FIELD EVALUATIONS/INPUTS FOR GROWER REPLANT SETTINGS AND NEW LINES OF NEMATODE RESISTANCE

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ABSTRACT

These last five years our studies to replace methyl bromide have contained elements of soil fumigation plus elements of nematode resistance. The soil fumigation component now occupies less of our time with greater focus on nematode resistance/tolerance within selections already at hand. Studies on use of Garlon plus one year of fallow followed by methyl bromide, Telone or metam sodium are now completing their fourth year. Two-year studies involving walnut or other crops following high rates of 1,3-dichloropropene, and/or chloropicrin have now been completed in sandy loam soils. Iodomethane at 150, 200, and 235lb/ac was also included in some of these studies. The soil fumigation alternative to broadcast methyl bromide is Telone II and for medium to finer textured soils, common to walnut production, but the treatment rates must be 50 to 100 % greater than that currently permitted in California. Based on other funding we have recently learned how to use: 1) Telone II plus chloropicrin, 2) Iodomethane plus chloropicrin and 3) Telone II at two depths. We continue to monitor tree growth and nematode development following a strip application of Telone II (now entering fifth leaf). Field studies with cloned VX211 at four different inoculation levels of *Pratylenchus vulnus* have now completed their second-leaf. Biomass of VX211 is now 60% greater than that for clones of Paradox AX1 but this growth benefit does not fit normal definitions for tolerance. We continue to refer to VX211 as exhibiting extra hybrid vigor but it needs additional testing to determine if it also carries a level of resistance. NX seedlings, now in their fourth-leaf near Rio Oso, CA, began to show nematode reductions in spring 2005 and continue to show significant growth benefit over DN rootstock. Confirmation of this finding with fall 2005 samplings is underway. In a mother block setting we have three UZ selections of Paradox originally identified to possess pre-infection resistance similar to that of NX. One of these trees continues to grow as well as our best NX selection and should now be cloned for field evaluations. Because we had an ideal setting, we screened in 2005 three new post-plant nematicides against *P. vulnus*. Treatments included: 1) 5000 ppm drench of NatureCur, a walnut tea-based nematicide, 2) A calcium-based Nutraphyte product applied as a foliar spray and as a drench and 3) Admire, a commercial insecticide that we have shown to have moderate nematicidal activity. Performance of these potential nematicidal agents on two-year old trees was not impressive but Admire performed best.

OBJECTIVES

1. Using the IPM-based guidelines for replanting of walnut we will become involved in grower replanting efforts to gain knowledge as to the performance of individual steps in the process.

2. Continue to evaluate the potential nematode resistance present in various Paradox and black walnut seedlings.

PROCEDURES

Replant Sites – Objective 1.

1. In 1998 we identified a replant site near Rio Oso high in root lesion nematode. Several strategies were implemented including Garlon in fall 1999 to the trunks to minimize the rejection component of the replant problem. In spring 2001 trees of a standard Paradox (DN) and the NX Paradox were planted across replicates of: MB, stripped Telone/Vapam, or Vapam drench. Beginning in the first leaf we have monitored nematode populations in 27 DN and 27 NX trees located across the fumigation treatments. Each winter we monitor tree circumferences of 125 representative trees located across each replicate.

2. In a second site near Winters, CA the grower had a replant site, no root lesion nematode but an abundance of ring nematode. This grower had several options after treating trunks with Garlon but finally chose Telone broadcast to very dry soil. Two years after planting the ring nematode populations average 33 per soil sample and these assessments will continue. Soil sampling is repeated each spring and fall.

3. A grower replanting near Capay Valley treated trunks with Garlon in 2000. After one year of fallow and a spring 2002 planting of safflower the field received a broadcast application of 33.7 gpa Telone. Soil counts of *P. vulnus* were assessed to the 12-foot depth before fumigation and after the fumigation to the five-foot depth. Trees were close-planted in 2004 as seeds.

4. A grower near Visalia did not use Garlon but dried his soil below 12% moisture using extensive soil ripping plus tree root removal and then treated with a dual application of 33.7 gpa Telone followed by 20 gpa metam sodium. Nematode return is being monitored from six sites across the 220-acre planting. These trees on Paradox are growing well.

5. At Kearney Ag Center in spring 2003 we planted NCB seedlings into replicated sites treated with 235 lb/acre iodomethane. Tree growth and nematode development will be monitored for two years to assess longevity of *P. vulnus* protection and eventual tree growth compared to methyl bromide or the untreated comparison.

Nematode Resistance Evaluated – Objective 2.

1. Assessment of the NX source of pre-infection resistance continues to receive field evaluation in Rio Oso. In summer 2004 we dug backhoe pits across each pre-plant fumigation site in an effort to follow roots below 4.5 ft depth following Vapam, below the 6 foot depth following methyl bromide, and beyond 5 foot in any direction following the strip treatment of 50 gpa Telone.

2. In February 2004 we planted 48 clones each of VX211 Paradox and a standard AX1 Paradox from Gale McGranahan/Wes Hackett. One tree of each was planted into each of 48 1/100th acre macroplots made available at Kearney Ag Center. These macroplots have concrete-lined walls that reach 5 feet into the soil with open bottoms. These non-fumigated, nematode-free sites were planted into 2.5 cubic feet of soil containing either 0, 1, 20, or 500

nematodes for each 250 cc of soil. These trees were only pencil-sized and roots were likely within the inoculated zone for at least 4 months until they grew past. Trees will be stressed by simulating grafting in 2005. We assessed tree growth in 2004 and this will continue at least another year. Development of the nematode populations is being assessed annually in the fall. Our goal is to determine if the exceptional growth of VX211 is due to hybrid vigor or nematode tolerance. These different inoculation levels should reveal the importance of different field population levels at time of planting, but without any rejection component whatsoever.

3. In February 2003 Janine Hasey and Tom Burchell provided us with 60 second-year rootings: Fifteen each of Chandler clones, 15 each of Vina clones, 15 NCB seedlings and 15 Paradox seedlings. Trees were planted into a 2.5 X 2.5 X 2.5 ft site containing 100 root lesion/250 cc and three root knot nematodes/250 cc soil. Trees were dug after 210 days using a backhoe which enabled quantification of tree biomass in addition to nematode population levels within roots of various size categories. In February 2004 Bob Beede and Tom Burchell provided us with an additional set of English and NCB rootings that included Serr, Chandler and Chandler on NCB. This latter planting was handled similar to those of Janine's except much more effort was placed in measurements of root sizes.

RESULTS AND DISCUSSION

<u>A. Field evaluation of pre-plant soil treatments</u>

Site 1 – Our standard July soil sampling of 27 NX and 27 DN trees revealed that nematode counts did not increase until fall 2004. Incidence of *P. vulnus* has increased across the treatments since that time. This sampling involves collection of soil along the microsprinkler-irrigated berms to the depth of 18 inches. However, this 8-acre planting can be viewed from a high levee bank and for the first time there appear to be occurrences of somewhat reduced growth within the Telone/Vapam strip-treated treatments compared to the broadcast-treated methyl bromide treatments. The effect is very subtle and erratic. This was a 50 gpa Telone treatment. Trunk circumferences for the trees in fall 2004 and 2005 reveal a significant decline in tree biomass associated with the strip treatment of Telone and particularly the drench application of metam sodium compared to that from methyl bromide treated soil (See Figure 1).

Site 2 – In a clay loam soil near Winters, CA the grower removed his old orchard in 1999 after treating all the stumps with Garlon. No *P. vulnus* were present in this site but *Mesocriconema xenoplax* was plentiful. This grower demonstrated in an adjacent block that he must treat this soil to attain adequate tree growth. In 2000 the site was planted to sudan grass to dry the soil deep (<12% soil moisture throughout the surface five feet of soil) and then treated with 332 lb/acre Telone II without metam sodium or a tarp utilized at the field surface. We have sampled across this field each fall after the new trees were planted in 2001. In 2004 population levels had reached 50 ring nematode/250 cc soil. In July 2005 (fourth leaf) the grower observed tree death and chlorosis associated with an elevated infestation of *P. vulnus* along one portion of the block. This damage appeared similar to that from Phytophthora. By fall 2005 the grower had removed 40 trees from the 8-acre block and prepared for spot fumigation. Affected trees will be replanted in 2006. In this clay loam soil the fumigation has been a failure in spots around the field.

Site 3 – In a 50-acre clay loam soil within Capay Valley, Garlon was applied in fall 2000. The field was idled in 2001 as old trees were removed. At the end of one year the *P. vulnus* population within soil samples collected to five-foot depth had reduced from 500/250 cc soil to 250/250 cc soil. In spring 2002 the site was planted to safflower. Following safflower the population levels of *P. vulnus* in the surface 2 feet averaged 60/250 cc soil. This site was left fallow in 2003. Soil samples collected at one-foot increments to the 12-foot depth in April 2003 revealed population levels deep in the soil as high as 400/ 250 cc soil. In this soil of higher clay content than the Rio Oso site, the nematode populations appear to be more resilient deep in the soil even though there has been nothing for them to feed upon. The grower became concerned about these high counts and applied 33.7 gpa Telone in June 2003. The soil moisture at that time ranged from 10 to 14%. Post-treatment soil samples collected in July to the five-foot depth revealed occasional surviving nematodes throughout the soil profile but with occasional pockets of *P. vulnus* in excess of 300/250 cc soil at the 4 and 5 foot depths. This field was close-planted with seeds in spring 2004 after: Garlon > tree removal > 2 years fallow > 1 spring of safflower > then Telone fumigation broadcast at 33 gpa rate. Nematodes in the first year are similar to what they were before planting and have a very spotty occurrence. First year tree growth is generally good except for an early problem with inadequate irrigation overlap that showed up as a row affect. This problem was corrected by late June 2004. Soil samples collected in 2005 reveal incidence of *P. vulnus* from five of five soil samples across the second-leaf trees.

Site 4 – A 220-acre fine sandy loam soil infested with 500 *P. vulnus* / 250 cc soil near Visalia, CA was selected. The grower did not use Garlon but chose to remove trees and then spend a full summer physically removing old tree roots while drying the soil. By late summer of 2001 the soil was dried below 12% soil moisture to the five-foot depth. The pre-treatment surface moisture requirement was met by delivering 1.5 inches water via sprinklers and 332 lb/acre Telone II applied. Metam sodium at 20 gpa was applied in front of a disk 7 days after the Telone was applied. This application utilized the entire Telone availability for the township. At the end of 2002 (first leaf) we could detect no *P. vulnus* in the surface five feet of soil. Trees were growing very well and with uniformity. At the end of 2003 soil samples from six locations reveal a trace detection of *P. vulnus* in one site. Trees are growing relatively uniform but there are locations several partial rows wide where trees are somewhat less impressive. This author would refer to these occurrences as part of the rejection component that Telone did not completely control. In 2004 the incidence of the weak areas had not disappeared but actually coalesced into a single meandering strip occurring across as many as 5 acres of the field. Its pattern was that of an old stream bed and occurs within a quarter mile of the Kaweah River. The soil had been ripped at least three times to the 5 ft depth in order to dry the soil but that did not alter soil patterns beneath. By fall 2005 the grower had demonstrated his ability to remedy poor growth in the weak areas using nutrient applications. This field is now the single example we have of excellent tree growth after five years and it was also the only field that had less than 12% soil moisture content at time of soil fumigation.

Site 5 – Near Visalia, CA a 6-acre orchard with 500 *P. vulnus /* 250 cc sandy loam soil was removed in June 2000 without Garlon. After 4 full years of non hosts soil samples collected for the surface 3 feet in August 2004 revealed 36 *P. vulnus* / 250cc soil. The orchard was planted to prunes in 2005. Prunes are a poorer host for *P. vulnus* compared to walnut.

Site 6 – At Kearney Ag Center we have planted 2 yr old NCB into a sandy loam soil that had received 235 lb/acre iodomethane 4 months earlier. With eight trees planted across each of four replicates we did not see phytotoxicity in any. Growth measurements compared to methyl bromide and the untreated were collected in winter 2003 and 2004. As a comparison, plum and prune trees planted to these sites did exhibit phytotoxicity. Final growth measurements are being collected at time of this writing but the walnut seedlings appeared unaffected by the iodomethane compared to methyl bromide. In a separate treatment these walnut seedlings were negatively impacted if the pre-plant treatment was sodium azide. This study was concluded in early 2005.

<u>B. Evaluation of rootstocks with potential nematode resistance</u>

Paradox selection VX is planted into several rootstock trials from the Paradox diversity studies. In no case has a large tree or group of VX trees appeared across those plots. Yet, VX211 appeared to exhibit tolerance or high vigor in the presence of *P. vulnus* screens conducted at Kearney. In February 2004 Wes Hackett and Gale McGranahan provided us with 48 clones each of VX211 and AX1 in 2004. One of each clone was planted into 1/100th of an acre inoculated with either 0, 1, 20 or 500 *P. vulnus* per 250 cc soil. The tree trunks were pencil-sized in February, grew similarly into May and then the superior growth of VX211 became evident. The leaflets are somewhat broader and greener from VX211 compared to AX1. By mid September some of the trees were 10 feet in height. Tree heights were measured and VX211 had grown 31% taller than AX1 where no nematodes were present; 30% taller where the inoculum was 500 *P. vulnus* / 250 cc soil (Figure 1, 2, and Tables 1 and 2). In the presence of 1 or 20 *P. vulnus* growth measurements were intermediate and not significant but generally reduced from that of the no nematode treatment. This first year of data indicates that VX211 stood out in our early screening most likely because of hybrid vigor, not tolerance to nematode pressure. In 2005 we simulated grafting of these trees (trunk pruned at 24 inch height) thereby increasing the opportunity for greater nematode damage. We also irrigated and fertilized only sparsely throughout 2005. VX211 continues to exhibit greener leaves than AX1. Trunks of VX211 in the second leaf were generally 60% larger than those of AX1 (see Figures 2 and 3). Compared to VX211 the AX1 leaves became relatively chlorotic by July 2005 but particularly where nematodes were present. Interestingly, with or without the nematode present, VX211 abscised its leaves 2 weeks ahead of AX1 in 2005.

Figure 1.

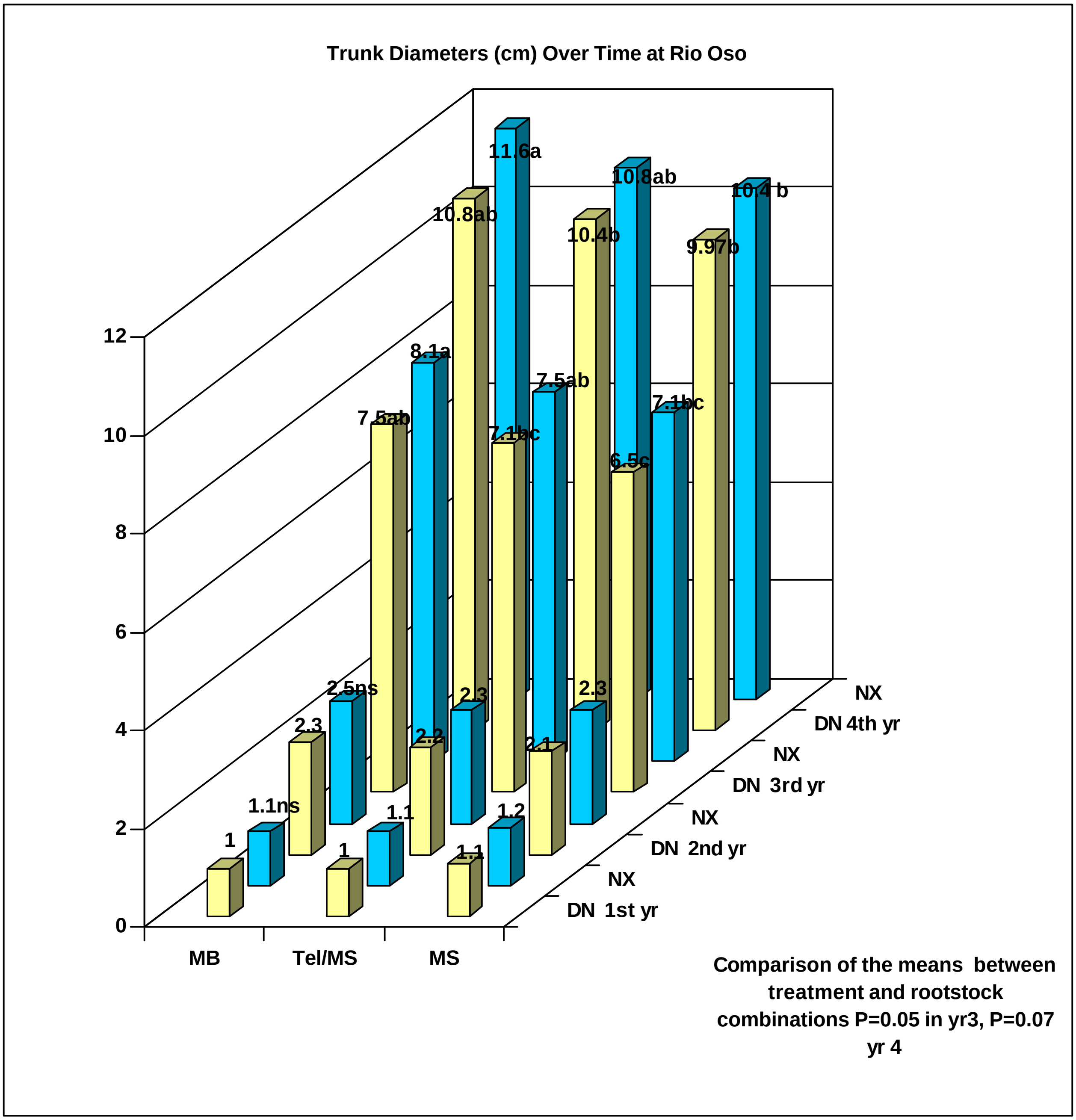
Trunk Diameters (cm) Over Time at Rio Oso
0
2
4
6
8
10
12
MB
Tel/MS
MS
DN 1st yr
NX
DN 2nd yr
NX
DN 3rd yr
NX
DN 4th yr
NX
1
1.1ns
2.3
2.5ns
7.5ab
8.1a
10.8ab
11.6a
1
1.1
2.2
2.3
7.1bc
7.5ab
10.4b
10.8ab
1.1
1.2
2.1
2.3
6.5c
7.1bc
9.97b
10.4 b
Comparison of the means between treatment and rootstock combinations P=0.05 in yr3, P=0.07 yr 4

Figure 2. First-year trunk diameters (cm) from VX211 and AX1 rootstocks at various nematode inoculation levels

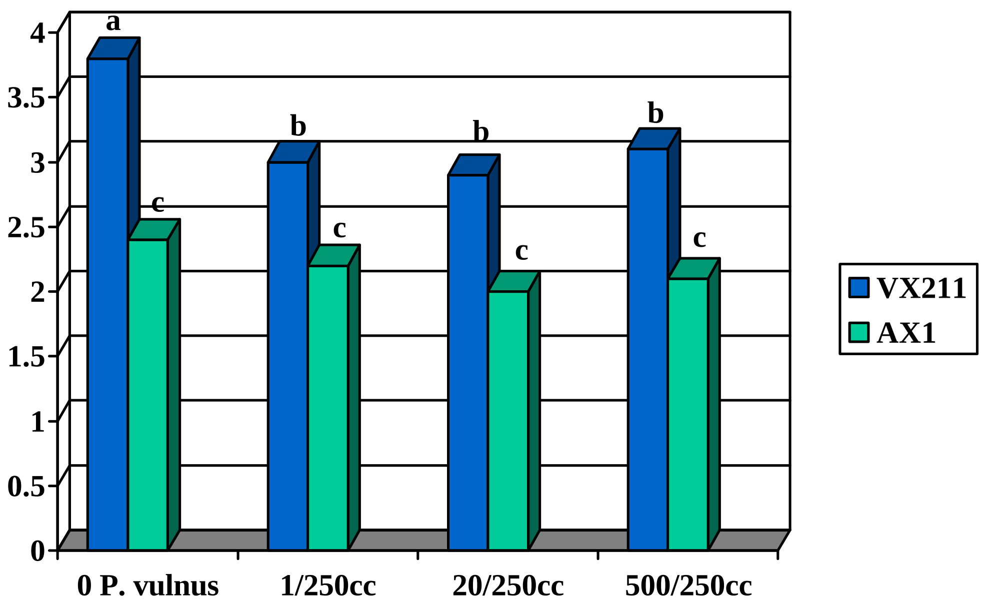

Figure 3. Second-year trunk diameters (cm) from VX211 and AX1 rootstocks at various nematode inoculation levels