UPDATE ON A PREPLANT METHYL BROMIDE ALTERNATIVES TRIAL IN A WALNUT REPLANT SITE

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Walnut growers, unlike other commodity producers, remain walnut growers for generations. Hence, re-establishment of this nut crop commonly occurs. Walnut re-establishment is an arduous task because of their extreme tree size, and their sensitivity to nematodes (principally P. vulnus and M. incognita). Walnuts are also significantly affected by the "replant problem", identified by some scientists as a disease, and others as a biological disorder associated with root exudates and substances from the previous and same plant species. Due to the cost and regulatory difficulty of fumigation, many growers chose not to fumigate. Consequently, some of the second generation orchards grow so poorly without fumigation that they threaten the economic survival of smaller farms. Others grow sufficiently well to tempt producers into thinking the benefits of fumigation do not justify the cost. The expanded buffer zones and pending loss of methyl bromide have also increased the need to evaluate the efficacy of alternative fumigants.

To address these issues, a walnut pre-plant fumigation trial was established in northern Kings County, CA (southern San Joaquin Valley). The field site is a uniformly deep, well drained Nord fine sandy loam formed from alluvial parent material. Permeability is moderate. Water holding capacity is high. Effective rooting depth is greater than 60". The 40 acre site was a 50 year-old walnut orchard consisting of Hartley and Franquette cultivars which were removed and chipped during the winter of 2006-2007. The site was repeatedly chiseled to a depth of 4 feet to bring up as much of the old root system as possible. Following root removal, the field was laser leveled, bordered every 40 feet, and irrigated in preparation for planting silage corn that was harvested in early August of 2007.

Preliminary nematode sampling in February of 2007 consisted of four composite samples, each consisting of ten subsamples taken at a depth of 24". Results showed 136, 363, 233, 281 *P. vulnus* per 250 cc of soil using sieve/mist chamber extraction.

Prior to fumigation, the site was ripped to a depth of six feet on four-foot centers in the direction of the tree rows (east/west). This was then followed by a six foot slip plow which used the ripper shanks to achieve maximum penetration and modification. Measurement of soil moisture and assessment of pretreatment nematode populations occurred one week after soil modification and two days prior to fumigation. The fumigation treatments were applied November 2, 2007.

The experimental design is a six by six Latin Square with a plot size of 75' x 160' and 12 trees/plot (13.14 acres). The following six treatments were established:

- 1. Untreated Control (2.07 acres)
- 2. Methyl Bromide broadcast @ 400 lb/ac (2.07 acres)
- 3. Telone II @ 33.7 gal/ac strip treated (10' swath) & shanked at 28" (2.07 acres)

- 4. Telone II @ 33.7 gal/ac broadcast & shanked at 28" (2.07 acres)
- 5. Telone II @ 33.7 gal/ac broadcast and shanked at 20", Plus 175 lb/ac Chloropicrin broadcast and shanked at 28" (2.07)
- 6. Telone II @ 33.7 gal/ac broadcast and shanked at 20", Plus Methyl Bromide @ 125 lb/ac at 28" depth (2.07 acres)

The selection of the above treatments was based upon comparison of the recognized standard, methyl bromide, against the most likely replacements. Researchers involved in the selection felt that comparison of Telone II strip fumigation to broadcast had more immediate practical value than testing unregistered materials, such as methyl iodide, which met considerable resistance in California, and may never see registration in this state for orchard use. The Kings County agricultural commissioner was also unwilling to allow methyl iodide application at this site, due to its unregistered status and the proximity of the test site to a rural school. The committee also felt the need to verify improved Telone efficacy with the addition of economic levels of chloropicrin and methyl bromide shanked at deeper depths to minimize off-site emissions. A total of 13.14 acres is involved in this project. The plots were cultipacked with a ring roller and tractor immediately following fumigation.

In addition, each plot was split to test the performance of newly developed walnut clonal rootstock material, VX211 against conventional seedling paradox. A third clonal rootstock source, Vlach, was also included as the buffer between each plot.

On February 28, 2008, each plot was sampled in one-foot increments to a depth of five feet for both nematodes and phytopathogenic agents. The only detectable nematodes recorded in the fumigated plots were in the upper foot of soil, where fumigant efficacy is affected by volatilization without tarping. The untreated plots averaged 50 lesion nematodes per 250 cc of soil per foot, or 250 from one to five feet.

Prior to planting, the orchard was marked for a tree spacing of 25' between the rows, and 40' between the trees going down a given row. The trial wound up being planted twice. The first planting employed plant material provided by Duarte Nursery, Hughson, California. I was directed to them by the UC Davis walnut breeder, Dr. Gale McGranahan in July of 2007. At that time, the director of the Duarte cloning program saw no problem in being able to provide me with plant material suitable for the field. They were also kind enough to locate a paradox seed source and propagate it for the project. However, as the scheduled date for pickup approached, my conversations with the director became more unsettling, since he indicated his success in getting the plant material out of the two or three leaf stage was minimal. I was met with the extreme small size on April 15, 2008, the agreed day for pickup. The director indicated that planting in the field may be the solution to initiating vigorous growth. The seedling paradox were in sharp contrast to the clonal material, since they were already about 10 inches tall.

The first planting was performed April 16, the day after pickup. Daily observations and moisture monitoring increased my concern about the potential of the clonal plant material. My apprehension was confirmed when Dr. McGranahan informed me after planting about the need for several weeks of dormancy before clonal material grows uniformly. A rapid search for

replacement plant material ensued, which lead me to Burchell Nursery and clonal material supplied them by Vitrotech, Spain.

Shortly after the first planting, the grower planted six-row corn for silage between the tree rows.

On April 23, 2008, I initiated an emergency request to the USDA for post-entry quarantine site status for the Hanford project. Their rapid action resulted in my being able to secure relatively robust plant material of both Vlach and VX211 from Burchell Nursery, who also provided superb cooperation in facilitating the paperwork and plant delivery.

On May 8, we replaced the Vlach and VX211 plants from Duarte with those from Burchell. The plants were then monitored daily for the first six weeks to insure optimal growing conditions. By early July, the corn was so tall and humid, that work could only be performed from dawn to about mid-morning. During this time, hand weeding, fertilizing, and monitoring of the irrigation system was conducted.

On August 12, 2008, the trees were measured for height. The untreated trees were significantly shorter (36 inches) than the fumigated trees (an average of 41 inches). The data also showed the paradox seedlings and VX211 clonal trees to be similar in height (38 versus 41 inches), but the Vlach were significantly taller (48 inches).

On December 17, 2008, and May 7, 2009, the trial was re-sampled for nematodes and biological populations. The fumigated plots were almost completely free of any parasitic nematodes, even though the untreated plots are distributed uniformly across the experiment. The untreated plots show injurious lesion nematode levels above 100 per 250cc of soil throughout the five feet sampled.

On December 18, 2008, the trial was measured for height and trunk caliper. Results showed highly significant tree height differences between the treated and untreated plots (65.6 inches versus 48.7 inches). There was no height difference within the fumigated treatments. Caliper diameters were also significant (20 mm for the untreated versus 24.8 mm for the treated). Significant height and caliper differences were also recorded for the three rootstocks. Paradox seedlings were the smallest (55.5 inches tall and 21 mm in diameter), and VX211 was the largest (70.6 inches tall and 27 mm in diameter). The clonal Vlach used as buffer trees between the plots was 65.6 inches tall and 24 mm in diameter.

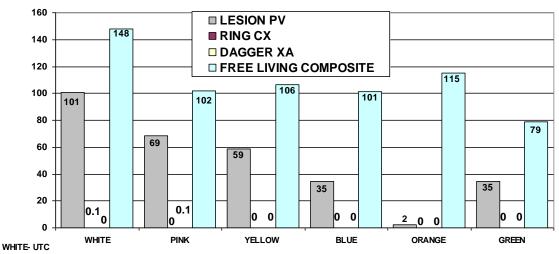
On November 9, 2009, trunk caliper measurements were taken again to compare treatment and rootstock growth effects. Tree height data could not be taken for comparison to data taken in 2008, due to the in-season tipping required to prevent breakage. The largest rootstock was Vlach (33.2 mm) followed closely by VX211 (31.0mm). The trees on paradox seedling were significantly smaller than the two clonal stocks (22.1mm). No significant differences in tree caliper size were recorded between the five fumigated treatments (average 32.4mm). However, the untreated trees are significantly smaller than those receiving fumigation (28.6mm).

On December 14, 2009, each of the 36 plots was soil sampled for nematode analysis in one-foot increments to a depth of five feet. Each sample represented a composite of three locations next to the more susceptible paradox rootstock. Chart 1 shows that lesion nematode is still highest in the control treatment, but all the fumigated treatments except the Telone II in combination with

chloropicrin are beginning to experience some infestation 750 days after treatment, based on this sample.

Chart 1. Effect of various fumigation treatments on parasitic and free living nematode populations 750 days following treatment on November 2, 2008. Averages based on three composite samples taken in one-foot increments to a depth of five feet.

GOLD TOP RANCH SOIL NEMATODE SAMPLES AVERAGES PER TREATMENT



PINK- Mehtyl Bromide Broadcast @400 lb/ac

YELLOW- Telone II @ 33.7 gal/ac strip treated (10' swath) & shanked at 20"

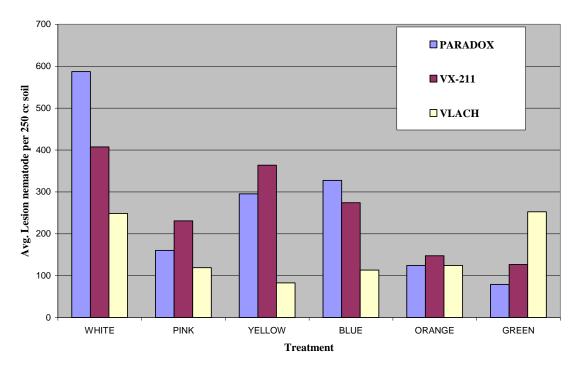
BLUE- Telone II @ 33.7 gal/ac broadcast & shanked at 20"

ORANGE- Telone II @ 33.7 gal/ac broadcast & shanked at 20" + 175 lb/ac Chloropicrin broadcast & shanked at 28"

GREEN- Telone II @ 33.7 gal/ac broadcast & shanked at 20" + Methyl Bromide @ 125 lb/ac 28" depth

Beginning November 17, 2010, an extensive three-day nematode sampling was performed on each of the three rootstocks and 36 plots in one foot-increments to a depth of five feet. Samples from each stock were submitted separately and daily to the laboratory to prevent any chance of misidentification. The results are shown in Chart 2.

Chart 2. Effect of fumigation on Pratylenchus nematode populations in three walnut rootstocks 2.96 years following treatment. Sampled November 17, 2010. Averages represent three composited sites per stock and six replications to five feet.



White: Untreated Control

Pink: Methyl Bromide broadcast at 400 lb/ac.

Yellow: Telone II at 33.7 g/ac strip-treated (10' swath) and shanked at 20 inches.

Blue: Telone II at 33.7 g/ac broadcast and shanked at 20 inches.

Orange: Telone II at 33.7 g/ac broadcast and shanked at 20 inches plus Chloropicrin at 175 lb/ac shanked at 28 inches.

Green: Telone II at 33.7 g/ac broadcast and shanked at 20 inches plus Methyl Bromide at 125 lb/ac shanked at 28 inches.

On January 27, 2011, trunk caliper diameters were taken approximately 12 inches above the soil surface on all trees within the trial. Chart 3 shows the results by treatment and excludes trees replanted after trial establishment. Chart 4 compares the performance of the three different rootstocks averaged over the treated and untreated plots.

Chart 3. Effect of fumigation on the average growth of three walnut rootstocks 994 days (2.72 years) following planting. Averages represent trunk caliper measurements of twelve trees in each of six replicated plots. January 27, 2011.

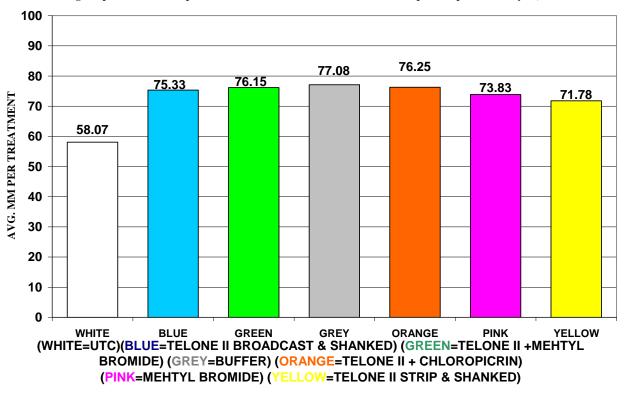
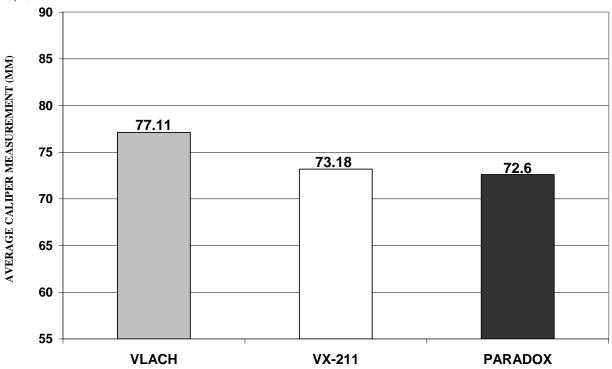


Chart 4. Growth comparison of Tulare English walnut on Vlach,VX211, and paradox rootstocks 994 days (2.72 years) after planting. Averages represent rootstock trunk diameter taken from six untreated plots and 30 fumigated plots. Sampled January 27, 2011.



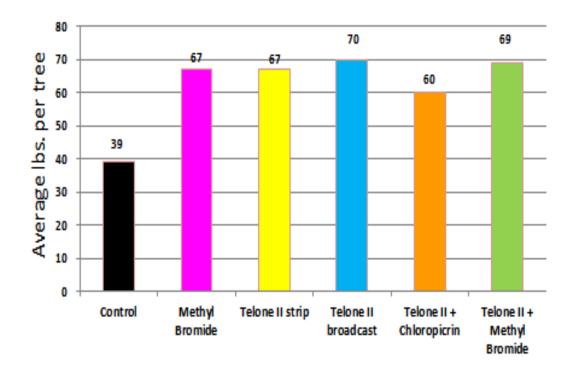
The most recent nematode data shows that the populations have raised substantially in all plots since the previous sampling at 244 days (see Chart 1). The untreated plots remain highest in lesion, and the Telone II plus chloropicrin plot remains the lowest. The methyl bromide, Telone II plus chloropicrin, and Telone II plus methyl bromide treatments are generally similar and lower in lesion populations than the Telone II only treatments. Thus far, the Telone II strip and broadcast treatments are no different in lesion nematode number. The Vlach clonal paradox rootstock shows a trend towards lower lesion levels than the VX211 clonal paradox. These data are surprising, since Dr. Michael McKenry, UC Nematologist, Riverside/Kearney Agricultural Center, Parlier, has reported VX211 tolerance to Pratylenchus under tank-trial conditions. His finding is supported by the levels recorded between VX211 and seedling paradox in the untreated plots of this trial, but there is no difference between them in the treated plots. The low lesion levels recorded in the Vlach may also be an anomaly created from fumigation overlap in the buffer area where these trees were planted. Unfortunately, space did not allow all three rootstocks to be included in the actual plot area.

Chart 3 shows the highly visible reduction in tree size from not fumigating. The untreated plots also had a very high percentage of the tree loss after the first two growing seasons (77.5%). Tree size in all the treated plots is very similar, with the strip treatment of Telone II being 5.3% smaller than the average of the other treated plots.

Chart 4 compares the average growth of each rootstock over all the treatments. It shows the danger of lumping the untreated trees with the treated, since the general average masks the very visible field effect of greater survival and uniformity of high vigor from fumigation. However, this chart does show that the surviving paradox seedlings are presently growing almost as well as the other stocks. Photoelectric light bar scanning of the entire trial has been performed in August of 2011 and September of 2012 to further delineate any differences in rootstock and treatment canopy size. Ultimately, yield data will serve as the great and final integrator of fumigant performance.

The results of the first harvest are shown in Chart 5. It should be noted that the English scions of these walnut trees are only four years old, and thus these yields are substantially above those anticipated by most growers. In fact, most young walnut orchards not harvested until the fifth year. The yield data from the first harvest shows fumigation provided an average 71% increase in production over the non-fumigated trees. This data has not yet been correlated with the percent shaded area measurements taken, so the yield differences between fumigation treatments may not be significant.

Chart 5. Effect of pre-plant fumigation on first-year commercial production of "Tulare" walnuts in a replant situation. Yield averages per tree within six, 0.35 acre plots in a Latin Square Design. Harvested October 10, 2012.



In summary, fumigation has thus far significantly increased tree development, uniformity, and yield compared to the unfumigated plots. Clonal selections of paradox rootstock have also provided significant increases in tree growth and canopy size over standard seedling paradox. To date, no differences in tree development exist between the fumigated treatments, but the addition of chloropicrin to Telone has shown greater nematode suppression during the first four years of development compared to the other fumigated treatments. Telone under strip or broadcast also thus far performed as well as 400 pounds of methyl bromide broadcast. It is hoped that future crop production will separate differences inherent in the fumigation treatments.

The results of this project have been presented at several field days and regional grower meetings. Fumigation of replant sites also seems to be more prevalent, after growers saw the dramatic difference between the treated and untreated plots in this important Area Wide project.