METHYL BROMIDE ALTERNATIVES, FOCUS ON ROOTSTOCKS

Michael McKenry

ABSTRACT

At Rio Oso, CA, Chandler scion was grafted onto two different one-year-old Paradox seedlings in the presence of three different pre-plant soil treatments. As reported earlier, the soil treatments resulted in varying volumes of nematode-free soil available for root exploration. Broadcast treatments of methyl bromide or metam sodium supplied a larger volume of nematode-free soil compared to strip applied Telone II. The vigor imparted by these two rootstocks in methyl bromide treated soil is relatively similar. However, a mechanism for pre-infection nematode resistance in NX compared to DN rootstock has enabled NX to fare better when planted after soil treatments that were restricted in distribution. NX seedlings and UZ229 clones are the only two Paradox rootstocks known to possess a mechanism for pre-infection nematode resistance, the commercial value of which was not previously known. Paradox clone VX211 imparts high vigor plus tolerance to nematode feeding. Its tolerance appears to be associated with reduced nematode presence within the terminus 12 inches of each root. In field settings where nematode populations are already low VX211 rootstock does provide nematode relief. Cloned Serr, an English walnut cultivar, is resistant to *Meloidogyne incognita* but remains a good host for *Pratylenchus vulnus.* Seedlings of *Juglans cathayensis* were grafted with Chandler in spring 2008 and 6 of 15 graft sticks were successful through the end of 2008. There is an abnormal swelling that surrounds each graft union, its practical impact not yet known. Five clones of *J. cathanensis* #21 were received in fall 2008, enough to hopefully confirm its putative resistance to *M. incognita* and *P. vulnus*. The walnut hull tea available as NatureCur does not control *P. vulnus* but is effective against other nematode species. At 20,000 ppm as a pre-plant treatment NatureCur is detrimental to growth of young walnut trees. Fosthiazate is too short-lived to be used as a pre-plant treatment, however drenched Telone EC can be highly nematicidal through fall of the first year.

OBJECTIVES

1) Maintain the Rio Oso trial for yield data, rates of nematode return and tree growth.
2) Remove up to half the trees (6 reps) from the VX211 and AX1 trial at Kearney Ag Center and graft over to Chandler for yield data.
3) Obtain for further nematode and field evaluations the clones of RX032 and UZ229 (Wes Hackett).
4) Continue nematode evaluations of various *Juglans* species from the USDA Davis Repository.
5) Compare all *Juglans* selections in adjacent replicated fumigated and non-fumigated sites in two walnut replant orchards, one following NCB the other Paradox.
6) Quantify the number of root tips/tree, their length, and the length of each root tip that will host *P. vulnus* and *Meloidogyne* spp.
7) Explore at our KAC trial site the value of Garlon + one year of chemical drenches and/or non-hosts as methods for reducing soil populations of *P. vulnus*, *Mesocriconema xenoplax* and *Meloidogyne* spp. down to various soil depths.

PROCEDURES

Objective 1. The Rio Oso site is now 7th-leaf and involves approximately 8 acres with every other tree being either NX or DN paradox. These rows have been planted over soil that received either methyl bromide to the 6-foot depth, Telone II at 50 gallons per acre stripped with delivery to the 4.5-foot depth or a broadcast application of Metam sodium delivered to the 4-foot depth.

Objective 2. Within 48 macroplots (1/100th acre each) at KAC we now have 4 reps each of VX211 and AX1 in each without nematodes plus 12 reps with nematodes. In fall 2006 we cut the trunks within 1/3 of these macroplots and applied Garlon. The remaining 4 or 8 reps will be grafted to Chandler for purposes of yield collection. Nematode sampling of the soil and trunk measurements will continue twice yearly.

Objective 3. Selection RX032 is a *Juglans microcarpa* that we selected because it performed well in our earliest nematode screens. A second *J. microcarpa* was also saved but its vigor level after 7 years is half that of RX032 so it is being terminated. UZ229 is of interest because it is now the largest of three saved UZ trees. These three trees were originally selected because three out of ten appeared to keep *P. vulnus* away from their roots, very similar to what was observed with the NX seedlings. Wood will be submitted to Wes Hackett again this year for further nematode evaluations. Approximately 13 UZ229 were received from Dr. Hackett last year and they are currently receiving evaluation against *P. vulnus* on a lath house bench. RX032 is of interest for its host status against nematodes but also for its growth habit against the rejection component.

Objective 4. In winter 2005-06 we received small amounts of *Juglans* seed from Ed Stover of the Germplasm Repository. These were planted into nematode inoculated soil at KAC. The most vigorous trees today include *J. hindsii* and *J. californica.* In addition we planted varying numbers of *J. regia, J. microcarpa, J. major, J. ailantifolia, J. mandshirica, J. cathayensis* and *J. nigra.* The *J. nigra* trees did not survive and more *J. microcarpa* are needed.

Objective 5. Any trees worthy of future evaluation plus all the trees received from the repository will be sized for uniformity and then planted into adjacent fumigated and non-fumigated sites within a walnut replant site for two years to determine size differences. We usually require six trees for each rep and reps are needed because the intensity of the rejection component varies across a field. It will be difficult to find the rejection component without the presence of *P. vulnus*. Each year we will compare visible differences in growth as well as trunk diameters to determine if trees grow similar whether fumigated or not. We refer to this measurement as tolerance to the rejection component.

Objective 6. By carefully removing entire first-year trees with a backhoe we are able to capture most of the root system. We dissect the root system into: 1) root initials, their number and length, 2) fibrous roots that support the tips <7 mm in diameter and 3) roots larger than 7 mm diameter. Walnut roots whether English, Black or the hybrid tend not to support more than three *P. vulnus*/gram of root. Our interest is in the number of initials, their size and the portion of the root that does not support high population levels. Plant data are tabulated and each column analyzed statistically. Nematode counts are collected from each of these root groupings and also analyzed for differences.

Objective 7. Milo and true sudan grass are non hosts of *P. vulnus* in field settings. Many other plants are as well but following a year of milo, for example, it is not unusual to find very few *P. vulnus* in the surface 2 feet of soil. This is the reason we recommend soil samples to 3-feet deep when searching for *P. vulnus* if the orchard has been out for a while. Acrolein is a weed killer that is used in aquatic settings such as ditches, lakes, ponds. It is also a good nematicide when applied to the 5-foot depth at 250 ppm in 6 inches of water. Further, NCB grows very well in the years following such applications.

RESULTS AND DISCUSSION

The value of pre-infection resistance

The two sources identified in 1999 to have pre-infection resistance mechanisms were NX and UZ seedlings. Three to four of the 10 seedlings examined from these two sources were unique within the Paradox Diversity Study, but we did not know the value of this type of resistance mechanism within an orchard setting. This mechanism has been reported in the literature but what is it worth? An 8-acre soil fumigation experiment at Rio Oso, CA provided an opportunity to compare NX Paradox seedlings with seedlings of DN Paradox.

Seven years after replanting with these two rootstocks into a methyl bromide (MB) fumigated site has provided trunk circumferences of 22.6 inches compared to 21.6 inches between NX and DN seedlings, respectively. After seven years, trunk size differences following MB are not significant and not consistently observable across the orchard (See Table 1a). The vigor of these two stocks appears similar if their root systems are grown in soil free of nematodes down to a depth of six feet.

The second best soil treatment at Rio Oso involved a strip application of 50 gpa Telone II (1,3-D) into this soil of 14% moisture content. It impacted nematodes and the rejection component of the replant problem to a depth of 4.5 feet deep within a treated zone 11 feet wide. In fact, the first 2 - 3 years of tree stature and girth following 1,3-D was similar to that of MB treated regardless of rootstock choice (refer to earlier California walnut board research reports). In 7^{th}-leaf the tree trunks of NX are 4% smaller than that of NX trees planted into MB and this difference is not significant. By contrast, the tree trunks of DN are 10% smaller than that of DN grown in MB treated soil (see Table 1b). This reduction in growth is significant at the 5% level of confidence.

Our third treatment was to drench the soil with 332 lb/acre metam sodium (MS) within 7 inches of water. This provided slightly reduced nematode control (99.9% instead of 99.99% control) as well as relief from the rejection component of the replant problem to a depth of 4 feet deep, broadcast. It was following this treatment that nematode populations began to return after two years (see earlier CWB reports). At 7^{th} leaf the trunk sizes are 16% reduced where DN is grown in MS treated soil versus MB treated soil. By comparison, trunk size of NX is only 6% reduced from that of MB treated soil. For NX stock there is no major growth difference when planting into MB treated or MS treated soil. However, for DN rootstock the benefit of MB compared to MS is significant at the 5% confidence level.

A springtime frost at Rio Oso destroyed the 2008 walnut crop. Thus we do not yet have any commercial harvest data from this experimental site to correlate the importance of trunk size with economic return to the grower.

We now have at least one metric that demonstrates a field level benefit to the planting of a rootstock identified to exhibit pre-infection nematode resistance because we can state that the increased trunk size is not a result of the slightly additional vigor associated with NX. Our interest in this trial continues but more importantly we have renewed interest in the publically available Paradox clone UZ229. This clone is frequently the Paradox rootstock that exhibits lowest root lesion population levels in our field trials, however greater attention needs to be given to the lack of vigor associated with rootings we currently receive compared to the vigor level expressed by the UZ229 mother plant at Kearney Ag Center.

Screening of the most recent walnut rootstocks: 'Starve and Switch' + spot nematicides

In fall 2006 we applied Garlon to 16 macroplots that had previously been planted to VX211 and AX1 for three years. Sixty days later trees were removed for root evaluation. In fall 2007 nine planting basins within each of 12 macroplots were treated with various nematicidal products. Four of the macroplots were previously non-inoculated checks and still free of nematodes so no nematicides were added to these. Treatments were all spot applied within three-foot diameter shallow basins along with 6 inches of water. Treatments included: 1) emulsified Telone at 250 ppm, 2) NatureCur (tea from walnut hulls) at 20,000 ppm, 3) fosthiazate at 100 ppm and 4) no nematode, untreated comparison. Clonal material planted into this site was less than successful with an approximate 15% tree death occurring among all the individual clones. Missing trees resulted in missing information but three of the nine clones provided some significant findings particularly for VX211, UZ229 and Burbank rootstocks. It is noteworthy that the other six clones tended to follow growth patterns and nematode build-up similar to that observed with the three clones where significant differences were evident.

Collection of soil samples from within the 1,3-D drenched soil provided complete nematode control for one year, regardless of the walnut clone planted. Soil samples from the NatureCur drenched soil showed a 7-fold stimulation of the *P. vulnus* population in July (not shown) and by fall of the first year had achieved a mean population level of 742/250 cc of soil (see Table 2). Trees planted to NatureCur treated sites showed poorest growth, not just because of the *P. vulnus* population explosion but because walnut trees do not grow well even 6 months after additions of walnut tea. This experiment and others have indicated NatureCur is nematicidal to most nematode species but not nematicidal to *P. vulnus*. Collection of soil samples one full year following fosthiazate revealed no long-lasting nematicidal value to the treatment, however trees planted to fosthiazate treated soil did receive short-term nematode control and grew quite well. Soil collected from the originally non-inoculated, untreated check grew as well as others in the trial, thus no major nematode problems and no rejection component were detected following removal of trees that had been in the ground only three years prior to this replanting.

The value of a selection exhibiting superior vigor

VX211 and Vlach are the two clonal Paradox rootstocks currently recognized as having greatest vigor. In general, our lab cannot confirm that Vlach has consistently superior vigor but there are locations where we too have viewed the superior vigor of Vlach. Beyond these two several other Paradox clones currently under study also exhibit superior vigor at least in their first and second year of growth. At our lab these clones have included RX1, RX032 and more recently RR4. By contrast walnut clones such as WIP3, AZ025 and UZ229 are generally not of superior vigor.

Our focus has been VX211 compared to the moderately vigorous clone AX1. Throughout the first three years we evaluated these two stocks VX211 has exhibited approximately 45% more vigor than AX1. We grafted both clones to Chandler in their 4th-leaf and we now observe in the 5th-leaf that their extra vigor transfers to the Chandler scions they support. In Table 3a we present the first yield data from Chandler on VX211 versus AX1. All yields are less than one pound per tree and there are no significant yield differences related to rootstock choice or the presence or absence of nematodes. We expect these yield values to change as these trees age.

In Table 3b we present the nematode counts per 250 cc of soil collected at the end of the 5th-leaf from around AX1 and VX211 trees. As quantified since the first year of their planting the VX211 trees display 30 to 50% greater vigor than AX1 but they almost never support more *P. vulnus* when compared to AX1. In some cases soil population levels were significantly lower on VX211 but even more often these population reductions were not significant. Note in Table 3b that from four reps of VX211 we do not yet have any root lesion nematodes contaminating our non-inoculated plots. This is in contrast to the situation with AX1 where *P. vulnus* is now beginning to contaminate one replicate. In 2007 we reported a mechanism of resistance in VX211 that occurs within the root tip but that *P. vulnus* could still feed from the outside of the root tip. We also reported several years ago that five years after treating walnut trees with Garlon herbicide we could still detect 5% of the *P. vulnus* population (~20 P. vulnus/250 cc soil) surviving without a food source and as deep as 9 feet below ground. The long survival of non feeding *P. vulnus* plus the relative obscurity of the resistance mechanism within VX211 lead us to the conclusion that we could have resistance mechanisms at work and not even be aware of their presence. As reported numerous times but more clearly elucidated in 2007: "VX211 is a paradox clone of superior vigor that exhibits tolerance to nematodes due to a mechanism for resistance present within its root tips".

Unlike other Paradox clones of superior vigor VX211 has at least one mechanism for fending off nematode development.

Resistance to root knot but not root lesion

Clones of Serr are currently our best example of an English walnut with resistance to root-knot nematodes. It is not resistant to root lesion nematode.

In 1984 this lab screened several English seedlings provided by Dr. McGranahan. In our one-year evaluation of ten replicates none of the English cultivars was resistant. Those English cultivars included Amigo, Eureka, Serr, Tulare, Waterloo and a few others. In those days, when cloning was not considered an option, we averaged our nematode data across 10 reps and the result was our finding of too many nematodes detected among the mean to even consider that any were resistant. We were shortsighted! There were several individuals among the ten reps of Serr that did not support root knot. As luck would have it, the Serr tree chosen for cloning and now commercially available happens to be resistant to *Meloidogyne incognita*. Thank you Bob Beede for encouraging root studies of Serr. Future attempts to find root knot resistance among English should begin with Waterloo which happened to show the lowest mean of nematode numbers of the group.

During our 2-year PDS evaluations recall that we evaluated the Paradox but also the Black Walnut parent of many of those hybrids. Ninety percent of the seedling Black Walnuts and an equal number of the Paradox seedlings did not support *M. incognita.* Eventually, we became most interested in the Rawlins cv of *J. hindsii* because we noted it to offer some protection against *P. vulnus.* As we have repeated screens of many Rawlins seedlings we have noted that about 2/3 of the seedlings offer resistance to *M. incognita* but that 1/3 of them are highly susceptible. Breeders searching for NCB parentage with resistance to *M. incognita* are guaranteed to find some in Rawlins but the reason I suggest Rawlins is because there may be some useful *P. vulnus* resistance also present.

Interestingly, we found that 90% of the Paradox seedlings we screened also had apparent resistance to *M. incognita*. We further noted that some of the most vigorous Paradox were the best hosts for *M. incognita*. For example, after five years we have not yet detected any *M. incognita* on the medium vigor AX1 but we have found them on the more vigorous selections VX211, Vlach and Wingnut.

Resistance to root knot and root lesion nematodes

This goal is the most challenging but also the most needed. To complicate our search, our goal is to find tolerance to the rejection component of the replant problem while searching for complete nematode resistance. My thesis is that we will find solutions to the rejection component by looking among *Juglans* spp that have parentage quite different from *J. regia* and *j. hindsii.* To reach this goal and with the help of the National Germplasm Repository we expanded our search among elite Juglans species. In 2007 we reported the finding of complete resistance (<0.2 nematodes/gram of root after two-year evaluations) within *Juglans cathayensis*. Future reports from our lab will include nematode evaluations from this species. In spring 2008 John Slaughter of Burchell Nursery grafted 15 sticks of Chandler into three-year-old limbs of *J. cathayensis*. The cuttings on six of the limbs have survived to this date. At each of the unions there is a narrow enlarged tree circumference. We do not yet know the practical implications of this.

Our lab has now received five cloned *J. cathayensis* #21 rootings from Wes Hackett and we will initiate conformational studies of nematode resistance within this rootstock in 2009. We should have an answer by next year's report. Phenotypically, *Juglans cathayensis* is a very different tree from *J. regia* or *J. hindsii.*

Table 1a. Tree growth differences of two rootstocks following three pre-plant soil fumigation treatments: 7th-leaf trunk circumferences in inches.

Fumigant	**NX rootstock**	**DN rootstock**
MB @400 lb/acre' broadcast	22.6 a	21.6 a
Telone II 50 gpa strip applied	21.8 a	19.6 b
Vapam drench to 4', broadcast	21.3 a	18.2 b

Read DMRT values across at *P*=0.05

Table 1b. Trunk circumferences expressed as a percent of that obtained following methyl bromide.

Treated soil zone	**NX rootstock**	**DN rootstock**	**circumference difference ***
99.99% control to 6'	100% a (22.6 in)	100% a (21.6 in)	1 inch
99.99% control to 4.5' by 11' strip	96% ab	90% c	2.2 inches
99.9% control to 4'	94% b	84% d	3.1 inches

* compared to MB treated DMRT, *P* = 0.05

Table 2. First-year growth one year after Garlon, then four spot treatments that impacted *P. vulnus*.

	Tree height in centimeters at one year after replanting				
	1,3-D ec	NatureCur	fosthiazate	uninocc./untreated	P=0.05
	height (cm)	height (cm)	height (cm)	height (cm)	height cm
RX1	37.1	17.4	32.2	28	ns
RR4	34.8	19.7	36.9	36.4	ns
VX211	34.2 a	13.5 b	35.8 a	35.1 a	
Burbank	30.8 a	14.9 b	24.3 ab	15.2 b	
J1A cont	29.8	8.4	24.8	25.9	ns
UZ229	26.3 a	5.8 b	19.9 ab	12.8 ab	
WIP3	25.3	16.3	22.8	18	ns
AZ025	24.3	14.2	30.2	29	ns
Vlach	20.1	10.6	36.5	19.5	ns
Ave. Pv.	0/250 cc soil	742/250 cc soil	240/250 cc soil	99/250 cc soil	

Table 3a. Yield of Chandler nuts at 5th-leaf after planting VX211 and AX1 clones into one cubic yard of soil containing 0, 1, 20 or 500 *P. vulnus* /250 cc of soil.

Initial Innoc.	Rootstock	nut weight / tree
0	VX211	206 grams
	AX1	343
1	VX211	163
	AX1	186
20	VX211	220
	AX1	307
500	VX211	161
	AX1	303

non significant at *P*=0.05

Table 3b. Root lesion nematodes/250 cc soil around VX211 and AX1 Paradox clones five years after planting (Sampled November 2008).