Workgroup/Department Walnut/Plant Pathology

University of California
Division of Agricultural Sciences
Project Plan/Research Grant Proposal

Project Year 2008/09 (May 1, 2008-April 30, 2009) **Anticipated Duration of Project** Yr 2 of 2

Project Leader G.T. Browne **Location** USDA-ARS, Dept. of Plant Pathology

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Project Title Biology and management of Phytophthora crown and root rot on walnut

Keywords Walnut, *Phytophthora*, crown rot, root rot, genetic resistance, rootstock, Paradox,

Commodity Walnut **Relevant AES/CE Project No**.

Problem and its Significance:

The problems addressed by this proposal are: 1) a need for horticulturaly desirable walnut rootstocks with resistance to *Phytophthora cinnamomi* and *P. citricola*, the most aggressive species of *Phytophthora* causing crown and root rot on California walnuts; and 2) a need to determine additional, apparently unknown causes of crown rot on Paradox rootstock. Phytophthora crown and root rot results in serious economic loss in many California walnut orchards. More than 10 species of *Phytophthora* are known to cause the disease, but among them *P. cinnamomi* and *P. citricola* are particularly aggressive and difficult to manage (Mircetich et al., 1998). Northern California black walnut (NCB) (*Juglans hindsii*) and English walnut seedling rootstocks (*J. regia*) are highly susceptible to most species of *Phytophthora* that affect the crop. Paradox seedling rootstocks (typically *J. hindsii* x *J. regia*) are more tolerant than NCB or English rootstocks to most species of *Phytophthora* but still are moderately to highly susceptible to *P. cinnamomi* and *P. citricola*. *Phytophthora cinnamomi* typically initiates attack through the roots and gradually advances towards the root crown, whereas *P. citricola* tends to invade the root crown or the base of the tree trunk directly. Both pathogens are capable of killing trees within a year or two of infection. The suspicion that there may be additional unknown causes of crown rot on Paradox rootstock (i.e., the focus of objective 2 of this research) is based mainly on: 1) occurrence of Paradox trunk and root crown cankers in the apparent absence of a *Phytophthora* or *Armillaria* sp., 2) the fact that the symptoms appear different than those associated with waterlogging damage and, at least in some orchards, occur in absence of waterlogging conditions.

The assessments of genetic resistance to *P. cinnamomi* and *P. citricola* will build on previous work. For several years we have collaborated with the Walnut Improvement Program (WIP) to evaluate resistance to *P. citricola* in diverse clones of Paradox hybrids (Browne et al, 2006; research reports to the Walnut Marketing Board [WMB] for 2001, 2004-2007). We selected clones from hybrid seed families for putative tolerance to the pathogen or for genetic backgrounds of interest. Generally, in subsequent repeated greenhouse evaluations of the resistance, RX1 (*J. microcarpa* × *J. regia*) and VX211 (*J. hindsii* × *J. regia*), have exhibited moderate tolerance to *P. citricola* while other clones have been moderately to highly susceptible

(Table 1). In 2007, we completed our first evaluation of resistance to *P. cinnamomi* and found RX1 to be resistant to the pathogen (Table 1). The proposed assessments of resistance to *P. cinnamomi* and *P. citricola* (Objective 1) will "round out" information available to growers and nurserymen on tolerance of the rootstocks to two most aggressive *Phytophthora* spp. infecting walnuts in California.

The proposed examination of orchard decline on Paradox rootstock is a response to continuing problems in the Sacramento and San Joaquin Valleys. Compared to NCB, Paradox typically offers superior tree vigor, resistance to *Phytophthora* spp., and tolerance to parasitic nematodes, but in several orchards, the rootstock has suffered decline and death due to root system failure. Some of the cases appear linked to waterlogging, while others appear to involve pathogen(s) (Browne et al., 2006 and 2007 reports to WMB). The former cases 1) have exhibited general, unfocused damage to the root system (i.e., as opposed to well-defined cankers on the root crown or major roots), 2) have occurred at sites subject to poor soil-water drainage, and 3) have exhibited decline incidence that varies gradually in the orchards and follows changes in the soil texture and profile. The latter cases 1) have been associated with focused crown rot, 2) have occurred and sites not apparently affected by high water tables or flooding, and 3) have exhibited "spotty" incidence (i.e., affected trees are scattered among healthy trees). Effective management of these problems requires an understanding of their causes. It is important to determine the causes of this crown rot on Paradox, because it appears to be associated with the death of English walnut trees statewide and may become more prevalent as use of Paradox rootstock continues.

Objectives

1. Evaluate elite Paradox hybrid clones for resistance to *Phytophthora cinnamomi and P. citricola*.

2. Examine contributions of pathogens to decline of English walnut orchards on Paradox rootstock.

Plans and Procedures

Objective 1

Greenhouse trials. It is proposed to evaluate resistance of approximately 25 hybrid clones to *P. cinnamomi* and *P. citricola* in greenhouse trials based at UC Davis. Nineteen of these clones were included in our previous tests and future plans (Table 1), and an additional six clones (UZ229, DAR, James 1 control, James 21 control, WIP6 and WNB x GRZ 1a) are new selections that we have not previously tested as clones. Each of our evaluations of resistance will include AX1 and PX1 (clonal hybrids of *J. californica* × *J. regia* and *J. hindsii* × *J. regia*, respectively) and NCB as standards that are moderately to highly susceptible to *P. citricola*, RX1 (clonal hybrid of *J. californica* × *J. regia*) as a standard moderately resistant to *P. citricola*, and Chinese wingnut seedling (*Pterocarya stenoptera*) as a standard highly resistant to both *P. cinnamomi* and *P. citricola*.

The experimental Paradox selections will be prepared for greenhouse evaluations of resistance by the WIP under supervision by Wes Hackett as indicated in the 2007 WMB proposal "*Clonal propagation of walnut rootstock genotypes for genetic improvement*" from McGranahan et al. The selections will be cycled through dormancy induced by refrigerated storage (several months at 42 °F) or by overwintering in a lath house, depending on the experiment. A cycle of

dormancy tends to equalize the physiological status of the diverse clones and increases "physiological susceptibility" to the pathogen after the plants resume growth. After the chilling, the plants will be moved to a greenhouse and watered and fertilized as needed as they break dormancy and establish vigorous shoot and root growth.

Two to 3 months after emergence from dormancy, under the supervision of Browne, the plants will be transplanted into 2-liter pots of non-infested UC potting soil mix (UCM) and UCM artificially infested with the appropriate *Phytophthora* sp. (40 ml of colonized V8 juice-vermiculite-oat medium per liter of soil). The experiments will employ a split-plot design with the inoculum treatments randomly allocated to main plots (i.e., groups of individual potted plants that include each hybrid clone and standard) in complete blocks and the rootstock plants randomly allocated to subplots (individual pots, each to hold a plant). In each experiment, 5 to 20 plants per rootstock will be inoculated with *P. cinnamomi*, another 5 to 20 with *P. citricola*, and 5 will serve as noninoculated controls. Starting 1 week after transplanting, the plants will be subjected to 48-hr episodes of soil flooding, once every 2 weeks. Between flooding episodes, plants will be watered and fertilized to optimize growth. Plants will be rated for severity of aboveground disease symptoms every 2 weeks using a 0-to-5 scale (i.e., 0=healthy, 5=dead, 1,2,3, and 4=progressive increments of disease). Disease progress curves will be calculated using the biweekly disease severity ratings. About 3 months after transplanting, the plants will be washed free from soil and evaluated for severity of crown and root rot. The proportion of the root crown length and circumference rotted will be measured on each plant and expressed as percentages. The percentage of roots rotted will be visually estimated. Root and shoot fresh weights will be determined.

All data will be subjected to analysis of variance and appropriate mean separation procedures. Clones exhibiting relatively little crown and root rot in repeated greenhouse trials will be considered tolerant to the pathogens and, barring unacceptable vulnerabilities or attributes, worthy of intensive field trials and orchard evaluations.

Objective 2.

We will examine walnut orchards exhibiting incidence of decline on Paradox rootstock in the Sacramento and San Joaquin Valleys. In cases suggesting involvement of soilborne pathogens rather than poor soil-water relations (e.g., as described above, spotty incidence of decline, incidence of distinct cankers), samples of necrotic crown and root tissue will be collected and cultured in semi-selective and non-selective media to detect oomycetes (e.g., *Phytophthora* and *Pythium* spp.), fungi, and bacterial pathogens. Potentially pathogenic isolates of *Phytophthora* and fungi associated with the disease will be tested for pathogenicity on Paradox hybrids and NCB in the greenhouse using methods similar to those of greenhouse screens for resistance. Isolates of bacteria will be tested for pathogenicity by inoculating suspensions of them from pure tryptic soy broth agar cultures onto wounds on stems of Paradox and NCB rootstock seedlings. A cork borer will be used to remove a small patch of bark from greenhouse and field plantings of the plants, and a quantified volume of bacterial suspension will be placed on the wounds and wrapped to prevent drying. The wounds will be allowed to incubate for approximately 4 weeks and then examined for evidence of necrosis. Controls will be inoculated with sterile broth. Isolations and identifications will be conducted from the necrotic tissues to complete Koch's postulates. Dan Kluepfel will collaborate with us in characterizing the bacterial isolates and their pathogenicity.

Literature Cited

Browne, G.T., Hackett, W.P., McLaughlin, S.T., Leslie, C.A., McGranahan, G.H. 2006. Evaluation of Resistance to *Phytophthora citricola* among diverse clones of paradox hybrid rootstocks. Acta Horticulturae 705:395-400.

Mircetich, S.M., Browne, G.T., Matheron, M.E., and Teviotdale, B.L. 1998. Armillaria and Phytophthora root and crown diseases. Pages 221-232 in: Walnut Production Manual, D.E. Ramos, Ed.. University of California, Division of Agriculture and Natural Resources, Oakland, CA. Publication 3373.

Table 1. Overview update on status of evaluations of resistance to *Phytophthora* in clonal hybrid walnut rootstocks[a]

Genotypes			Status and proposed future of clonal screening efforts					
Parental background (species of *Juglans*)			Previous years' trials with *P. citricola*			Recently completed with *P. citricola*	Recently completed with *P. cinnamomi*	Proposed with *P. citricola and P.cinnamomi*
Maternal	Paternal	Clone	2001	2004	2006	2007	2007	2008/09
(major x hindsii) x nigra	*regia*	NZ1		MR	MS	*MS*		
(major x hindsii) x nigra	*regia*	AZ025				*MS*	*MS*	*proposed*
(major x hindsii) x nigra	*regia*	AZ1	MS		MS	*MS*		
(major x hindsii) x nigra	*regia*	AZ2	MR	MR	MS	*MS*		
(major x hindsii) x nigra	*regia*	AZ3		MR	MS	*MS*		
californica	*regia*	AX1	MS	MS	MS	*MR to MS*	*MS*	*proposed*
californica	*regia*	AX2	MS			*MS*	*S*	*proposed*
californica	*regia*	AX3						
californica x nigra	*regia*	UX022			MS	*MS*		
californica x nigra	*regia*	UX1	MR		MS	*MS to MR*	*MS to MR*	*proposed*
californica x nigra	*regia*	UX2	MS		MS	*MS to MR*	*S to MR*	*proposed*
hindsii	*hindsii*	BUR				*MR*	*MR*	*proposed*
hindsii	*regia*	GZ1		MS	MS	*MS*		
hindsii	*regia*	GZ2			MS	*MS*	*MR*	*proposed*
hindsii	*regia*	GZ3				*MS*	*MS to MR*	*proposed*
hindsii	*regia*	JX1						
hindsii	*regia*	JX2	MR	MR	MS	*MS*		
hindsii	*regia*	PX1		MS	MS	*MS*	*MS*	*proposed*
hindsii	*regia*	RR1				*HS*	*MS to MR*	*proposed*
hindsii	*regia*	RR4				*HS*	*MS*	*proposed*
hindsii	*regia*	UZ1						
hindsii	*regia*	UZ2						
hindsii	*regia*	UCD-121	MS					
hindsii	*regia*	Vlach			MS	*HS*	*MS*	*proposed*
hindsii	*regia*	VX211		MR	MR	*MS to MR*	*MS to MR*	*proposed*
hindsii x regia	*regia*	WIP2		MS	MS	*MS*		
hindsii x regia	*regia*	WIP3			MS	*MS*	*MS*	*proposed*
microcarpa	*regia*	RX1	R	MR	MR	*MS to MR*	*R*	*proposed*
nigra	*regia*	CW1				*MS*	*MS*	*proposed*
nigra	*regia*	MW1				*MS*	*MR*	*proposed*
nigra	*regia*	RW2				*MS*	*MS*	*proposed*
nigra	*regia*	CR				*MS*	*S*	*proposed*

[a]Abbreviations: MS=moderately susceptible, with more than 50% crown length and circumference girdled; MR=moderately resistant, with less than 50% of crown length and width girdled; R=resistant, with 0 to 10% crown length and crown circumference girdled' S= susceptible with 90 to 100% or root crown length and circumference girdled with crown rot.

Budget Request

Budget Period: May 1, 2008 to April 30, 2009
Funding Source: Walnut Marketing Board

Item	**Amount ($)**
Post graduate researcher / SRA salary and benefits:	16,000
Supplies and Expenses:	2,000
Travel:	500
Total:	**18,500**

Signature of Principal Investigator: _____

Signature of Authorized Institution Rep,
USDA-ARS, Davis; Location Coordinator: _____

Signature of Authorized Institution Rep.,
University of California, Davis; Dept. Chair:_____