

CLONAL PROPAGATION OF WALNUT ROOTSTOCK GENOTYPES FOR GENETIC IMPROVEMENT 2007

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ABSTRACT

U. S. patent applications have been submitted for clonal rootstocks RX1 (*Phytophthora* resistant) and VX211 (nematode tolerant) and several entities have been licensed to produce and sell them in California. We continued to micropropagate candidate pest and disease resistant or tolerant genotypes for greenhouse screening with liner-sized plantlets and for orchard trials with bare-root nursery-grown trees. We produced over 4500 liner-sized plantlets of 28 genotypes for greenhouse screens and growing in the nursery row to a size large enough for grafting and use in orchard trials. We also produced over 900 liner plantlets of 45 lines transformed for resistance to crown gall for greenhouse tests for susceptibility to gall formation and establishment of a small field plot at UC Davis for testing their horticultural characteristics. Using our *ex vitro* rooting and acclimatization protocols, the efficiency of micropropagation continued to be high with 52% success with rooting and 82% survival and rapid growth of rooted plantlets. An experiment with root pruning of newly rooted microshoots indicates that root kinking in liners can be eliminated without reducing growth by severe pruning at the time of potting. Experiments at two commercial nurseries demonstrate the potential for using root grafting for producing grafted walnut cultivars on clonal rootstocks in one growing season. Evaluation of the orchard trial in Butte County with Chandler on *Phytophthora* resistant RX1 rootstocks and seedling paradox rootstocks after two seasons shows that diameter growth of RX1 trees is very good but somewhat smaller than for the seedling paradox trees.

GOAL AND OBJECTIVES

The goal of this project is to provide the California walnut industry with new clonal rootstocks selected or designed to combat the most threatening pests and diseases. The overall objective is to devise clonal methods of propagation for candidate genotypes and to provide clonal plantlets so that they can be evaluated in greenhouse and field replicated disease and pest challenge tests.

PROCEDURES AND RESULTS

Propagation

We have continued to use three approaches to clonally propagate candidate rootstock genotypes with nematode, crown gall, *Phytophthora*, or blackline tolerance or resistance:

- A. Tissue culture micropropagation with *ex vitro* rooting of microshoots.
- B. Dormant hardwood cuttings on bottom heated beds.
- C. Bench grafted root cuttings during the dormant season.

Tissue culture micropropagation: In our laboratory and greenhouse at UC Davis, we produced over 4500 liner plantlets of 28 genotypes for replicated disease and pest screening tests by Greg Browne, Dan Kluepfel and Mike McKenry and for growing on and grafting in nurseries for subsequent orchard trials (Table 2). We also produced over 900 liner plantlets of 45 lines transformed for putative resistance to crown gall plus non-transformed control lines for use in greenhouse screening tests for crown gall resistance by Dan Kluepfel (Table 2). These transformed liner plantlets and controls were grown on to a size large enough for screening in 1.5 gallon containers in the greenhouse. Some of these larger sized plants will be used for planting in a small field trial at UC Davis to determine their horticultural characteristics. By use of *ex vitro* rooting our efficiency in producing micropropagated liner plantlets remained quite high with 52% success in rooting microshoots and 82% survival and growth of the rooted microshoots (Tables 1 and 2).

To have an inventory of liner plantlets of the many genotypes available for use throughout the year we have been storing them in cold boxes in a dormant state at about 42°F. After about five to six months in storage at this temperature they begin to sprout etiolated shoots which subsequently deteriorate. To alleviate this storage problem, we are doing an experiment using 32°F storage conditions in comparison to 42°F. After two months storage at 42°F and five months in storage at 32°F, all of the plants of the seven genotypes being used are fully dormant with no etiolated shoot growth. After storage at 32°F for a total of six months the plantlets will be tested for re-growth in the greenhouse.

In an experiment to study the influence of growing newly rooted microshoots of four genotypes in 1.5" x 7" tapered tree tubes, we varied the degree of root pruning performed at the time of transplanting to tree tubes. As shown in Figure 1, the number of kinked roots that were found when the root systems were washed free of soil after three months of growth in the greenhouse was greatly affected by the degree that roots were pruned before transplanting. When the roots were left intact with no pruning, a mean of 1.0 to 1.5 roots per plant, depending on genotype, were kinked. However, if all but 0.25 inch of the roots were pruned of before transplanting, there were no kinked roots on any of the plants of the four genotypes examined. Pruning roots to about one half of their original length reduced the number of kinked roots as compared to the unpruned controls. Surprisingly, as shown in Figure 2, even severe pruning had little or no affect on shoot height growth. In some genotypes, it appeared that root pruning promoted shoot growth slightly. These bare-rooted liner plantlets have been transplanted to 1.5 gallon containers for further growth. Their root systems will be evaluated again at the time they are transplanted to a nursery row.

Hardwood cuttings: Work on rooting hardwood cuttings was limited to producing clonal plants for a Mike McKenry nematode susceptibility experiment and WIP backcross clones that do not propagate well by tissue culture micropropagation. We produced about 50 plants each of UZ229 and RX032 and 40 plants of WIP5 for growing on for grafting in a commercial nursery.

Bench grafting root cuttings: We performed experiments on root grafting at two commercial nurseries this past year. One experiment was initiated February 28, 2007 using a suspended heating cable system in plastic tubs filled with wet wood shavings that kept the graft union and the bottom of the root piece at 27°C. Chandler scions were whip-and-tongue grafted on paradox

seedling root pieces of varying diameter. The bottoms of the root pieces were treated with a quick dip of 8000 mg/l potassium indolebutyric acid (KIBA). When rooting data were taken on April 3, 2007, we found (Table 3) that nearly 100% of the root pieces had formed adventitious roots. However, root counts showed that the number of roots formed increased with increasing diameter of the root piece. All 87 of the root grafts that were rooted were transplanted to a nursery row. When survival and growth data were taken on December 12, 2007, it was determined that 22 of the 87 rooted root grafts had survived and grown. Observation indicated that it was mostly the small and medium sized root grafts that had died in the nursery row. When the largest diameter of the shoots growing from the Chandler scions on the paradox seedling root grafts was measured, it was found to have a mean of 22.0 mm (0.87 inch). In comparison, the mean of the largest diameter of shoots of June-budded Chandler scions on paradox seedling rootstock was 12.6 mm (0.5 inch).

The second root grafting experiment was initiated March 22, 2007 using a suspended heating cable system in plastic tubs filled with wet wood shavings that kept only the graft union at 27°C. Although the bottom of the root piece was not heated, it was treated with a quick dip of 8000 mg/l KIBA. Three different cultivar scions were whip and tongue grafted onto six different rootstock clones (Table 4). Observations at the time of planting of the grafted root pieces from the six clonal rootstock indicated that they had quite large adventitious root systems with the exception of those from WIP3 which had many but short adventitious roots. This may indicate that WIP3 needs heat provided to the base of the root piece to obtain greater adventitious root elongation. At the time of planting in the nursery row, graft-take was about 90% with the exception of grafts involving rootstock GZ2 where graft-take was 50% or lower dependent on the scion cultivar. Tables 4 and 5 show the survival and scion shoot mean maximum diameter data collected on December 14, 2007. Survival was quite variable depending on rootstock genotype with grafts on RX1 (74%) having the best survival and those on GZ2 having the worst survival (36%). Survival varied much less with scion cultivar with Tulare having the highest survival at 62% and Howard the lowest at 50%. Overall survival for the three scion cultivars on the six rootstock clones, a total of 234 grafts, was 55%. Growth of scion shoots was also quite variable with grafts on RX1, VX211 and GZ2 having the largest diameter (17.7 mm) and WIP3 the smallest (12.3 mm). Shoots from Tulare scions had the largest diameter across rootstock clones (17.4 mm). Overall shoot diameter of grafts of three scion cultivars on six rootstock clones was 16.0 mm (0.63 inch).

These two experiments show good potential for using root grafting to produce grafted walnut cultivars on clonal rootstocks in one growing season (March 1 to December 15). Improvements are needed in transplanting to the nursery row so that survival is higher. Caliper of shoots from root grafted scions on paradox seedling rootstock appeared to exceed that of shoots from June-budded scions on paradox seedlings in the experiment reported here.

Field Trials

The first two field trials were planted with one year old nursery-grown clonal paradox in 2004 and 2005 in replant situations. These trials were planted as ungrafted trees and grafted on site. Grafting on site introduced substantial variation because not all grafts were successful on the first attempt. Reports of these trials are given by Joe Grant and Janine Hasey in this edition of the Walnut Research Reports.

A third field trial established in a newly planted orchard in Butte County is now in its 2nd leaf. It consists of 80 RX1, some remaining AZ2, and 80 seedling paradox, all nursery grafted to Chandler. The AZ2 failed to thrive at transplanting and either died or had to be cut back severely. About half have been replanted. RX1 and paradox seedling rootstock trees transplanted with nearly 100% survival and have grown well in both seasons except for a few RX1 which had a small lean from vertical. Diameter measurements taken (on a subset, n=40, of the rootstocks) directly below the graft union on December 14, 2007 were very similar: RX1=87 mm (S.E.=1.3); seedling paradox=91mm (S.E.=1.6). However, in retrospect, measuring just below the graft union is somewhat misleading because in general the seedling paradox were grafted higher than the RX1.

No new rootstock grower trials were initiated this year due to our decision to only plant grafted trees unless otherwise requested. Rootstocks of VX211, RX1, Vlach, AZ025 and WIP3 as well as several less characterized rootstocks have been grown at several nurseries and grafted to Chandler. Our appreciation goes out to these nurseries.

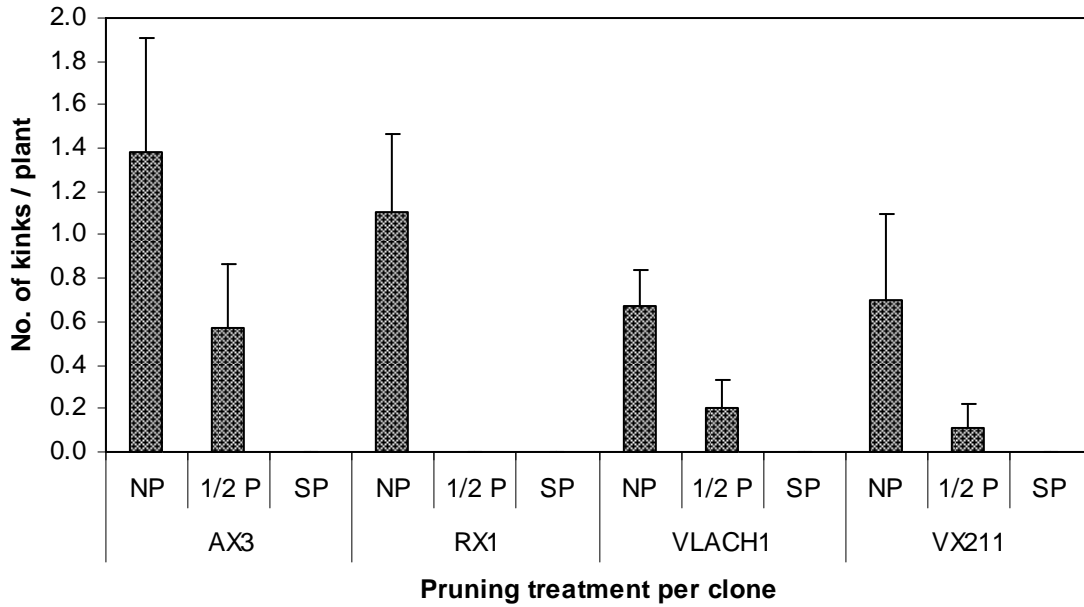
The largest orchard trial to date will be planted this year in San Joaquin County under the direction of Joe Grant. Chandler-grafted nursery trees using five rootstock clones and seedling paradox will be dug in January 2008 and planted along with own-rooted Chandlers.

A one acre field plot at UC Davis has been has been methyl bromide fumigated in preparation for field-planting transgenic putatively crown gall resistant lines for assessment of their horticultural characteristics.

Nursery Propagation and Commercialization

U. S. patent applications have been submitted for clonal rootstocks RX1 and VX211. Several tissue culture micropropagation laboratories and tree nurseries have been licensed to produce and sell RX1 and VX211 clonal rootstock in California. We can provide cultures of microshoots to any laboratory or nursery that wants them for licensed production of plants. We can also provide microshoots of Vlach, a public domain clone, to any laboratory or nursery that wants to produce it.

Figure 1. The influence of severity of root pruning rooted microshoots at time of planting on root kinking in four rootstock clones. Data taken three months after planting in 1.5" x 7" tapered tubes.

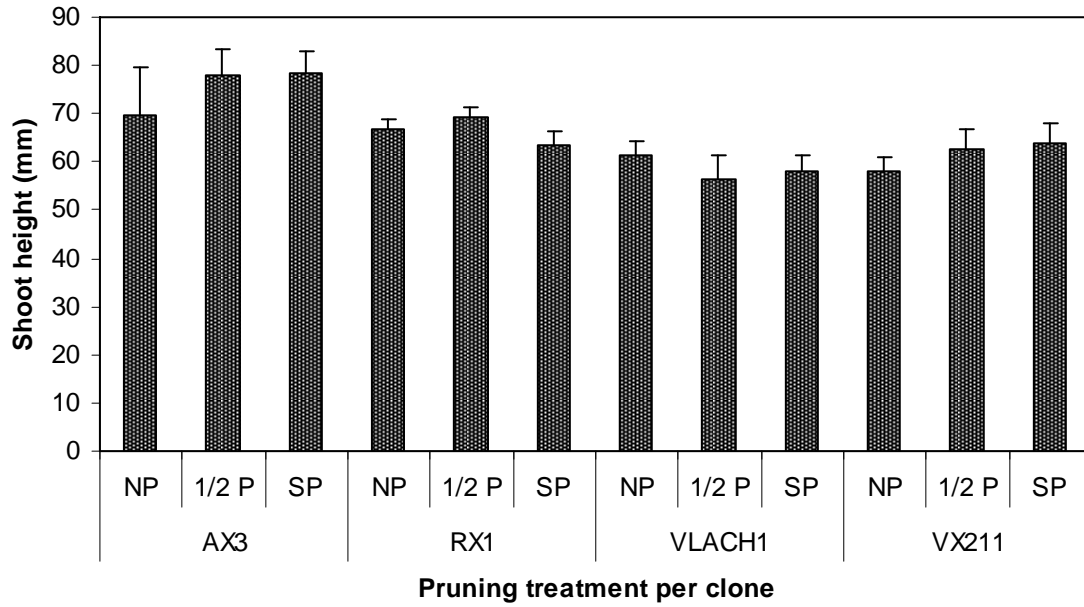


NP=Not pruned

1/2P – half of root length removed

SP = severely pruned (0.25 inch of root retained)

Figure 2. The influence of severity of root pruning rooted microshoots at time of planting on shoot growth in four rootstock clones. Data taken three months after planting in 1.5" x 7" tapered tubes.



NP=Not pruned

1/2P – half of root length removed

SP = severely pruned (0.25 inch of root retained)

Table 1. Greenhouse survival of ex vitro rooted rootstock genotypes in 2007

Genotype	# Alive	# Dead	% Survival
<u>Paradox selections</u>			
84-121	44	21	68
AX1	256	32	89
AX2	442	36	92
AX3	155	44	78
AZ025	157	33	83
Burbank	276	49	85
DAR	52	23	69
GZ2	15	4	79
GZ3	82	38	68
JX1	374	78	83
MW1	55	31	64
Px1	126	6	95
RX1	269	45	86
UX1	37	15	71
UX2	364	63	85
UZ 229	60	21	74
UZ1	324	64	84
UZ2	169	39	81
Vlach	401	84	83
VX211	293	39	88
XZ1	322	75	81
WNBxGRZ 1a	45	28	62
WNxW 10.05 b	27	0	100
<u>Backcrosses</u>			
WIP1	4	3	57
WIP2	26	6	81
WIP3	70	9	89
WIP4	0	1	0
WIP6	59	17	78
<u>Transgenic crown gall resistant</u>			
J1 control	84	17	83
J1 transgenic (23 lines)	180	61	75
J21 control	130	52	71
J21 transgenic (7 lines)	99	22	82
RR4 control	130	22	86
RR4 transgenic (15 lines)	298	76	80
<u>Total</u>	5425	1154	82

Table 2. Ex vitro rooting percentage for rootstock genotypes in 2007

Genotype	# Tried	# rooted	% Rooted
<u>Paradox selections</u>			
84-121	112	62	55
AX1	417	316	76
AX2	606	457	75
AX3	735	267	36
AZ025	307	169	55
Burbank	623	417	67
DAR	293	94	32
GZ2	58	17	29
GZ3	522	218	42
JX1	828	490	59
MW1	530	92	17
Px1	289	213	74
RX1	902	532	59
UX1	261	69	26
UX2	509	387	76
UZ 229	358	116	32
UZ1	745	400	54
UZ2	491	254	52
Vlach	886	495	56
VX211	788	479	61
XZ1	593	364	61
WNBxGRZ 1a	140	75	54
WNxW 10.05 b	17	10	59
<u>Backcrosses</u>			
WIP1	143	10	7
WIP2	318	46	14
WIP3	318	165	52
WIP4	45	11	24
WIP6	163	75	46
<u>Transgenic Crown gall resistance</u>			
J1 control	259	81	31
J1 transgenic (23 lines)	271	128	47
J21 control	418	222	53
J21 transgenic (7 lines)	125	60	48
RR4 control	317	174	55
RR4 transgenic (15 lines)	236	130	55
<u>Total</u>	13623	7095	52

Table 3. The effect of root piece diameter on rooting success and root number for Chandler on seedling Paradox root grafts stuck in wood shavings at 27°C. Data taken four weeks after treatment with 8000 mg/L K-IBA.

Root diameter (inches)	Grafted (n)	Rooted (n)	Roots (n)
< 3/8	28	27	5.4
3/8-5/8	32	29	6.5
5/8-7/8	31	31	9.7

Table 4. Mean survival percentage of root grafts of three scion cultivars on six rootstock clones (n=13). Whip-and-tongue grafts made 3/22/07 and planted in a nursery row 5/8/07. Data taken 12/14/07.

Rootstock Clone	Scion Cultivars			Overall Rootstock
	Chandler	Howard	Tulare	
AX2	92	69	54	72
WIP3	46	8	62	38
RX1	46	92	85	74
VX211	54	38	67	51
UX022	46	62	69	59
GZ2	44	31	44	36
Overall Scion	54	50	62	--
Grand Mean	--	--	--	55

Table 5. Mean maximum diameter (mm) of scion shoots of root grafts of three scion cultivars on six rootstock clones (n=13). Whip-and-tongue grafts made 3/22/07 and planted in a nursery row 5/8/07. Data taken 12/14/07.

Rootstock Clone	Scion Cultivars			
	Chandler	Howard	Tulare	Overall Rootstock
AX2	15.7	14.3	16.1	15.3
WIP3	11.2	8.0	13.6	12.3
RX1	18.5	15.8	19.2	17.7
VX211	14.4	18.2	20.3	17.7
UX022	15.8	12.1	15.9	14.6
GZ2	17.4	16.0	19.4	17.7
Overall Scion	15.5	14.8	17.4	--
Grand Mean	--	--	--	16.0