DETERMINING UNKNOWN CAUSES OF REPLANT DISORDER ON *PRUNUS* SPECIES IN CALIFORNIA

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Replant disorder (RD) of *Prunus* species (i.e., stone fruits and nuts) can occur when the trees are planted without precautions at sites previously devoted to a closely related crop. The symptoms include poor growth, delayed crop production, and, in severe cases, tree death. Some known causes for *Prunus* RD include parasitic nematodes, oak root fungus, and *Phytophthora*. Additional, but presently unknown, biological causes are likely, however, because *Prunus* RD can occur in absence of the known pests, and several diverse fumigants can reduce incidence and severity of RD effects.

The project reported here focuses on determining unknown causes for RD on almond in the upper Sacramento Valley (near Chico, California) and on peach in the central San Joaquin Valley (near Parlier, California). Our approach in this research has been to: 1) characterize key symptoms associated with RD in replicated field plots (by contrasting RD-affected and healthy trees); 2) sample and isolate from RD-affected and healthy root systems in the field plots to determine whether culturable microbes are associated with the symptoms; 3) conduct greenhouse plant bioassays with the RD soils, following general and selective soil treatments designed to suppress groups of soil microbes; and 4) test pathogenicity of microbes associated with RD on *Prunus* species.

Replicate fumigated and non-fumigated plots were established at an almond replant site near Chico, CA (in cooperation with Paiva Farms, Chico; Tri-Cal, Inc., Hollister) and at peach and plum replant sites near Parlier, CA (by the USDA-ARS WMRL; Tri-Cal cooperating). The Chico plots were set up in fall 2000 at the site of an old almond orchard, where a previous replanted block of almonds on Marianna 2624 rootstock had failed to grow properly in spring-summer 2000. Experiment 1 at Chico included pre-plant treatments of methyl bromide (MBr) (360 lb/A), chloropicrin (374 lb/A), Telone (360 lb/A), and a non-fumigated control, each imposed on four replicate 19- x 22-m replicate (18-tree-site) plots in randomized complete blocks. The fumigants were injected by shank over entire plots on 10/21/00 without plastic mulch. The plots were replanted in January 2001 with almond on Marianna 2624. Experiment 2 at Chico involved pre-plant tree-site fumigation with MBr or Telone at 1.0 and 1.8 lb per tree site, respectively (10/21/00), compared to a non-fumigated control; the plots were replanted in January 2001 with almond on Marianna 2624, or in the case of the control, also with almond on Lovell peach rootstock. Experiment 2 had four replicate three-tree-site plots per treatment in randomized complete blocks. At the

time of fumigation, soil water content at 8 to 46-cm depth was 0.14 to 0.17 kg water per kg soil, and soil temperature was 19 to 20° C. Details of the Parlier pre-plant fumigation treatments and plots have been given previously (1).

In experiments at Chico, replanted trees grew poorly or died in the non-fumigated plots (Table 1). Pre-plant shank fumigation with chloropicrin in Experiment 1 strongly improved performance of replanted almond trees, whereas the shank treatment with MBr was only slightly beneficial and that with Telone had no significant effect (Table 1). In Experiment 2, in which pre-plant treatments were concentrated at tree sites, both MBr and Telone improved 2001 replant performance compared to the control (Table 1). In non-fumigated plots, almond on Lovell peach performed better than almond on Marianna 2624. We excavated roots from the healthy and diseased trees in the Chico trials on 4/26, 5/14, 5/31, and 8/13/01. The healthy trees in chloropicrin-fumigated plots produced many fine roots that were initially white in color, later turning darker on the outside; whereas the diseased trees produced few new roots, and most of them were necrotic. Among the fungi that were isolated from healthy and diseased roots on the experimental trees sampled on 5/14/01, only incidences of *Aspergillus*, *Cylindrocarpon* and *Fusarium* varied significantly according to root health class (Table 2). *Cylindrocarpon* was isolated more frequently from necrotic roots (incidence 18%) than from apparently healthy roots (2%) (P=0.005). Similarly, *Fusarium* was detected more frequently from diseased roots (46%) than from apparently healthy roots (2%), but this effect of root health interacted significantly with the pre-plant fumigation treatment (P=0.001, Table 2). *Aspergillus* was infrequently isolated. Many other fungi were detected, but the incidences were generally low (i.e.,<5%) and had no measurable association with symptoms of root disease (Table 2, also others not listed). Quantification of nematodes from replicate soil samples collected on 8/13/01 from non-fumigated and chloropicrin-fumigated plots did not reveal an association between numbers of plant parasitic nematodes and disease.

For the Parlier plots, Trout and Ajwa have previously quantified significant positive growth responses of peach and plum to pre-plant MBr and alternative fumigation treatments, including chloropicrin (1). On multiple occasions during 2000 and 2001, we sampled roots from typical vigorous trees in the MBr fumigated plots and typical stunted trees the non-fumigated plots at Parlier. The most obvious difference between root systems from the two treatments was that those in fumigated plots had more small and more large roots, compared to root systems in non-fumigated plots. In some samples, there appeared to be more fine roots ($\leq$1 mm diameter) with discolored cortex from non-fumigated than from MBr-fumigated plots. However, this difference could not be objectively quantified. Among fungi isolated from the sampled roots, increased incidence of *Fusarium* spp. and *Cylindrocarpon* spp. occasionally, but not consistently, has been associated with root discoloration or necrosis in non-fumigated plots. Many other fungal species have been detected at relatively low incidence (i.e., <5%).

Selected isolates of *Cylindrocarpon*, *Cylindrocladiella*, *Fusarium*, *Humicola*, and *Pythium* induced root cortex necrosis on Nemaguard peach seedlings in greenhouse pathogenicity tests, but smaller root and shoot masses usually did not accompany the cortex disease symptom (data not shown). Pin nematode counts (S. Schneider, M. McKenry) have suggested a possible association between RD symptoms and populations of the nematode at the Parlier plots, although an important parasitic role of this nematode species has generally been discounted in California.

Soil collected from Chico and Parlier replant plots was subjected to semi-selective treatments with difenoconazole, fludioxonil, mefenoxam, streptomycin + chloramphenicol, fenamiphos, or a non-treated control before replanting with Marianna 2624 (Chico soil) or Nemaguard peach (Chico and Parlier soils). For both soils, the plants grown in non-treated samples of soil developed significant amounts of root cortex necrosis. Only the fludioxonil treatment (or pre-plant soil heating) significantly reduced severity of the root cortex symptom.

To date, our results suggest partial fungal involvement in *Prunus* RD in California. Additional work is needed and in progress to further characterize fungal, bacterial, and nematode populations associated with the RD symptoms, as well to test pathogenicity and growth effects of selected isolates. An improved understanding of *Prunus* RD, once achieved, should improve control strategies for the problem.

Literature Cited

1. Trout, T., and Ajwa, H. 2000. Fumigation and fallowing effects on replant problems in California peach. Pp. 16-1 to 16-4, 2000 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions.

Table 1. Effects of pre-plant fumigation treatments and rootstock on first-year performance of almond trees replanted at the site of an old almond orchard near Chico, CA[a]

Experiment	Pre-plant fumigation treatment	Rootstock	Increase in trunk diameter (mm) (2/1/01-8/13/01)	Tree height (m) (8/13/01)	Disease rating (0-5 scale) (8/13/01)
1	None (control)	Marianna 2624	1 a	1.0 a	3.4 a
	MBr	Marianna 2624	4 b	1.2 a	2.1 b
	Chloropicrin	Marianna 2624	10 c	1.7 b	0.3 c
	Telone	Marianna 2624	2 a	1.1 a	2.9 a
2	None (control)	Marianna 2624	1 a	0.9 a	3.5 a
	None (control)	Lovell peach	6 b	1.3 b	1.2 b
	MBr	Marianna 2624	7 b	1.4 b	0.9 b
	Telone	Marianna 2624	8 b	1.3 b	1.4 b

[a]The pre-plant treatments were applied on 10/21/00; by shank over entire plots in Experiment 1and by one probe per tree site in Experiment 2; details given in text. All plots were planted in January 2001. Means within columns and within an experiment but without letters in common are significantly different according to 95% confidence intervals.

Table 2. Incidence of selected fungi on roots sampled from replanted almond trees on Marianna 2624 rootstock in Chico Experiment 1

Pre-plant soil fumigation treatment	Status of root sample	Surface sterilization treatment	Incidence of isolation per root piece (%)[a]						
			Aspergillus	*Cylindrocarpon*	*Fusarium*	*Mortierella*	*Penicillium*	*Rhizoctonia, Monilliopsis*	*Tricho-derma*
None	Healthy	None	0	0	0	0	0	0	0
		Bleach	0	0	0	0	0	0	0
	Diseased	None	4	4	75	4	17	0	4
		Bleach	4	33	67	0	0	4	0
Chloropicrin[b]	Healthy	None	0	8	25	25	17	0	8
		Bleach	0	0	0	0	8	0	0
	Diseased	None	8	21	8	25	13	8	29
		Bleach	0	13	33	0	0	8	0
Significant statistical effects[c]:			Root health status (P=0.03)	Root health status (P=0.005); fumigation x surf. ster.; (P=0.03)	Fumigation x root health status; (P=0.001)	Fumigation x surf. ster.; (P=0.02)	Surf. ster. (P=0.03)	None	None

[a]Heatlhy and diseased or discolored roots were sampled on 5/14/01 from trees in three replicate chloropicrin-fumigated and non-fumigated plots in the Chico field trial. In the lab, fine roots (≤1 mm diameter) were segregated by health status (healthy=white; diseased=dark and sunken), cut into segments (0.5 to 1 cm length), subjected to rinsing in sterile water or bleaching (10% commercial bleach, adjusted to pH 7.2), and cultured on water agar amended with tetracycline, 100 ppm.). Eight water-rinsed and eight bleached root segments were cultured per medium per tree. Three days after culturing the roots, all fungal isolates were transferred individually to one-fifth strength PDA amended with tetracycline and identified to genus according to morphology. Additional fungi, not listed above, were isolated at low incidence (generally less than 5%).

[b]374 lb/A, pre-plant shank injected on 10/21/01, no tarp.

[c]According to analysis of variance, SAS Version 8.