"Performance of 'UCB-1' pistachio rootstock selections and pre-plant soil fumigation in a sandy location with high counts of plant parasitic nematodes."

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<u>Summary</u>

Pistachio plantings in Merced County are expanding onto sandier soils (>90% sand) known to have high counts of soil nematodes. The necessity of pre-plant fumigation for control of nematodes in these soils conditions is unknown. To evaluate the efficacy of pre-plant fumigation, this study compares growth performance and soil nematode counts among five clonal and one seedling rootstock lines grown in 1,3-dichloropropene (1,3-D) pre-plant fumigated and non-fumigated soils. Preliminary nematode analysis indicated high populations (>75/500g of soil) of Ring (*Mesocriconema xenoplax*), moderate populations (<50/500g of soil) of Lesion (*Pratylenchus vulnus*) and low populations (<25/500g of soil) of Rootknot (*Meloidogyne* spp.) nematode. 1,3-D was applied at 33 gallons/treated acre applied as a 11' strip centered on the tree row in March 2013. Rootstocks were planted in May, 2013. Trunk growth, grafting success, soil nematode counts, and leaf nutrient content were studied. There was no significant difference in trunk growth between rootstocks planted in fumigated (8.75 mm) and non-fumigated soil (8.50 mm). Differences were observed, however, in rootstock lines. Rootstock D110P exhibited greater mean change in trunk diameter (10.7 mm) than rootstock D415W (5.7 mm). A smaller, second trial is evaluating five other UCB-1 rootstocks in comparison to UCB-1 seedlings, and was established at the same time as the first trial but only in pre-plant fumigated soil. In this trial, rootstocks did not vary significantly in growth in 2014. Nematode analysis from soil samples taken in 2013 indicated stubby root nematode counts were higher in non-fumigated soil than in fumigated soil, but counts did not vary among rootstocks, nor were other plant parasitic nematodes detected. Most rootstocks exhibited low bud grafting failure rates, but D90W and UCB-1 Clone rejected 33% and 40%, respectively, of Kerman bud grafts. Preliminary analysis of nutrient content in rootstock and scion leaves indicates variation in nutrient concentration between leaf source, and variation in nutrient allocation among rootstocks, though further research and analysis is needed before the meaning and significance of these differences can be properly assessed.

<u>Introduction</u>

Pistacia vera 'Kerman' is the primary fruit bearing cultivar of pistachio grown in California (Ferguson *et al.* 2005). 'Kerman' is vulnerable to verticillium wilt, Phytopthora, and nematodes (Ferguson *et al.* 2005), necessitating grafting onto rootstocks tolerant to these disease pressures. *Pistacia integerrima* x *P. atlantica* 'UCB I' is a widely used rootstock exhibiting good overall tolerance to fungal pathogens, frost, salinity, and (historically) nematodes, while still producing high yields (Ferguson *et al.*2005).

The UC Statewide IPM Program identifies four nematode species that can occur in pistachio orchards (Ohlendorf 2012). *Paratylenchus hamatus* (pin nematode) and *Xiphinema americanum* (dagger nematode) are migratory ectoparasites. Pin nematode causes general loss of vigor in most of its host species, but major declines in crop production require heavy infestations that are generally favored in fine textured soils (Shurtleff and Averre 2000). *Meloidogyne* spp. (root-knot nematodes) are sedentary endoparasites forming galls that can disrupt root growth, development and function. Although these nematodes are listed, there are no specific pest management recommendations.

Pistachio rootstocks have historically exhibited resistance to root lesion (*Pratylenchus vulnus*) and root knot (*Meloidogyne* spp.) nematodes (McKenry and Beede, 2011). Consequently, literature on pistachio-nematode interactions is exceedingly limited, and silent on the efficacy of pre-plant and post-plant management options. McKenry and Beede (2011) reported discovering loss of resistance to root lesion and root knot nematode in some UCB-1 (*Pistacia atlantica* x *Pistacia integerrima*) and *P. atlantica* rootstock lines. While initially suspecting that damage from high ring-nematode (*Mesocriconema xenoplax*) populations might increase rootstock susceptibly to secondary root-lesion nematode infection, McKenry and Beede (2011) instead identified a new biotype of root-lesion nematode (Pv. 30) that caused significant reductions in tree growth despite low ring-nematode populations in sampled soil. This aggressive new biotype therefore appears to bypass the resistance mechanisms of UCB-1 and *P. atlantica* rootstocks. Further, Pv. 30 was discovered in, and seems to prefer, sandy soils, but can cause appreciable damage on UCB-1 and *Prunus* rootstocks in sandy loams as well (McKenry and Beede 2011).

Although Pv. 30 has not yet been found in commercial orchards in Tulare and Kings Counties where McKenry and Beede (2011) conducted research, its preference for sandy soils should raise concerns for prospective pistachio growers in northern Merced County, where soils with over 90% sand predominate. Concerns about potential vulnerability to nematode infestation compel prospective growers to pre-emptively fumigate new orchards even though it is unknown if this pre-plant soil treatment is needed. This project will be the first to investigate the efficacy of pre-plant fumigation of pistachio orchards in California and the first to examine different clonal lines of 'UCB-1' to screen for rootstock resistance to plant parasitic nematodes.

Objectives:

1. To determine if pre-plant soil fumigation increases tree performance in comparison to non-fumigated soils in a soil infested with plant parasitic nematodes.
2. To determine performance differences between 'UCB-1' clonal lines and 'UCB-1' seedling rootstocks in soils infested with plant parasitic nematodes.

Procedures

This project contains two trials that are evaluating different selections of 'UCB-1' clonal lines in comparison to 'UCB-1' seedlings. These trials were established in a commercially planted orchard near Livingston, CA in May 2013 as part of a 10-15 year project. Like other potential pistachio sites in the county, the soils are comprised of more than 90% sand, have low cation exchange capacities (<4.0 meq/100g), high soil nematodes counts (>200 per 500g soil), and poor-quality groundwater for irrigation. Trees are planted at 22'x17' and are irrigated with a single line drip.

For Trial 1, five clonal lines or one seedling line of *P. atlantica* x *P. integerrima* 'UCB-1' were planted on May 22, 2013 as a split-plot, randomized complete block design, consisting of four blocks of two rows, each row containing all 6 rootstock treatments. Prior to planting, one row in each block was fumigated on March 25, 2013 with Telone® II (1,3-Dichloropropene) applied as a liquid injection at row center at a rate of 33.3 gallons/acre, while the remaining row in each block was not fumigated. Rootstocks include 'UCB-1' seedling, 'UCB-1' clone line 1, 'UCB-1' clone line D259P, 'UCB-1' clone line D364W, 'UCB-1' clone line D110P, and 'UCB-1' clone line D415W. Rootstocks were bud grafted with the scion *Pistacia vera cv.* 'Kerman' in the fall of 2013. Rootstocks that failed to successfully graft were grafted again in late spring and summer of 2014. Budding failure rates were determined after the third grafting attempt.

For Trial 2, five clonal lines and one seedling line of *P. atlantica* x *P. integerrima* 'UCB-1' were planted as a randomized complete block design, consisting of four blocks in two rows. Prior to planting, the soil in both rows was fumigated with Telone® II (1,3 dichloropropene) applied as a liquid injection at row center at a rate of 33.3 gallons/acre. Rootstocks for this trial include UCB-1' seedling, 'UCB-1' clone line 1, 'UCB-1' clone line D154W, 'UCB-1' clone line D#5, 'UCB-1' clone line D90W, and 'UCB-1' clone line D71P. Rootstocks were likewise bud grafted in the fall of 2013 and late spring and summer of 2014.

To determine the effects on tree growth, trunk diameters of all trees were measured in late November at a height of 24 inches using a digital caliper.

To compare nematode counts among treatments, soil samples were collected in mid-October 2013 using protocol similar to Shurtleff and Averre (2000). An auger was used to obtain 18-in. soil cores from within the drip line of three trees within each fumigation, rootstock, and block treatment combination. Three soil samples from each treatment were pooled and submitted to Nematodes, Inc. (Selma, CA) for analysis of number of nematodes per 500g of soil.

In mid-June, leaf samples from rootstock and successfully-grafted Kerman scions were taken to examine differences in nutrient content and partitioning between leaf sources. Leaves from three trees in each fumigation and rootstock treatment were pooled and submitted to Dellavalle Laboratory, Inc. (Fresno, CA) for analysis.

Statistical comparisons were evaluated using JMP® 11 (SAS Institute, Cary, NC). Data were tested for normality using the Shapiro-Wilk W test and for homoscedasticity using the Levene and Welch tests. For trunk growth, differences among means were analyzed by log10 transformed one-way ANOVA and the Tukey-Kramer HSD post-hoc test. Soil nematode counts were compared statistically by one-way ANOVA of ln (count +1) and Tukey-Kramer HSD. Leaf nutrient contents were compared by two-way ANOVA and LS Means Differences Tukey HSD or LS Means Differences Student's *t* test to examine interactive effects between fumigation treatment and rootstock. Data that could not be transformed to meet normality criteria for

ANOVA tests were analyzed using the nonparametric Steel-Dwass All Pairs test on JMP® 11. Unless otherwise noted, interactive effects of two-way ANOVA were not observed ($p > 0.05$).

Results and Discussion:

In Trial 1, a two-way ANOVA indicated there were no interactive effects of trunk growth between rootstock and fumigation treatment ($p = 0.1044$), therefore rootstock and fumigation treatment were analyzed separately. No significant differences in mean trunk growth were observed between fumigated (8.75 mm) and non-fumigated (8.50 mm) treatments (Table 1). Among rootstocks in both fumigation treatments, D110P showed greater average growth than D415W, but otherwise differences were not significant. Rootstocks in Trial 2 (where all trees were planted in fumigated ground) also exhibited similar growth rates (Table 3).

Stubby root nematode (*Paratrichodorus sp.*) was the only plant parasitic nematode species detected in soil samples collected near Trial 1 trees in October 2013; root knot, ring, root lesion, and pin nematodes were not present. Stubby root nematode occurred at significantly higher concentrations in non-fumigated soils (11 per 500g) than in fumigated soils (less than one per 500g), but not among rootstocks (Tables 4 and 5) . Free-living (non plant parasitic) nematodes occurred at similar rates between fumigated and non-fumigated soils, and varied slightly among soils around different rootstocks (Tables 4 and 5). Interactive effects among fumigation and rootstock treatments were not significant ($p = 0.57$ and $p = 0.33$, respectively).

Rates of successful graft unions of Kerman buds (through July 2014) varied among rootstocks. All Kerman buds grafted to D259P were successful, while D110P, D415W, and D364W exhibited lower grafting failure rates than D#5, D71P, UCB-1 Seed, and D154W (Table 6). D90W and UCB-1 Clone 1 rootstocks had the highest graft failure rate (33-40%). The UCB-1 Clone 1 rootstock was identified as being part of the population exhibiting bushy-top, short internode symptoms. Trees have been replaced and symptoms have been confined to this one clonal line.

Leaf tissue analysis revealed differences in nutrient concentrations between scion leaves and rootstock leaves, as well as variation in these differences among rootstocks. Potassium, zinc, magnesium, and chloride occurred at significantly higher concentrations in scion leaves than in rootstock leaves, while calcium, iron, and copper occurred at higher concentrations in rootstock than in scion leaves (Table 7). Among rootstocks, zinc content did not vary in rootstock and scion leaves, iron occurred at higher concentrations in UCB-1 Clone 1 rootstock leaves than in D415W rootstock leaves (Table 8). Copper content differences among rootstocks varied depending on leaf source, with significantly more in D364W rootstock leaves than in D71P, D90W, UCB-1 Clone 1, and UCB-1 Seed rootstock leaves, while D415W scion leaves showed greater copper concentrations than scions grafted to D71P, and UCB-1 Seed. Further statistical analyses and understanding of the meaning and impact of the observed differences in nutrient content among leaves collected from the rootstock and scion is ongoing.

Conclusion and Practical Application:

Initial data does not indicate fumigated significantly enhances growth rates in pistachio, though incidence of one species of plant-parasitic nematode is higher in non-fumigated ground. The rootstocks in this study are showing some variation in growth and bud take rates. Preliminary study of nutrient allocation suggests some nutrients are allocated differently between the rootstock leaves and scion leaves, and there may be differences in this allocation among rootstocks. Further study of all these effects are needed before practical recommendations can be made.

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Tables:

Table 1 Change in trunk diameter (2013-2014) between trees planted in non-fumigated and fumigated (Telone II) soil, inclusive of all rootstocks in Trial 1. Different letters indicate statistical significance.

Treatment	Mean change in trunk diameter (mm)
Non-fumgiated	8.50 A
Fumigated	8.75 A

Table 2 Change in trunk diameter (2013-2014) among rootstocks, inclusive of trees in non-fumigated and fumigated soils in Trial 1. Different letters indicate statistical significance.

Rootstock	Mean change in trunk diameter (mm)
D110P	10.7 A
D259P	7.54 BC
D364W	8.11 ABC
D415W	5.65 C
UCB-1 Clone 1	8.69 AB
UCB-1 Seed	9.49 AB

Table 3 Change in trunk diameter (2013-2014) among rootstocks in Trial 2. Different letters indicate statistical significance.

Rootstock	Mean change in trunk diameter (mm)
D #5	10.6 A
D71P	12.2 A
D90W	11.1 A
D154W	9.64 A
UCB-1 Clone 1	9.52 A
UCB-1 Seed	9.54 A

Table 4 Nematode counts from soil sampled (October 2013) near trees planted in fumigated and non-fumigated ground, inclusive of all Trial 1 rootstocks. Different letters indicate statistical significance.

	Nematodes per 500g Soil					
Treatment	Root Knot	Ring	Root Lesion	Stubby Root	Pin	Free Living
Non-fumigated	0	0	0	11.3 A	0	32.0 A
Fumigated	0	0	0	0.67 B	0	30.3 A

Table 5 Nematode counts from soil sampled (October 2013) near trees of different rootstocks, inclusive of all Trial 1 rootstocks. Different letters indicate statistical significance.

	Nematodes per 500g Soil					
Treatment	Root Knot	Ring	Root Lesion	Stubby Root	Pin	Free Living
D110P	0	0	0	4.86 A	0	54.4 A
D259P	0	0	0	1.00 A	0	15.4 AB
D364W	0	0	0	0.25 A	0	13.1 B
D415W	0	0	0	17.8 A	0	17.3 AB
UCB-1 Clone 1	0	0	0	7.42 A	0	52.9 AB
UCB-1 Seed	0	0	0	3.25 A	0	39.1 AB

Table 6 Percent of Kerman buds that failed to successfully graft to different rootstocks.

Rootstock	Kerman Graft Failure
D #5	18 %
D71P	11 %
D110P	4 %
D154W	17 %
D259P	0 %
D364W	8 %
D415W	4 %
D90W	33 %
UCB-1 Clone 1	40 %
UCB-1 Seed	13 %
Total	15 %

Table 7 Comparison of nutrient concentrations of rootstock and scion leaves, inclusive of all rootstocks. Leaves were sampled and analyzed simultaneously . Letters indicate statistically significant differences. Asterisks indicate two-way ANOVA interactive effects (p <0.05) between leaf source and rootstock, therefore leaf concentration differences cannot be attributed to leaf source alone.

Leaf Source	**N**	**P***	**K***	**Zn**	**Mn**	**Na**	**B***	**Ca***	**Mg***	**Fe**	**Cu**	**Cl***
	%	%	%	mg/kg	mg/kg	%	mg/kg	%	%	mg/kg	mg/kg	%
Scion	2.29 A	0.18 A	1.18 A	21.4 A	38.6 A	<0.01 A	56.5 A	1.50 B	0.42 A	173.0 B	7.24 B	0.19 A

Rootstock	2.25 A	0.17 A	0.80 B	16.8 B	37.0 A	<0.01 A	58.0 A	1.66 A	0.39 B	194.7 A	8.71 A	0.11 B

Table 8 Comparison of nutrient concentration between rootstock and scion leaves among rootstocks in both trials. Different letters indicate statistical significance. Nutrients that showed two-way ANOVA rootstock*leaf source interactive effects are not shown.

Rootstock	**Zn** mg/kg		**Fe** mg/kg		**Cu** mg/kg	
	Leaf Source					
	Rootstock	Scion	Rootstock	Scion	Rootstock	Scion
D #5	15.5 A	22.5 A	210.5 AB	148.2 A	9.80 AB	9.30 AB
D71P	14.0 A	15.3 A	174.7 AB	149.3 A	5.70 B	4.70 B
D110P	19.0 A	19.7 A	190.1 AB	178.0 A	9.60 AB	7.30 AB
D154W	14.8 A	19.5 A	213.8 AB	198.5 A	10.3 AB	7.80 AB
D259P	17.9 A	23.5 A	196.1 AB	180.1 A	9.90 AB	8.10 AB
D364W	16.3 A	22.1 A	181.0 AB	180.8 A	12.1 A	7.90 AB
D415W	21.1 A	21.9 A	173.3 B	163.3 A	9.60 AB	9.90 A
D90W	18.8 A	24.0 A	205.3 AB	186.0 A	6.80 B	5.70 AB
UCB-1 Clone 1	15.7 A	25.7 A	209.9 A	170.9 A	6.40 B	6.50 AB
UCB-1 Seed	14.7 A	17.2 A	195.3 AB	171.4 A	7.30 B	5.40 B