METHYL BROMIDE ALTERNATIVES, FOCUS ON ROOTSTOCKS

Michael McKenry

ABSTRACT

In 2009 we collected yield data from six-year-old Chandler scion on VX211 vs AX1 rootstocks. The number and total weight of nuts produced per tree were significantly greater on VX211 compared to AX1 whether inoculated with nematode-free soil or soil containing 1, 20 or 500 *P. vulnus*. A manuscript detailing the finding and testing of VX211 vs AX1 is currently in press and the abstract of that publication is attached herein. We also collected yield data from 8-year-old Chandler scion on NX vs DN, with 40% of the NX rootstock expected to possess pre-infection resistance to *P. vulnus*. Planted to ground fumigated with 400 lb/ac methyl bromide the DN trees produced yields slightly better but not significantly better than NX. NX trees planted to ground strip-treated with 500 lb/ac Telone II produced the same yields as those treated with methyl bromide, however yields from DN were reduced by 20%. Trees planted to soil drenched with 330 lb/ac metam sodium suffered yield losses of 9% and 37% for NX and DN, respectively. The mechanism of nematode resistance in NX and UZ229 appears to provide practical field value. UZ229 needs greater attention relative to its reduced vigor and its ability to strike roots. Hackett has just provided us with 150 clones of UZ229 for future studies. Beyond the paradox hybrid selections listed above our newest research is focused on *Juglans cathayensis* because of its high resistance to *P. vulnus* and *Meloidogyne* spp. Clones from *J. cathayensis* #21 planted in spring 2009 enable us to now confirm that it is truly nematode resistant; the only *Juglans* spp. known to be a complete non-host of root-knot and root-lesion nematodes. This selection also provides additional features. Live *P. vulnus* survive deep within soil without feeding for five years but when growing in the vicinity of roots of *J. cathayensis* #21 they cannot be found after two years. Roots of this selection are in some manner actively nematicidal. Another feature of this selection is that it is a very different parentage when compared to commercially available *Juglans* spp or hybrids. We search for such differences when selecting for tolerance to the rejection component of the replant problem. On the negative side, grafting Chandler onto *J. cathayensis* seedlings has been only 50% successful and after two years there continues to be a physically expansive graft union in every case. We now have 50 seedlings of second-leaf *J. cathayensis* that will be planted into a replant setting in 2010.

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) 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 8th-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 but it has not rooted well to this date. We have also observed it to support high populations of Ten-lined June Beetle. 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 NX rootstock. RX032 is of interest for its host status against nematodes but also for its potential growth habit against the rejection component.

Objective 4. Evaluation of more exotic selections of Juglans from the National repository initiated in 2005. We evaluated varying numbers of *J. regia*, *J. microcarpa*, *J. major*, *J. ailantifolia*, *J. mandshirica*, *J. cathayensis* and *J. nigra*. Our focus is on *J. cathayensis*. In 2010 we will receive numerous first-leaf trees of *J. cathayensis* collected during an expedition by Gale McGranahan.

Objective 5. Fifty *J. cathayensis* will be planted into a walnut replant setting at Kearney in 2010 for evaluation of their tolerance to the rejection component of the replant problem.

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 its worth? An 8-acre soil fumigation experiment at Rio Oso, CA provided an opportunity to compare NX Paradox seedlings with seedlings of DN Paradox seedlings. Yields were collected in 2009 and those findings parallel with the trunk circumferences of earlier years.
These yield data plus our earlier reports on nematode build-up and trunk diameters indicate the practical value of having even a moderate amount of nematode resistance within Paradox rootstocks. Rootstock breeding programs must include nematode resistance, particularly to *P. vulnus*, if walnut yields are to be maintained over a number of years. In addition, these findings provide quantification of the short sightedness of a strip application compared to a broad application of pre-plant fumigants when walnut is the replant and *P. vulnus* is present. Strip applications have value when the major soil pests are not present or there is durable resistance to them within the rootstock.

**Screening of the most recent walnut rootstocks: ‘Starve and Switch’ + spot nematicides**

In fall 2005 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. Except for use of emulsified Telone as a pre-plant none of the “softer” chemicals provided adequate pre-plant protection.

**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 have evaluated these two stocks VX211 has exhibited approximately 40% more vigor than AX1. We grafted both clones to Chandler in their 4th-leaf and we now observe in the 6th-leaf that their extra vigor transfers to the Chandler scions they support. In Table below we present the yield data from Chandler on VX211 versus AX1.
These yield data parallel our findings from annual trunk girth measurements and the reader must recognize that these were not replant settings; total yield losses being due only to the presence of the nematode. Although not significant, trunk measurements as well as these yields indicate that higher inoculum levels resulted in improved tree growth and yield. Our hypothesis for this discrepancy is based on our method of providing inoculum in this experiment. Our inoculation procedure involved a serial dilution of soil collected from around plum trees infected with *P. vulnus*. Every 250 cc of this well-mixed soil contained 500 *P. vulnus* plus any egg stages or biological control agents present therein. Bent et al. recently reported on the nematode control value of various organisms within this soil. The mass of soil used to inoculate 500 *P. vulnus* was 25 times larger than the mass of soil used to inoculate 20 *P. vulnus*. Likewise, the mass of soil used to inoculate 20 *P. vulnus* was 20 times larger than the soil mass used to inoculate with 1 *P. vulnus*. There were bio control agents with our inoculum and those offered nematode relief when they were in adequate abundance.
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. After two years there continues to be an enlarging tree circumference at the graft union. We do not yet know the practical implications of this.

First-year nematodes / 250 cc soil from various *J. cathayensis* selections

<table>
<thead>
<tr>
<th>Tree Selection</th>
<th>n</th>
<th>root-lesion</th>
<th>root-knot</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.03 B:2-12</td>
<td>5</td>
<td>601</td>
<td>0</td>
</tr>
<tr>
<td>442 C:5-13</td>
<td>5</td>
<td>142</td>
<td>6.2</td>
</tr>
<tr>
<td>11.01 A:2-26</td>
<td>12</td>
<td>554</td>
<td>14</td>
</tr>
<tr>
<td>11:02 A:2-25</td>
<td>5</td>
<td>57</td>
<td>37</td>
</tr>
<tr>
<td>11:04 B:2-13</td>
<td>1</td>
<td>1414</td>
<td>0</td>
</tr>
<tr>
<td>J. cat #21</td>
<td>5</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>PI 159568 Afghan</td>
<td>5</td>
<td>993</td>
<td>0</td>
</tr>
</tbody>
</table>
First-year soil counts from each of five
*J. cathayensis* selections

<table>
<thead>
<tr>
<th>Rootstocks</th>
<th>$P. vulnus / 250$ cc soil</th>
<th>Mean $P=0.05$</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.cat #21 clones</td>
<td>25 24 7 0 27</td>
<td>16.6 a</td>
</tr>
<tr>
<td>J. cat siblings</td>
<td>652 436 944 491 484</td>
<td>601 b</td>
</tr>
<tr>
<td>English PI 159568</td>
<td>748 1444 274 1188 1312</td>
<td>993 b</td>
</tr>
</tbody>
</table>

REFERENCES


Abstract: VX211 is a highly vigorous Paradox hybrid clone that outgrew other walnut seedlings in the presence of nematodes. A four-year macroplot trial involving Paradox VX211 and a standard Paradox selection, AX1, demonstrated that the damage threshold level of *Pratylenchus vulnus* on commercially available walnut rootstocks is $<1$ nematode/250 cm$^3$ of soil. Using 1 as the initial population level (Pi) within an inoculation zone of 80 L of soil, the $P. vulnus$ population level increased 2,500-fold in the first year of growth. Three years after inoculation soil population levels of $P. vulnus$ on VX211 were significantly reduced compared to that of the moderately vigorous AX1. Growth of VX211 was 35% greater than that of AX1 regardless of the Pi. Examination of stained roots revealed that feeding and reproduction by $P. vulnus$ on VX211 was primarily ectoparasitic. This is the first report on a new walnut rootstock that can be readily cloned, has high vigor; exhibits tolerance to low population levels of $P. vulnus$, reduces nematode feeding and reproduction within the root terminus, and is currently available to California growers.