University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year 2008-2009 Anticipated Duration of Project 2 of 2 years

Project Leader: Gale McGranahan Location: Davis

Cooperating Personnel: Wesley Hackett, Chuck Leslie, Bruce Lampinen, Abhaya Dandekar, Dan Kluepfel, Greg Browne, Janet Caprile, Mike McKenry, Bill Coates, Joe Grant, Janine Hasey, Kathy Kelley and Nicolas Manterola

Project Title: Clonal Propagation of Walnut Rootstock Genotypes for Genetic Improvement

Keywords: Disease and pest resistance, micropropagation, hardwood cuttings, root cuttings, grafting

Commodity(s) Walnuts Relevant AES/CE Project No.

Problem and its Significance:

During the past several years as a result of Walnut Marketing Board and IAB funding, we have developed methods to clonally propagate many paradox, backcross and several *Juglans hindsii* genotypes using *in vitro* micropropagation, hardwood cutting and/or root grafting techniques. We have produced over 20,000 plantlets of 40 genotypes. Over 3600 of these have been grown in four commercial nurseries to a size large enough for grafting in one growing season. Some of these genotypes have shown a degree of resistance or tolerance to root lesion nematode, *Phytophthora citricola*, *Agrobacterium tumefaciens* and cherry leaf roll virus (blackline). Five genotypes showing moderate resistance to *P. citricola* in greenhouse tests, one with tolerance to root lesion nematodes and moderate resistance to *P. citricola* and one with tolerance to cherry leaf roll virus have been clonally propagated in sufficient numbers for orchard tests. Two of these genotypes have been grafted with Chandler scions and have been planted in an orchard trial in Butte County along with grafted seedling paradox to assess horticultural and disease and pest resistance characteristics. U. S. patent applications have been submitted for RX1 which has moderate vegetative vigor and *Phytophthora citricola* resistance and VX211 which shows high vegetative vigor, lesion nematode tolerance and moderate *P. citricola* resistance. A genetically engineered paradox appears immune to *A. tumefaciens* induced gall formation and one non-transgenic genotype shows slower gall formation as compared to Px1 when tested with greenhouse inoculations using a very virulent *A. tumefaciens* strain.

Our success with clonal propagation and the results of rootstock selection demonstrate the feasibility and provide the opportunity for developing genetically improved walnut rootstocks for the California walnut industry. With the phase-out of methyl bromide as a soil fumigant and the lack of a good alternative for control of soil borne pests and diseases, developing improved rootstocks with both desirable horticultural characteristics and resistance to pests and diseases becomes of paramount importance. Based on the success in identifying or genetically engineering and clonally propagating pest and disease resistant or tolerant genotypes, the outlook is very good for developing and clonally propagating commercially desirable walnut rootstocks.

Objectives And Approach:

The goal of this project is to provide the California walnut industry with new rootstocks selected or designed to combat the most threatening pests and diseases. To accomplish this goal, methods for clonal propagation of the selected or designed genotypes are required. Promising candidate genotypes will be provided by some of our collaborators who are plant pathologists, nematologists or molecular biologists. The overall

objective of the project is to devise clonal propagation methods for the candidate genotypes and provide clonal plantlets so that they can be screened in replicated disease and pest challenge tests. Those genotypes passing the initial screening tests will be propagated in sufficient numbers for nursery production and orchard trials by our farm advisor collaborators. As rootstock clones are shown to have desirable pest and disease and horticultural characteristics, we will work with nurseries and commercial laboratories to commercialize propagation and production technologies.

Plans And Procedures:

Our results over the past several years indicate that most paradox, backcross and black walnut genotypes can be clonally propagated by *in vitro* micropropagation, hardwood cuttings on bottom heated beds in the dormant season or root grafting during the dormant season using a system that heats the bottom of the root piece and the graft union simultaneously. These methods have been described in detail in the Walnut Research Reports for 2001-2006. Micropropagation is generally the most efficient method for rapidly obtaining sufficient numbers of plantlets for replicated pest and disease resistance screens. We have made it more efficient by developing an *ex vitro* rooting protocol. Hardwood cuttings have been more successful for some genotypes than micropropagation but are quite genotype specific. Root grafting may be more feasible for nursery production of many selections where micropropagation facilities are not available. In summary we will use three approaches for clonal propagation of candidate rootstock genotypes with nematode, crown gall, *Phytophthora* or blackline tolerance or resistance.

A. Tissue culture micropropagation with *in vitro* or *ex vitro* rooting. Emphasis will be on *ex vitro* rooting because of the greatly enhanced survival and growth of plantlets obtained from *ex vitro* rooting.
B. Dormant hardwood cuttings on heated beds. Emphasis will be on previously untested genotypes and genotypes for which tissue culture micropropagation is not very successful.
C. Grafted root cuttings during the dormant season. Emphasis will be placed on the time of grafting and rooting during the dormant season and the timing and method of transplanting to the nursery row. We will assist nurseries in implementing the root grafting techniques.

Highest priority will be given to 1) producing Chandler-grafted plants of the most promising genotypes for orchard trials with farm advisors, 2) producing plants of genotypes which show resistance or tolerance to one pest or disease so that they can be greenhouse cross-tested for resistance to the other pests and diseases of concern and 3) producing plants for re-testing of transgenic, putatively crown gall immune or resistant selections to verify their immunity or degree of resistance.

Specific Plans:

Improvement of propagation and growing protocols to enhance efficiency and plant quality for re-testing and orchard trials

A. Consolidate production of plants for re-testing by Greg Browne and Dan Kluepfel in one greenhouse facility in order to enhance plant size and quality for use in pathogen screens and re-tests.
B. Test procedures for transplanting liner plants to the nursery row to improve the quality of root systems of grafted, bare root trees ready for orchard planting.
C. Test procedures for improving nursery survival and stand establishment of root-graft-derived plantlets in the nursery row.
D. Develop a method for obtaining adventitious buds and/or somatic embryos from selected genotypes such as VX211 and RX1 in order facilitate the generation of transgenic plants of these genotypes and other clonal selections.

Orchard trials

A. Establish and evaluate a farm advisor orchard trial with Chandler-grafted VX211, RX1, WIP3, Vlach own-rooted Chandler and paradox seedlings in San Joaquin County (with Joe Grant). AZ025 may be included.
B. Establish and evaluate a farm advisor orchard trial with Chandler-grafted RX1, Vlach and paradox seedlings at the West Side Research and Extension Center to study salt (chloride and boron) tolerance using irrigation water from deep wells (high salt) vs. water from the California Aqueduct (with Bob Beede).
C. Establish and grow liners of VX211, RX1 and Vlach at the Suchan Nursery for Cilker's Orchards.

Suchan will also be growing seedling paradox rootstocks for the same orchard.

D. Establish and grow liners of VX211, RX1 and Vlach commercially for use in producing Chandler-grafted trees for orchard trials.

Nematode resistance and tolerance

A. Propagate and grow liners of VX211, UZ229, RX032, Vlach, Burbank, Tramell, James1 and 21 controls, Rawlins controls, DAR (*J. ailantifolia x regia* hybrid) and *J. cathayensis* using micropropagation and hardwood cutting methods for nematode screens and re-testing by Mike McKenry and for production of grafted nursery trees for additional orchard trials by farm advisors.

B. Devise clonal methods of propagation for re-testing of seedling genotypes of 15 Juglans species from the National Clonal Repository selected by Mike McKenry for lesion and root knot nematode resistance.

Crown gall resistance

A. Micropropagate AZ025, VX211, RX1, Vlach, and WIP3 for re-testing for crown gall resistance by Dan Kluepfel.

B. Propagate promising genotypes from initial crown gall screens of Paradox Diversity Study seed sources and new screens by Kluepfel and the USDA Clonal Repository of new paradox seedlings for re-testing for resistance.

C. Continue micropropagation of the most promising James and Rawlins paradox transgenic lines (J1 19A, RR4 2A, RR4 6A) putatively resistant to crown gall for re-testing by Dan Kluepfel.

D. Establish a one acre field plot to test crown gall resistant transgenic James and Rawlins genotypes for horticultural characteristics.

Phytophthora resistance

A. Continue to provide Greg Browne with micropropagated plants of 20 genotypes for re-testing for *P. cinnamomi* and *P. citricola* resistance using greenhouse screening procedures.

B. Provide Greg Browne micropropagated plants of six new genotypes (UZ229, DAR, James 1 control, James 21 control, WIP6 and WNB x GRZ 1a) for greenhouse screening for *P.* cinnamomi and *P. citricola* resistance.

C. A putative Chandler graft compatible wingnut genotype will also be propagated by hardwood cuttings from basal sprouts (suckers) for further graft compatibility tests.

Blackline tolerance

A. Use hardwood cuttings and grafted root cuttings for clonal propagation of WIP1, 4 and 5 which have not been successfully propagated from microshoots.

B. Initiate *in vitro* multiplication and rooting of WIP genotypes 85-117-.Continue hardwood cutting propagation of these genotypes. Root cuttings with grafts of cultivars will also be tried with these genotypes.

TABLE 1 INVENTORY OF CLONAL WALNUT FOR FIELD TRIALS AND TESTING

	Nursery A	Nursery B	Nursery C	Total
Nematodes and Phytophthora				
VX211	67	12	50	129
Vlach	164	112	50	326
Phytophthora				
AZ2	0	1		1
AZ3	6	15		21
JX2	63	14		77
NZ1	8	0		8
RX1	284	83	50	367
Crown Gall				
AZ025	47	151	50	198
Blackline				
WIP2	235	1		236
WIP3	547	53	50	600
WIP6	9	8		17
Under Test				
AZ1	28	2		30
CW1	40	181		221
GZ2	178	59		237
GZ3	0	87		87
UX1	49	6		55
UX2	68	21		89
Phytophthora susceptible controls				
AX1	143	88		231
AZ1	2	0		2
GZ1	13	33		46
Px1	731	70		801
Total	2682	997	250	3929

BUDGET REQUEST Budget Year 2008-2009

Funding Source

Salaries and Benefits

Jr. Specialist (Hirbod) 75%		$ 24,464
Agric. Technician (Manterola) 50%		$ 19,020
Lab/Field Assistance		$ 10,000
Subtotal	Sub 2	$
Employee benefits	Sub 6	$ 24,466
	SUBTOTAL	$ 77,950
Supplies and Expenses	Sub 3	$ 1,000
Equipment	Sub 4	$
Travel	Sub 5	$ 1,050
	TOTAL	$ 80,000

Department account number _____

_____ Date _____

Originator's Signature

AGRICULTURAL EXPERIMENT STATION

Department Chair_____ Date _____