Stakeholder Relevance Statement

Project Title: Development of Disease-Resistant Walnut Rootstocks: Integration of Conventional and Genomic Approaches

Project Type: This pre-proposal is a renewal submission to the SREP category.

Statement of problem being addressed, its importance, a list of hypotheses to be tested and a list of project objectives. Our proposed research addresses six economically important diseases affecting commercial walnut nurseries and orchards and native walnut stands. Among these, crown gall disease caused by *Agrobacterium tumefaciens*, lesion nematode damage caused by *Pratylenchus vulnus*, crown and root rot caused by *Phytophthora* species, and root rot caused by *Armillaria mellea* are major sources of loss in English walnut production and collectively reduce orchard and nursery revenues by an estimated 18% ($241 million/yr based on a 2011 walnut crop value of $1.34 billion, USDA NASS). Roughly 5% of this loss is due to direct costs of soil fumigation. Soil fumigation has been cost-effective, but is increasingly regulated to protect human and environmental health. The long-term availability of soil fumigation is uncertain, and even under the best circumstances it provides only partial control of soilborne pathogens. Due to the severe economic losses inflicted by these particular pathogens and the strategic limitations of soil fumigation, the California Walnut Production Research Advisory Council (PRAC) of the California Walnut Board (CWB) classified the four diseases they cause as "highest priority" problems for the industry.

The remaining two diseases, thousand cankers disease (TCD), which is caused by the fungus *Geosmithia morbida*, and Paradox canker disease (PCD), which has unknown etiology, are important "emerging" walnut problems. TCD has attracted national attention due to the extreme threat it poses to eastern black walnut, *Juglans nigra*, a valuable nut and timber species in the eastern US. TCD is also adversely impacting black walnut species native to California, which are widely used to supply walnut rootstocks. The Walnut Council, the primary stakeholder group for *J. nigra*, identified TCD as "the primary threat" to black walnut. It is estimated that losses due to TCD could total over $50 million in MO and KS alone. PCD, a lethal cause of crown rot and trunk cankers in English walnut trees on Paradox rootstock, occurs statewide in CA. Surveys during the first 2 years of this project indicate that TCD and PCD are becoming widespread and economically significant diseases.

Seedling Paradox rootstock (*Juglans hindsii* × *J. regia*), the current industry standard, is sensitive to all six of these disease problems. We propose to address this vulnerability by testing two key hypotheses: 1) genes mediating resistance to the pathogens of interest exist in wild species of *Juglans* (in fact, our research in the first 2 years of this project provides initial support for this hypothesis); and 2) genes conferring resistance to the pathogens can be mapped and bred into useful new well-adapted commercial walnut rootstocks. Objective 1 will test the first hypothesis by challenging diverse wild walnut species germplasm with the five known rootstock pathogens of interest (i.e., the unknown agent of PCD will not be included). This will involve greenhouse and field trials employing seedlings and selected clones to identify and confirm novel sources of resistance. The second hypothesis will be tested under Objective 2 through genetic and association mapping approaches using SNPs developed in a genotyping-by-sequencing strategy.

We also will address two non-hypothesis-driven objectives. Objective 3 will focus on extension efforts, including transfer of new rootstock genotypes and technologies to stakeholders and assessment of emerging rootstock threats such as TCD and PCD. In fact, we have already

begun: 1) transfer of some promising disease-resistant genotypes identified in our SCRI project to a commercial nursery to initiate propagation for orchard trials and 2) statewide TCD and PCD surveys in CA. Objective 4 will include cost-benefit analyses of: 1) clonal propagation of the improved rootstocks and 2) impacts of the clonal rootstocks on orchard productivity and soil fumigation reliance.

Objectives

1. Identify and characterize *Juglans* germplasm with resistance to key rootstock pathogens.
2. Identify genetic markers in *Juglans* species and map genes for resistance to the key rootstock pathogens.
3. Conduct extension efforts that: a) demonstrate and deliver disease-resistant rootstocks to stakeholders and b) assess emerging threats to walnut rootstocks.
4. Quantify economic impact of disease-resistant rootstocks on the US walnut industry.

Statement of the how the proposed research approach will address each objective. Our multi-disciplinary SCRI team will continue integrating a conventional rootstock breeding approach with novel genomic technologies, adaptive extension outreach, and rootstock impact assessment. Work under each objective will build on accomplishments made by the team in its first 2 years. Under Objective 1, we have already assembled and screened a large collection of seedling and clonal genotypes of diverse *Juglans* species, interspecific hybrids, and mother tree (MT) genotypes for resistance to the pathogens under study. We will build on this work in our proposed renewal project by: a) expanding the number and diversity of rootstock genotypes generated, and b) testing these additional genotypes and re-testing initially promising genotypes for resistance to the five key pathogens. As previously, the additional genotypes will be generated from multiple wild species and interspecific hybrids of *Juglans* and challenged with the five key pathogens in greenhouse and field trials. Seedling selections with promising resistance will be clonally micropropagated for confirmatory testing, and mother trees with progeny segregating for resistance to one or more pathogens will be identified and used to produce hybrid F1 mapping populations for use in Objective 2. Objective 2 will use state-of-the-art genomic technologies to find genetic markers for resistance identified in Objective 1. The mapping populations will be characterized using a genotyping-by sequencing strategy for development and mapping of SNP markers. Linkage disequilibrium analysis will be used to find associations between genetic markers and resistance. Validation of linkage between markers and pathogen resistance will enable marker assisted selection for rootstock breeding, facilitate efficient use of germplasm, and serve as a model for rootstock development in other woody perennial crops. Our extension efforts in Objective 3 have transferred five rootstock genotypes with promising levels of pathogen resistance to a commercial nursery in preparation for advanced propagation, future orchard testing, and demonstration trials. An educational walnut rootstock handout featuring new rootstock releases has been distributed statewide in CA to walnut stakeholders. Our proposed continued work under Objective 3 will involve University of California Cooperative Extension (UCCE) Farm Advisors, commercial nursery representatives, and growers in adaptive research trials and supportive outreach activities. As the project proceeds, nursery and orchard demonstration trials will be used to facilitate adoption of new rootstocks, and orchard surveys will continue to examine the biology and impacts of TCD, PCD, and other emerging threats to walnut rootstocks. In Objective 4, we will assess economic impacts of: improved rootstock genotypes, clonal production technologies, and emerging rootstock threats. The analyses will use data gained from our extension trials and surveys and involve an agricultural economist, horticulturalists, pathologists, and nursery and nut production

representatives. Results from the economic analyses will be shared with stakeholders via Objective 3 outreach.

Statement of the process to obtain stakeholder input to identify proposed project objectives. Our stakeholders were and continue to be actively engaged in development of this SCRI project and its renewal. English walnut stakeholders provided input through the California Walnut Board's (CWB's) four PRAC working groups, which meet annually. The "Genetic Improvement" and "Plant Pathology" PRAC groups identified five of the diseases considered for this project (crown gall, Phytophthora crown and root rot, lesion nematode damage, Armillaria root rot, and PCD) as "highest priority" problems. The Walnut Council, the primary stakeholder group for culture and utilization of *Juglans nigra*, identified TCD as "the primary threat" to its industries.

In addition to the input described above, we continue to receive advisory support from the Walnut Rootstock Advisory Panel (WRAP), a committee of walnut growers, walnut processors, and walnut nursery operators; the panel meets twice a year and provides direct input to the PIs of our SCRI project and executive oversight for all of our proposed research and extension activities. The research proposed here also has been highly endorsed by participants of University of California Annual Walnut Research Conferences and county-wide walnut days, each of which are attended by commercial walnut growers, nursery operators, UCCE Farm Advisors, and researchers.

Statement of process to be used for continued stakeholder engagement to achieve project objectives, including a description of how stakeholders will be involved in project evaluation. As discussed above, our stakeholders were and continue to be engaged in this SCRI project through CWB's four PRAC working groups and the WRAP. Representatives from these advisory groups, as well as additional walnut nursery and walnut grower stakeholders, will provide executive oversight for our proposed research and extension activities. Each relevant PRAC group (i.e. "Genetic Improvement" and "Plant Pathology" groups) meets annually, and in these gatherings the results of our Walnut Rootstock SCRI grant-supported research are presented and evaluated. Our industry-led WRAP, which has oversight authority for all aspects of this project, including its inception, has met and will continue to meet twice a year, receiving and reviewing results and presentations from each of the PIs, and suggesting project improvements. The project director (PD) coordinates project responses to these suggestions and responds, in writing, to WRAP. The PD also will continue to present progress reports at the annual University of California Walnut Research Conference held in Bodega Bay, CA.

Statement of how the project will translate results into useable information that will be delivered to the entire stakeholder community in a timely fashion. We have a nationwide target audience of extension agents, growers, nursery operators, and researchers. Results of our project have been featured in industry extension meetings, regional field days, Plant Management Network webcasts (www.plantmanagementnetwork.org/edcenter/), social media and websites dedicated to informing the general public (e.g., Fruit and Nut Research Information Center; fruitsandnuts.ucdavis.edu/). The features have focused on results of rootstock resistance assessments, news of rootstock releases, orchard trials, and progress of TCD and PCD surveys.

We will continue to use the land-grant college system as the primary base of our SCRI project's outreach, adding outreach via commodity-based, trade-based, and additional formats where effective. UCCE Farm Advisors will conduct coordinated outreach activities including seven annual county walnut meetings and numerous field-day meetings across the state.

Additional UCCE-based outreach conduits will continue to include walnut newsletters, UCCE walnut cost studies, and the UC-IPM web site. County and regionally based walnut newsletters focused on TCD will be sent at monthly or quarterly intervals to clientele in CA, MO and TN. A walnut nursery workshop for stakeholders will be conducted by our SCRI research and extension team and the WRAP. We will continue to share project results through newspapers and trade magazines such as "*Pacific Nut Producer*," "*Diamond Foods Grower News*," "*Ag Alert*" and the CWB "*Walnut News*" newsletter. We will utilize the National Plant Diagnostic Network, which provides additional resources for extending new information to broad stakeholder groups on potential emerging threats. The Network includes educational programs and web-based resources already in place for TCD, which target both English- and Spanish-speaking audiences and can be amended to reflect new information. A website dedicated to our SCRI working group has been established and will be linked to social media through Facebook, Flickr, and YouTube. We will use quick response barcodes (QR codes) on printed outreach materials to facilitate smartphone access to our online outreach resources, and we will produce and "broadcast" webinars describing our advances in development of disease-resistant rootstocks. Peer reviewed discipline-oriented journals and web-based genomics databases will be used to extend products of the research to the academic community.

Brief documentation of the relevance of the Project Director's scientific background to project objectives. The Project Director has been examining the biology and ecology of *Agrobacterium tumefaciens* in walnut for over 10 years. This work has included an examination of wild *Juglans* and *Prunus* germplasm accessions for resistance to crown gall. As part of this effort, along with colleagues, he assembled a team of researchers to begin a systematic approach to identifying and characterizing new *Juglans* rootstock genotypes with resistance to the key soilborne diseases of walnut rootstocks. This team was awarded a 2-year USDA-NIFA-SCRI grant in 2012 to identify disease resistant *Juglans* genotypes, map the genetic loci mediating this resistance, and generate new hybrid rootstocks through a combination of traditional breeding and in vitro propagation techniques. The initial 1.6 years of this project have resulted in the identification of *Juglans* genotypes that exhibit high levels of resistance to crown gall, *Phytophthora*, or lesion nematodes. In addition, the PD and his team have generated a large number of new hybrid genotypes that are now ready to use in the search for genetic markers linked to disease resistance. To facilitate these efforts, this team also has developed improved and novel propagation approaches to produce clonal copies of genotypes exhibiting disease resistant phenotypes. In a very short time frame, the PD and his SCRI team have made tremendous progress towards their stated goals of identifying commercially viable disease-resistant walnut rootstocks.

Project logic model chart that illustrates scientific outputs and outcomes

Team: PIs	Inputs	Activities	Outputs	Short-term Outcomes	Long-term Outcomes	Impact
Germplasm assembly (Objective 1): Leslie Aradhya Preece Hackett	Walnut germplasm, including mother trees and pollen of diverse *Juglans* spp.	Generate, propagate, and curate open-pollinated (OP) and hybrid seedlings and clones. Coordinate w/ phenotyping and genetic mapping teams	OP and interspecific hybrid seedling and clonal plants; publications describing propagation technologies	Utilization of germplasm for disease resistance evaluations; development of rootstock breeding populations and technologies	Commercial availability of clonal rootstock selections; nursery adoption of clonal rootstock propagation technologies	Walnut nursery and orchard industries stimulated by new propagation technologies and superior rootstocks
Phenotyping (Objective 1): Browne Baumgartner McKenry Kluepfel Coggeshall Schlarbaum	OP and interspecific hybrid seedling and clonal plants; preliminary data on disease resistance	Evaluate resistance of the walnut rootstock germplasm to soilborne pathogens; communicate data	Quantitative data and publications on resistance of walnut rootstock germplasm to pathogens	SNP linkage mapping for disease resistance is facilitated; clones with putative resistance to pathogens are produced	Validated SNP markers for disease resistance; clones with resistance to pathogens established in field trials	Enhanced competitive-ness and efficiency of walnut industry due to improved resistance to key pathogens
Genetic mapping (Objective 2): Aradhya Dvorak Dandekar Neale	OP and interspecific hybrid seedling and clonal plants; data on disease resistance of germplasm; English walnut SNP map and genomic information	Create association & biparental maps of disease resistance based on SNP segregation and disease resistance in OP and hybrid populations	Comparative genetic maps of wild spp.; linkage of resistance loci & SNP markers; genetic information for use in breeding; genetic publications & databases	Mapped markers linked to genes conferring disease resistance; comparative linkage maps for examination of other traits in walnut germplasm	Marker assisted selection (MAS) used in rootstock breeding; gene pyramiding; combined resistance to several pathogens in single rootstocks	Genetic information will accelerate development of improved walnut rootstocks and improve breeding options
Extension and Education (Objective 3): Hasey Lampinen Browne Baumgartner McKenry Kluepfel	Disease resistance information; new clonal rootstocks	Partnering with farm advisors, stakeholders, and WRAP to disseminate new rootstock information; conduct new rootstock orchard demonstration trials	Extension publications and web-based outreach describing new rootstocks and their performance	Stakeholder involvement in rootstock evaluations and stewardship; awareness of genetic/ breeding technologies, environmental benefits	Rootstock performance information from long-term trials; publications summarizing orchard performance of rootstocks and validating resistance to pathogens	Informed grower and nursery adoption of new disease-resistant rootstocks; less dependence on soil fumigation

Project logic model chart that illustrates scientific outputs and outcomes (continued)

Team: PIs	Inputs	Activities	Outputs	Short-term Outcomes	Long-term Outcomes	Impact
Novel /Emerging Diseases (Objective 3): Bostock Browne Coggeshall Hasey Schlarbaum Seybold	Reports of new/emerging diseases from UCCE Farm Advisors and growers	Orchard surveys to characterize new and emerging threats; assess: 1) host susceptibility to *G. morbida*, (TCD), 2) host preference of insect vector of *G. morbida*, 3) Koch's postulates for PCD	Publications and newsletter articles assessing the incidence and severity of TCD and PCD in walnut	Broadened grower awareness TCD and PCD and their management; Broadened awareness of *Juglans* genotypes resistant / tolerant to TCD	Disease management strategies for TCD and PCD	Enhanced resiliency of walnut production due to effective threat management
Economic Analysis: (Objective 4): Klonsky Browne Baumgartner Hasey McKenry Kluepfel	Growth, survival and yield data from replicated orchard rootstock trials; nursery production data	Assemble and analyze actual and estimated cost and income data from industry, nurserymen, growers, and collaborating scientists	Extension publications and database entries summarizing cost-benefit analyses for adoption of clonal walnut rootstocks	Grower interest and initial plantings of new rootstocks will be facilitated by relevant economic analyses	Adoption of new clonal rootstocks with resistance to pathogens; and superior adaptation to new environ-ments	Enhanced economic efficiency and sustainability of walnut nursery and orchard production
Project Management: Kluepfel	Project and evaluation plan	Project Manager will coordinate research teams and determine progress towards objectives and short-term outcomes	Objective evaluation to document areas in need of improvement over time	Continued modifications and improvement of project to ensure successful conclusion.	Accomplish-ment of project objectives; effective delivery of genetic resistance and genetic knowledge	Public and industries will benefit from productive use of Federal grant dollars