 h (0.2” [0.5 cm] water). Molasses was “drip” applied (4.5 tons/ac [9 metric tons ha^{-1}]) 3 wk after the bran.

In the second KAC replant trial, all peach trees were removed in May, and the land was bare fallowed before replanting with almond on Nemaguard rootstock in January 2014. The treatments included: deep (4-ft.) and shallow (2-ft.) soil ripping in factorial combinations with 1) no treatment (i.e., “No-sudan control), 2) ASD (“No-sudan + ASD”), or 3) soil fumigation in October (“No sudan + Oct. fumigation”). In the second trial, ASD was implemented similarly as in the first trial, except without sudan grass and molasses.

Both KAC trials included non-treated “negative controls” and preplant-fumigated (Telone C35, 550 lb/ac [610 kg ha^{-1}]) “positive controls”. All treatments were applied in a randomized complete block design to five (experiment 1) or three (experiment 2) replicate 10’ x 90’ (3x27-m) plots centered on rows where the almond trees were later replanted.

To provide an initial assay of the KAC treatment efficacy, bags of soil (1 oz [30-ml]) infested with *Pythium ultimum* (an RD pathogen) were buried at 6 and 18" [30 and 45 cm] depths in ASD, fumigation, and control plots before the treatments were applied. After the treatments, the bags were retrieved and assayed for *P. ultimum* viability.

Both KAC trials were replanted on a "20 ft x 10 ft" spacing. Nemaguard seedlings were later established as interplants between the almond trees to facilitate destructive root sampling for microbial analyses. Efficacy of the treatments was assessed according to almond tree and interplant seedling growth.

Results and Discussion:

Objective 1. Determine causes of RD.

Interactions among *Trichoderma*, *Pythium*, and *Cylindrocarpon* species. In the greenhouse trial examining impacts of these organisms on Nemaguard rootstock, *P. ultimum* caused severe plant weight reductions, but *Cylindrocarpon* did not (**Figure 1**). *Trichoderma* did not significantly increase or decrease plant growth, with or without either of the pathogens. These results provided no support for a hypothesis that *Trichoderma* impacts growth of replanted orchards, but they did confirm contributions of *P. ultimum* to the RD complex. Nevertheless, it is very possible that the dynamics of microbial populations in field soil differ in important ways from those simulated in greenhouse trials, so it is not justified to completely discount a disease-suppressive role of *Trichoderma* in RD.

Pathogenicity of *P. helicoides*. Four of the five tested isolates of *P. helicoides* caused root and crown rot and reduced plant weights on Nemaguard rootstock in each of two experiments (**Table 2**). Biweekly episodes of soil flooding, intended to mimic prolonged soil water saturation resulting from over irrigation or inadequate drainage, dramatically increased severity of the disease, but the pathogenic isolates caused some disease even without prolonged soil saturation. In experiment 2, the pathogenic isolates caused disease on plants inoculated at 2 weeks of age (small plants, **Table 2**) and on plants inoculated at 10 weeks of age (big plants, **Table 2**).

Collectively, the experimental results suggest that, when it is present in a soil, *P. helicoides* can contribute to growth suppression and decline of almond trees and that soil water saturation can increase severity of the disease. In future work, we plan to determine whether phosphonate treatments may help to prevent disease losses to *P. helicoides* and other closely related RD pathogens.

Objective 2. Support development of strategic approaches to management of RD.

Use of a greenhouse bioassay to predict RD severity and examine RD causes in different soils. In bioassay experiment 1, the preplant soil pasteurization treatment increased total plant weights by 0 to 42%, compared to the non-treated control (**Figure 2**). The total plant weights exhibited significant soil treatment × location interaction (i.e., the response to pasteurization differed among soils from the different locations) (P<0.0001). Similar soil treatment × location interactions were apparent when top and root weights were analyzed

separately (data not shown; soil treatment × location interaction significant at *P*=0.004 to <0.0001). Based on non-overlapping confidence intervals, preplant soil pasteurization significantly increased total plant weights at locations 2, 7, 9, 10, 13, 14, 15, and 17 (**Figure 2**). In non-pasteurized soil from all locations, Nemaguard peach seedlings' roots exhibited a pronounced darkening of the root cortex tissues (i.e., the tissues external to the root stele) (**Figure 2**). Pasteurization dramatically reduced severity of root cortex necrosis in soil from all locations. The percentage of root cortex necrosis was affected by location (*P*<0.0001) and soil treatment (*P*<0.0001), but there was not significant interaction of location x soil treatment (*P*=0.14). Total plant weights in experiment 1 were negatively correlated with root incidence of *Pythium* spp. (r= -0.42, *P*=0.007) and of *C. macrodidymum* (r= -0.598, *P*=0.001) (**Figures 2, 3**). Across the soils, the percentage increase in plant weight resulting from pasteurization (i.e., as compared to plant weights in the non-treated control treatment) was not significantly correlated with pH of the collected soils (**Table 1**) (*P*=0.14). Soil fumigation was not included as a soil treatment in experiment 1.

In experiment 2, soil pasteurization increased total plant weights by 8 to 268%, and soil fumigation increased total plant weights by 19 to 351%, compared to the control (**Figure 4**). All of the measured plant growth variables, i.e., total plant weight (**Figure 4**), top weight (not shown), root weight (not shown) and percentage of root cortex necrosis (**Figure 4**) exhibited significant soil treatment x soil location interactions (*P*=0.0009 to <0.0001). Based on non-overlapping confidence intervals, preplant soil pasteurization and soil fumigation significantly increased total plant weights at locations 1, 2, 3, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17, and 20 (**Figure 4**). Total plant weights in experiment 2 were negatively correlated with root incidence of *Pythium* spp. (r= -0.67, *P*<0.0001) and of *C. macrodidymum* (r= -0.67, *P*<0.0001) (**Figures 4, 5**). Across all soils, the percentage increase in plant weight resulting from pasteurization and fumigation (i.e., compared to the control) was positively correlated with soil pH (**Table 1**) (r=0.43, *P*=0.05 and r=0.45, *P*=0.05 respectively).

The bioassay results to date indicate some potential for use of soil sampling to predict RD severity, and they indicate that *Pythium* spp. and *C. macrodidymum* may play at least a partial role in RD etiology in many almond replant soils. The fact that plants in the fall/winter bioassay (2013/14) benefited less from pasteurization than those in the spring/summer bioassay (2014) suggests that time of year may affect development of RD in Nemaguard seedlings in a greenhouse. We will continue our bioassay experiments to learn more about RD and its prediction statewide.

Comparing fumigant and non-fumigant treatments for management of RD. In the trials at KAC, ASD treatments quickly generated and maintained anaerobic conditions in the 6-wk treatment period (**Figure 6 A, B**); values of Eh (reduction potential) less than +200 mv are considered anaerobic. ASD in experiment 1 cumulatively generated 279,765 and 333,479 mv·h below the anaerobic threshold of +200 mv at 6 and 18" (15 and 45 cm) soil depths, respectively, while ASD in experiment 2 generated 187,232 and 323,378 mv·h below anaerobic threshold at 6 and 18" (15 and 45 cm), respectively.

All preplant soil fumigation and ASD treatments markedly reduced inoculum survival in bioassays with *P. ultimum* (**Table 3**) and strongly stimulated almond tree growth (**Figure 7 A-D**; *P*<0.0001). Significant growth suppression of the replanted almond trees resulted without

preplant remediation treatments in both experiments (**Figure 7 A-D**). The growth stimulation of ASD was especially pronounced in the Nemaguard rootstock seedlings interplanted between the almond trees. There was no significant impact of preplant soil ripping depth on plant growth in experiment 2 (*P*=0.5 to 0.3), and the sudan grass rotation had a relatively small stimulatory effect on replanted tree and seedling growth in experiment 1 (**Figure 7 A, C**). Molasses addition in Exp. 1 did little to intensify anaerobic conditions or control RD.

Our results indicate ASD has excellent potential as a non-fumigant method to prevent RD; further optimization of ASD is justified and will be pursued. A key focus in future ASD trials will be to reduce costs of administering the treatment (approx. $2400/acre as applied in this study, Browne, *unpublished*) while retaining its efficacy.

Research Effort Recent Publications:

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.

Browne, G.T., Schmidt, L.S., and Devengenzo, M.T. 2014. Evaluating the potential of anaerobic soil disinfestation for the control of Prunus replant disease. (Abstract 312-P, 2014 APS-CPS Joint Meeting, Minneapolis, MN).

Schmidt, L.S., Devengenzo, M.T., and Browne, G.T. 2014. Developing a bioassay to predict and characterize Prunus replant disease in California orchards. (Abstract 313-P, 2014 APS-CPS Joint Meeting, Minneapolis, MN).

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.

References Cited:

Shennan, C., Muramoto, J., M., B., Koike, S.T., Daugovish, O., Rosskopf, E. et al. 2007. Optimizing anaerobic soil disinfestation: an alternative to methyl bromide? In Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, pp. 40-41 to 40-44.

Tables and Figures:

Table 1. Sources and characteristics of soil used for greenhouse bioassay experiments 1 and 2.

Soil source (nearest city), code	Soil series and texture	Soil pH	Nematodes per 250 cc soil		
			Root knot	Ring	Lesion
1. Durham, MdS	Farwell loam	5.6	0	0	0
2. Durham, MdT	Farwell loam	8.0	0	0	0
3. Durham, MaT	Farwell loam	8.0	0	0	0
4. Arbuckle, NiT	Arbuckle sandy loam	6.0	0	0	16
5. Crows Landing, GoT	Capay clay	6.7	0	0	86
6. Snelling, DoT	Snel. & Whitn. sandy loam	6.9	0	0	0
7. Fresno, GuT	Hanford fine sandy loam	6.0	0	0	14
8. Firebaugh, PaT	Various, fine sandy loam, loam	8.2	0	0	0
9. Kerman, AvT	Grangeville sandy loam, fine sandy loam	8.0	0	0	0
10. Sanger, Ge	Hanford fine sandy loam	7.6	0	0	0
11. Sanger, Br	Various, loam to sandy loam	6.0	0	682	0
12. Parlier, KAC	Hanford fine sandy loam	8.2	0	0	0
13. Parlier, ARS	Hanford fine sandy loam	7.9	26	0	22
14. Dinuba, Kla	Greenfield sandy loam	7.4	0	9	176
15. Hanford, JWPl	Nord fine sandy loam	7.6	0	14	0
16. Hanford, JWPeD	Nord fine sandy loam	7.8	0	18	0
17. Hanford, JWPeH	Nord fine sandy loam	7.6	0	52	0
18. Wasco, PaT	Wasco sandy loam	7.6	0	0	0
19. Shafter, PaE99	Driver course sandy loam	7.9	0	0	0
20. Shafter, PaW99	Various, sandy loam	6.5	0	0	0

Table 2. Pathogenicity of *Pythium helicoides* on Nemaguard peach rootstock and effect of soil water saturation on disease development.

Exp.	Inoculum	Soil moisture	Small plants[a]			Big plants[b]		
			Root cortex necrosis (%)	Length of root crown rotted (%)	Total plant wt. (g)	Root cortex necrosis (%)	Length of root crown rotted (%)	Total plant wt. (g)
1	Control	No flood	17	3	39.7	--	--	--
		Flood	4	7	35.8	--	--	--
	P.he. 42a	No flood	24	2	25.3	--	--	--
		Flood	100	100	0.4	--	--	--
	P.he. 65b	No flood	21	17	35.4	--	--	--
		Flood	4	13	31.4	--	--	--
	P.he. 450c	No flood	29	8	28.3	--	--	--
		Flood	100	100	0.8	--	--	--
	P.he. 5796a	No flood	43	8	25.8	--	--	--
		Flood	100	100	0.9	--	--	--
	P.he. 6038a	No flood	34	10	31.1	--	--	--
		Flood	100	74	0.9	--	--	--
	95% Confid. interval:		(+/- 9)	(+/- 11)	(+/- 3.8)	--	--	--
2	Control	No flood	30	3	36.9	21	5	45.7
		Flood	7	5	38.7	6	1	36.7
	P.he. 42a	No flood	59	2	13.3	76	13	24
		Flood	100	98	1.5	97	53	5.8
	P.he. 65b	No flood	31	19	39.4	28	6	40.5
		Flood	4	6	33.9	12	7	35.9
	P.he. 450c	No flood	64	0	10.9	63	2	33.1
		Flood	100	96	1.5	98	23	7.6
	P.he. 5796a	No flood	78	3	10	68	0	36.6
		Flood	100	96	1.9	99	23	8.1
	P.he. 6038a	No flood	47	2	14.5	72	2	34.8
		Flood	98	78	1.9	92	22	12.8
	95% Confid. interval:		(+/- 9)	(+/- 12)	(+/- 3.1)	(+/- 12)	(+/- 13)	(+/- 6.6)

[a]2 weeks old when transplanted into infested soil.
[b]10 weeks old when transplanted into infested soil.

Table 3. Effects of selected KAC pre-plant treatments (in experiments 1 and 2) on survival of *Pythium ultimum*, which was buried in nylon bags in a bioassay.

Exp.	Inoculum set	Date of placement	Date of removal	Soil treatment	Depth of bioassay inoculum in soil (cm)	Survival of bioassay inoculum (cfu / g soil)	
						Mean	(S.E. of mean)
1	1	9/18/2013	11/18/2013	Sudan control	15	6140	(450)
					46	3180	(801)
				Sudan+ASD	15	0	(0)
					46	20	(20)
	2	10/29/2013	11/18/2013	Control (+/- sudan)	15	4010	(502)
					46	4345	(313)
				Oct. fum. (+/- sudan)	15	0	(0)
					46	0	(0)
	3	12/9/2013	1/4/2014	No-sudan Control	15	4300	(384)
					46	4392	(558)
				No-sudan + Dec. fum.	15	0	(0)
					46	0	(0)
2	1	9/18/2013	11/18/2013	No-sudan Control	15	5717	(994)
					46	6383	(2036)
				No-sudan + ASD	15	0	(0)
					46	0	(0)
	2	10/29/2013	11/18/2013	No-sudan Control	15	3667	(1135)
					46	4167	(775)
				No-sudan + Oct. fum.	15	0	(0)
					46	0	(0)

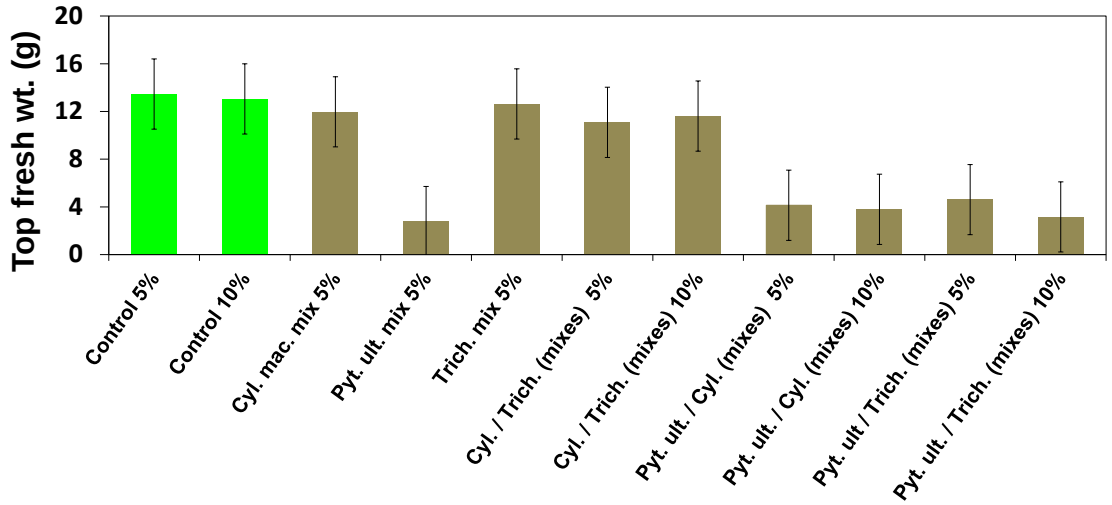

Figure 1. Results of greenhouse pot trial examining the potential for interactions of *Cylindrocarpon macrodidymum* and *Pythium ultimum* with *Trichoderma* spp. that affect severity of RD. Note that the results suggest that *P. ultimum* can be an aggressive RD pathogen but provide little evidence for direct of interactive impacts of *C. macrodidymum* or *Trichoderma* spp.

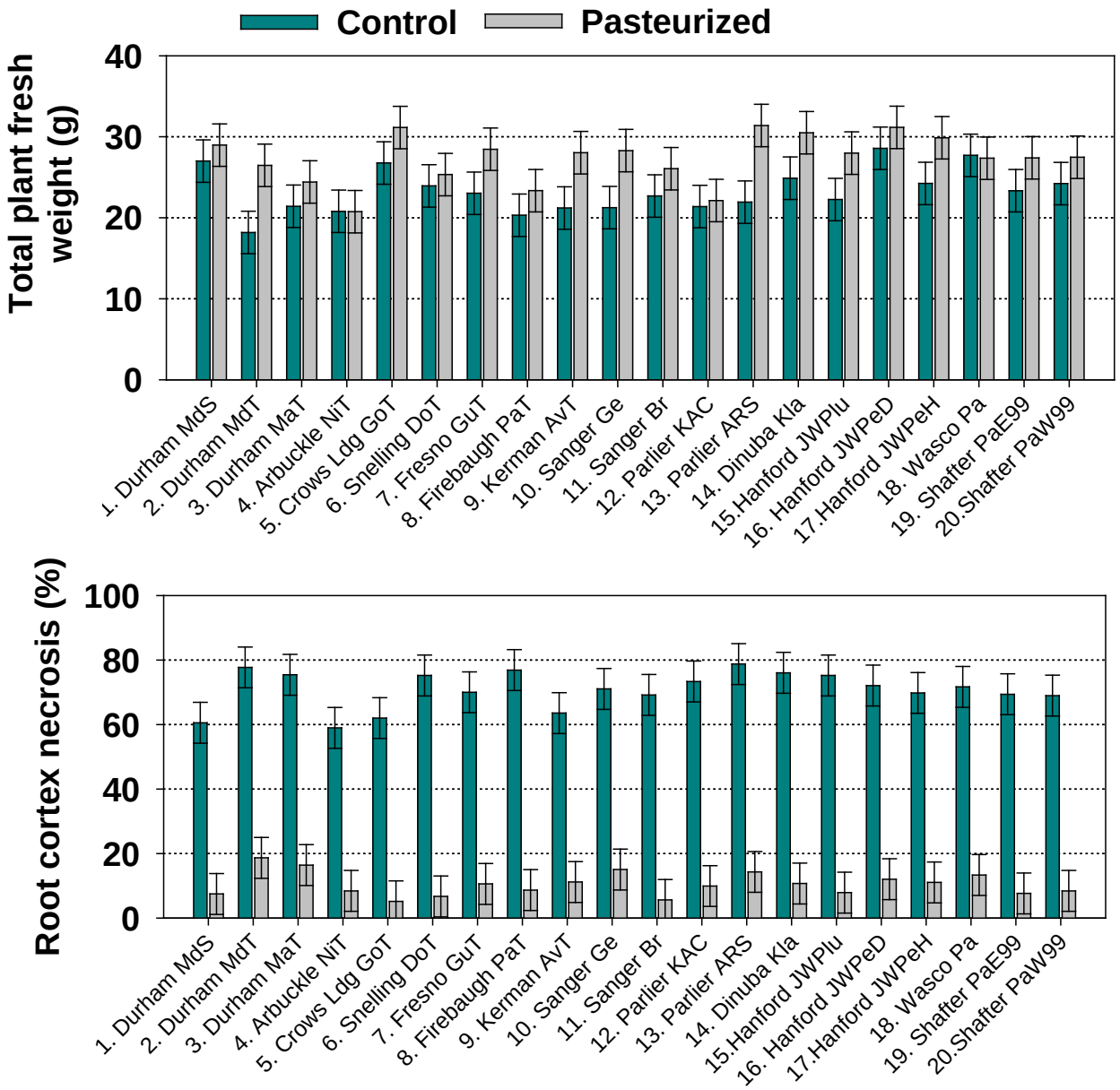

Figure 2. Plant responses to preplant soil treatments among 20 almond and stone fruit replant soils in greenhouse bioassay experiment 1, which was conducted from 29 Nov 2013 to 5 Feb 2014. Data were collected at the end of the experiment. Labels on *x* axis specify soils listed in **Table 1**. Error bars are 95% confidence intervals for means.

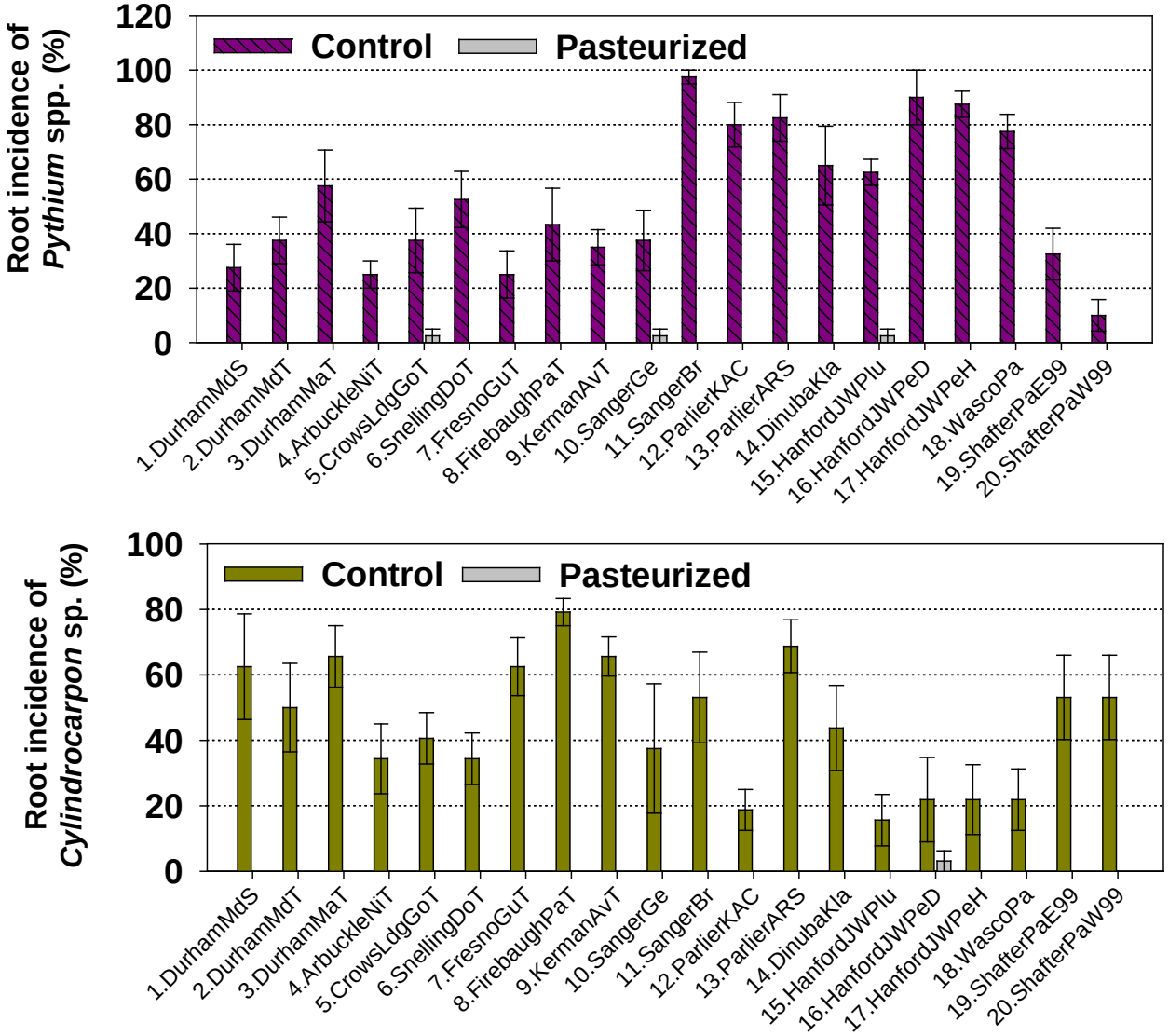

Figure 3. Impacts of preplant soil treatments on incidence of some RD pathogens among 20 almond and stone fruit replant soils in greenhouse bioassay experiment 1, which was conducted from 29 Nov 2013 to 5 Feb 2014. Data were collected at the end of the experiment. Labels on *x* axis specify soils listed in **Table 1**. Error bars are 95% confidence intervals for means. Bar absence indicates values of zero.

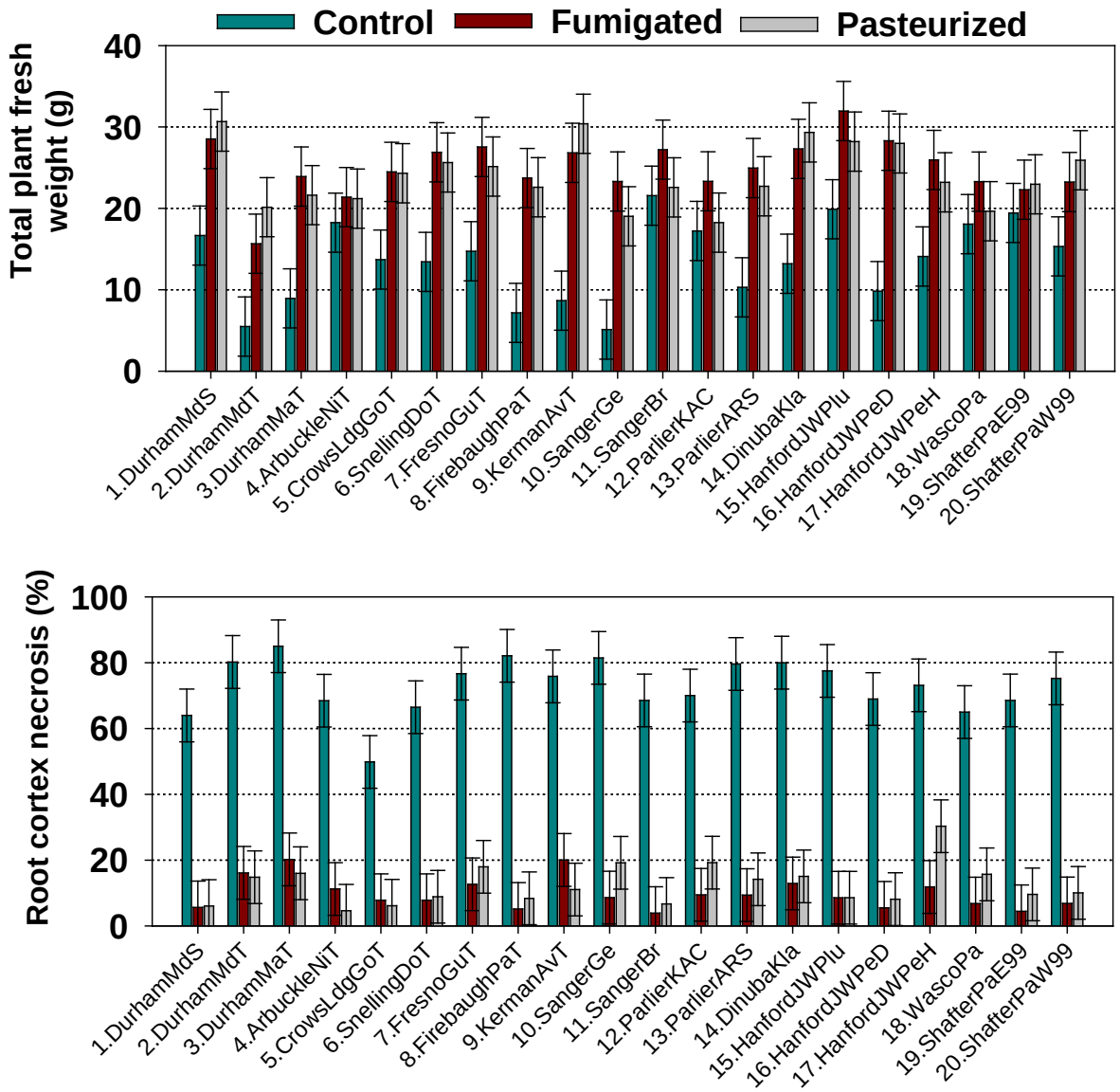

Figure 4. Plant responses to preplant soil treatments among 20 almond and stone fruit replant soils in greenhouse bioassay experiment 2, which was conducted from 23 April 2014 to 25 June 2014. Data were collected at the end of the experiment. Labels on *x* axis specify soils listed in **Table 1**. Error bars are 95% confidence intervals for means.

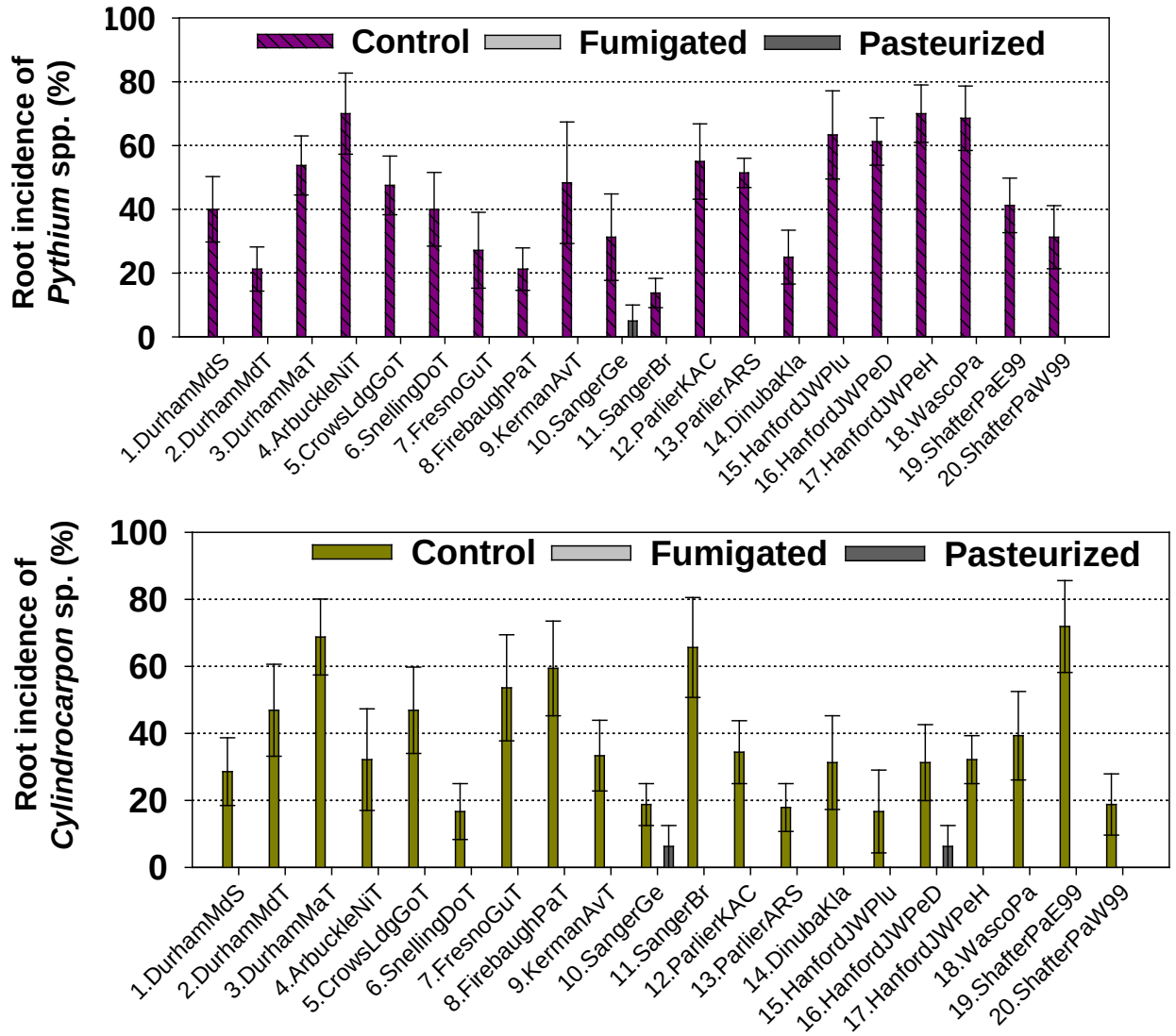

Figure 5. Impacts of preplant soil treatments on incidence of some RD pathogens among 20 almond and stone fruit replant soils in greenhouse bioassay experiment 2, which was conducted from 23 April 2014 to 25 June 2014. Data were collected at the end of the experiment. Labels on *x* axis specify soils listed in **Table 1**. Error bars are 95% confidence intervals for means. Bar absence indicates values of zero.

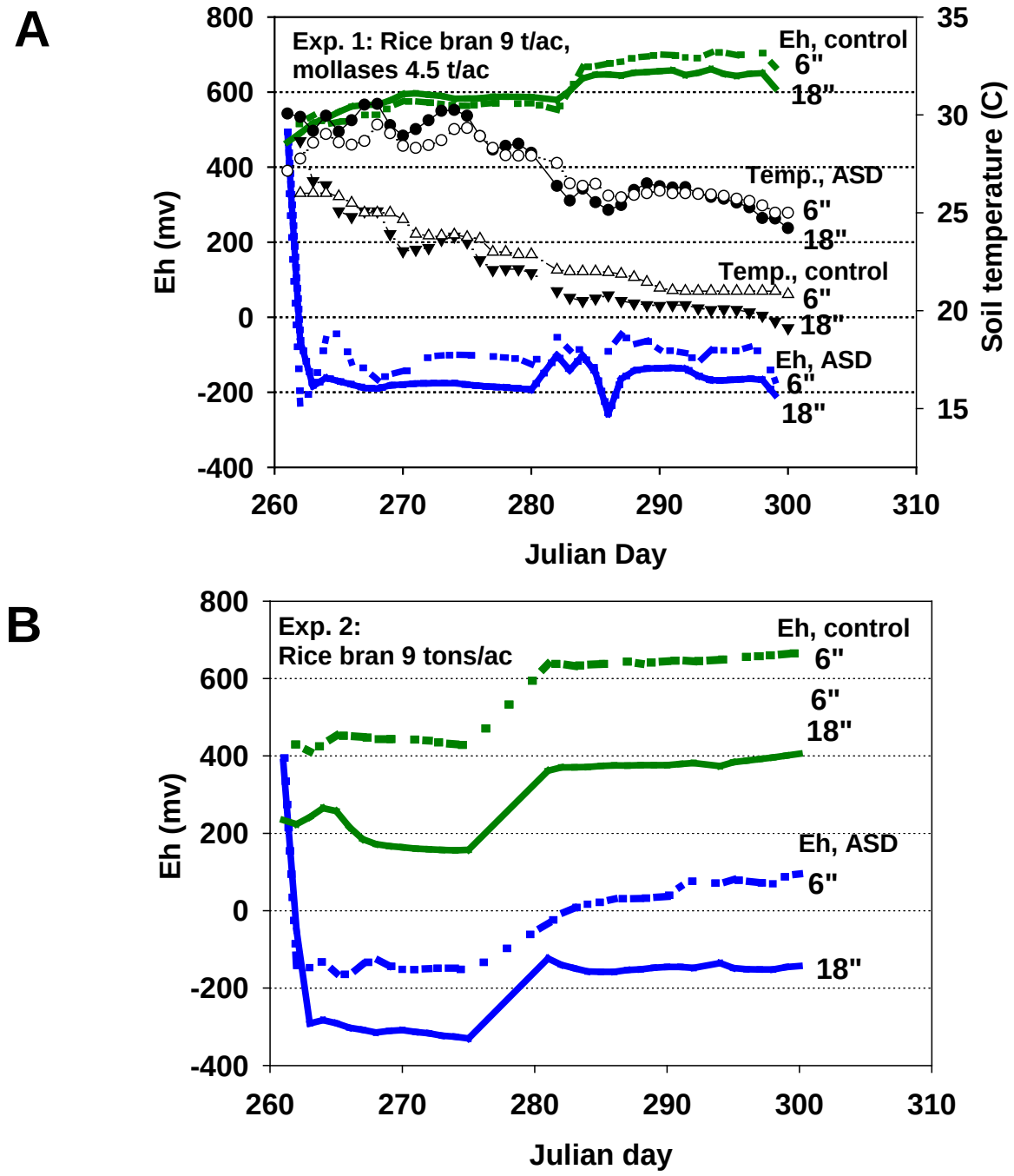

Figure 6. Effects of ASD and control treatments on soil environmental variables. **A**, Exp. 1, and **B**, Exp. 2. Eh readings less than +200 mv are considered to be anaerobic. Depths indicated in inches are below the soil surface.

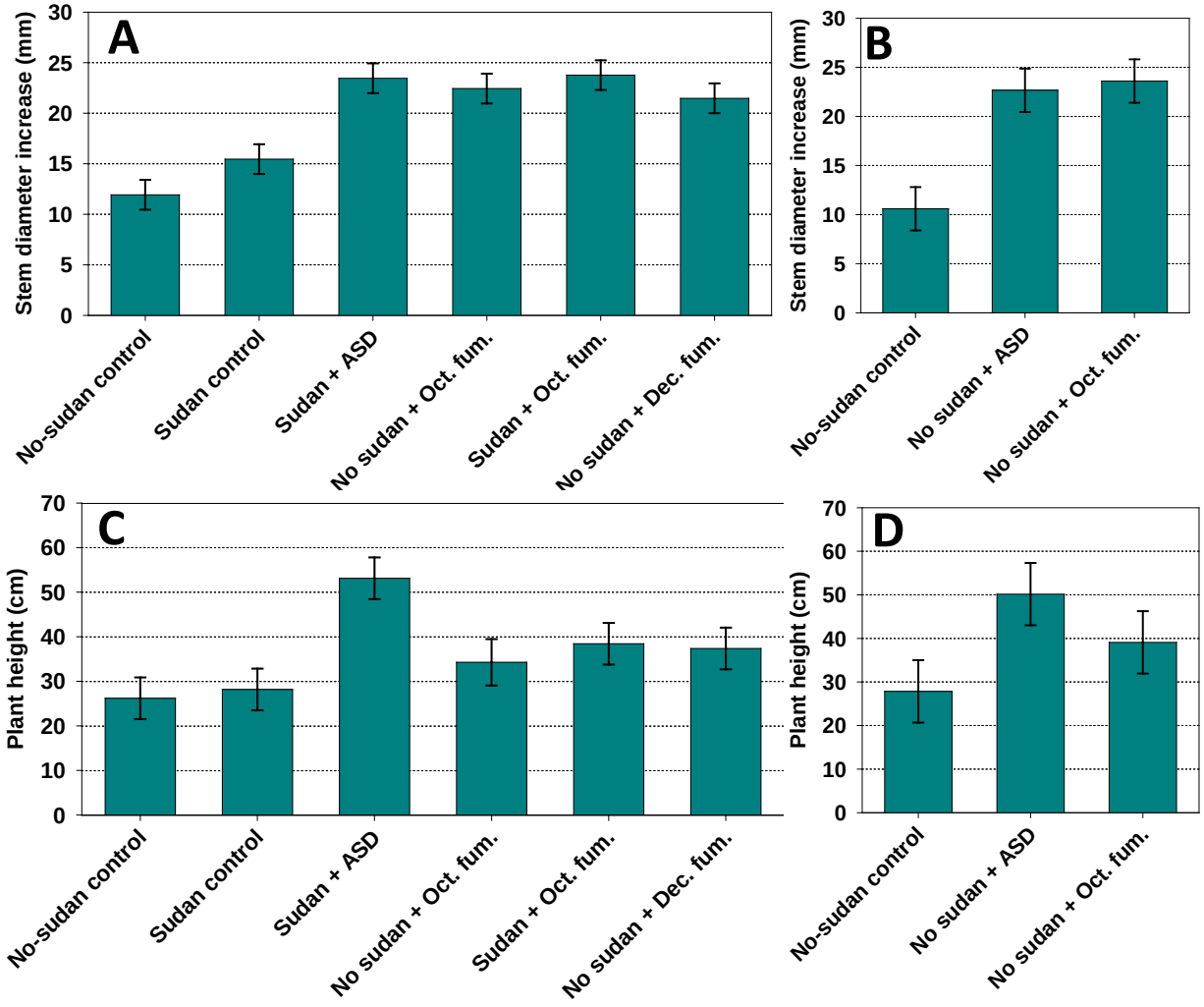

Figure 7. Effects of preplant soil treatments on replant growth. **A** and **B**, growth of replanted almond trees in experiments 1 and 2, respectively, as of 18 July 2014; and **C** and **D**, growth of interplanted Nemaguard peach seedlings in experiments 1 and 2, respectively, as of 18 July 2014.