

<b>Title:</b>	<b>DEVELOPMENT OF DISEASE-RESISTANT WALNUT ROOTSTOCKS: INTEGRATION OF CONVENTIONAL AND GENOMIC APPROACHES</b>		
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**Program Code:** SCRI

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**Non-Technical Summary**

In 2010, 4,800 growers in the US produced 503,000 tons of walnuts on 227,000 acres with a farm gate value of \$1.061 billion. Walnuts are the fourth largest U.S. specialty crop export which places the US as the world's largest exporter of walnuts with over 50% sold on the export market. Walnuts are among the most nutrient-dense whole-food sources of the short-chain omega-3 polyunsaturated fatty acid that protects the heart against cardiovascular disease and have been placed on "super food" list by the US Food & Drug Administration. In addition, walnut is an important hardwood with an estimated standing timber value of over \$539 billion. Most commercially cultivated walnut trees, grown for nut production, are grafted on rootstocks to limit root diseases which currently cause an estimated 18% annual loss worth \$191 million to the industry. The availability of new pathogen-resistant rootstocks is critical for the viability of the walnut industry and is ranked by the US walnut industry as its top research priority. We will use an integrated approach encompassing, disease resistance-screening, plant propagation, genomics, and conventional and novel breeding strategies to develop, characterize, and deploy clonal walnut rootstocks with

improved resistance to four major soilborne diseases caused by *Agrobacterium tumefaciens*, *Phytophthora* spp., *Pratylenchus vulnus*, and *Armillaria mellea*. This work will exploit the Juglandaceae germplasm collections in the USDA-ARS National Clonal Germplasm Repository in Davis, CA and the Missouri Horticulture and Agroforestry Research Center in New Franklin, Mo. Together, these collections comprise the largest assemblage of wild *Juglans* spp. in North America. In this process we will identify genes conferring resistance to these diseases and develop SNP markers for their selection in segregating populations and integration into commercial rootstocks. Emerging walnut rootstock diseases, such as Thousand Canker Disease and Paradox Canker, will be monitored by integrated outreach. A comprehensive economic analysis will optimize nursery propagation strategies and quantify the value to the industry of using pathogen-resistant clonal rootstocks developed here. Our project will enhance collaboration, communication, and exchange of information among the walnut research community and the broader USA tree crop industry.

## Accomplishments

### Major goals of the project

1) Assemble germplasm and generate mapping populations for discovery and mapping of novel genetic loci that mediate resistance to major soil-borne diseases of walnut. 2) Characterize *Juglans* species half-sib and interspecific hybrids for resistance to the soil-borne pathogens, *agrobacterium tumefaciens* (Crown Gall Disease, CG), *Phytophthora* spp. (crown and root rot, PHY), *Armillaria mellea* (*Armillaria* root disease, ARD), *Pratylenchus vulnus* (Root-lesion nematode, NEM), and threatening rootstock pathogens *Geosmithis morbida* (Thousand canker disease, TCD) and the walnut strain of cherry leaf roll virus (walnut blackline disease, WBD). #) Develop and map SNP markers associated with disease resistance. 4) Quantify economic impact of disease-resistant rootstocks on US walnut industry. 5) Conduct bi-directional extension efforts that: a) deliver disease-resistant rootstocks to stakeholder, and b) assess emerging threats to walnut rootstocks.

### What was accomplished under these goals?

**Impact Statement:** Commercial English walnuts (*Juglans regia*) in California are grown on a hybrid rootstock known as Paradox. Despite its popularity, Paradox is susceptible to three major wide-spread soil-borne pathogens which all cause serious losses to the industry. Our project is designed to deliver novel disease-resistant rootstock genotypes. Towards that end, we identified walnut selections which exhibit resistance to one or more of the target pathogens. These selections have been clonally propagated in preparation for large scale testing. The use of disease-resistant rootstocks will dramatically enhance yields and reduce our dependence on ozone-depleting soil fumigants which will put walnut production on a more sustainable path.

#### 1) Assemble germplasm and generate mapping populations for discovery and mapping of novel genetic loci that mediate resistance to major soil-borne diseases of walnut.

We produced 14,197 vitro propagated plants which entered disease resistance screening.

Approx. 10,000 open-pollinated seeds of *J. ailantifolia*, *J. cathayensis*, *J. hindsii*, *J. major*, and *J. microcarpa* were sown and entered disease resistance screening.

Using the air layering technique, we rooted several *J. microcarpa* mother trees.

More than 21,000 open and cross-pollinated seeds were collected. These are currently at 4C for stratification and will be placed into disease resistance screening.

#### 2) Characterize *Juglans* species half-sib and interspecific hybrids for resistance to the soil-borne pathogens, *Agrobacterium tumefaciens* (Crown Gall, CG), *Phytophthora* spp. *Pratylenchus vulnus* (Root-lesion nematode), and threatening rootstock pathogens *Geosmithis morbida* (Thousand canker disease, TCD).

##### **Crown Gall Resistance:**

We clonally propagated 13 open-pollinated (OP) *J. microcarpa* CG resistance seedlings. Using 11 of these CG-resistant progeny we generated 150 plantlets which will enter CG and *Phytophthora* resistance testing. Clone K3, continues to exhibit CG resistance.

We screened plants generated from OP seeds collected from "high value" mother trees. Six *Juglans* species were examined. Across all 6 species, we found approximately 17% of the progeny exhibit CG resistance at 2 months post-inoculation. At 4 months post-inoculation, 11% of the progeny exhibit limited gall formation. All genotypes exhibiting CG resistance were placed into in vitro culture.

Under greenhouse conditions, we examined approximately 350 OP progeny from 22 *J. microcarpa* mother trees. Two months after inoculation with *A. tumefaciens*, 69 seedlings exhibited low to no tumor formation. Of those 69 seedlings, 46 continued to exhibit CG resistance.

In an effort to map the genetic loci which mediate crown gall resistance in these targeted genotypes we screened at least 50 OP progeny from "high value" mother trees shown to throw CG-resistant progeny. All trees have been inoculated and will be rated when plants break dormancy.

##### **Phytophthora Resistance:**

Three greenhouse evaluations of rootstock resistance to *Phytophthora* were completed in 2013, two examining hybrid rootstock clones and one examining seedlings from selected mother trees. Clonal evaluations include *J. cathayensis* X *J. regia* hybrids and new *J. microcarpa* x *J. regia* clones. Twenty-four selections were included in the first clonal trial and 29 in the second. The seedling trial was designed to identify mother trees that offer superior resistance to *Phytophthora* as well as

to facilitate identification of genetic markers for resistance to Phytophthora in future genomics studies; seedlings from 32 different mother tree sources were included (there were 1,404 seedlings total, 948 of which were inoculated with Phytophthora cinnamomi, 456 of which served as controls). in the first clonal greenhouse trial J. cathayensis x J. regia hybrids expressed high resistance to both P. cinnamomi and P. citricola. Results of the second clonal trial in 2013 are being analyzed.

#### **Nematode Resistance.**

Clones RX032 and RX1 continue to tolerate replant rejection, (i.e., suppressed tree growth in replanted walnut orchards). We identified 16 clones that met or exceeded this benchmark for replant rejection.

At this time none of the selections in the 2012 experiment provided P. vulnus resistance

Thus far, susceptibility to M. incognita is present in 22 of the candidate hybrids. We will continue this test another year to identify clones that tolerate nematode feeding. Based on the two-year data set we can identify six of the 47 hybrids tolerant of root rejection, as well as the nematode component of the replant disorder. In 2012 we identified 7 J. cathayensis selections as nematode resistant. By July 2013 only a single J. cathayensis tree #27 remained nematode resistant.

#### **3)Develop and map SNP markers associated with disease resistance.**

#### **4)Quantify economic impact of disease-resistant rootstocks on US walnut industry.**

Nothing to report for goals 3 and 4. These goals will be address once goals 1 and 2 are completed. We anticipate accomplishing goals 3 and 4 in years 2 through 4 of this project.

#### **5)Conduct bi-directional extension efforts that: a)deliver disease-resistant rootstocks to stakeholder, and b)assess emerging threats to walnut rootstocks.**

We held industry oversight committee meetings where we extended research results to industry representatives attending. In addition we have held or participated in numerous extension/farm advisor meetings where the results of our project have been presented to growers and nursery representatives and county farm advisors. In addition we held multiple farm advisor workshop/training sessions to train advisors on TCD identification to aid with statewide surveys. Training sessions have been given on ID of Lethal Paradox Canker Disease. Two promising rootstock genotypes which exhibit CG resistance are being propagated for field trials in summer 2014.

We are assessing 12 walnut rootstock field trials established in the central valley of California. All plots have seedling Paradox rootstock as a standard. Ten clonal walnut rootstocks are being tested in various situations, i.e. fumigated and non-fumigated plots, areas with walnut blackline, Phytophthora or CG. Surveys of all these rootstock plots will be complete 1/2014. The survey of the oldest clonal rootstock plot was completed in 2012 and revealed clonal Paradox rootstocks had significantly lower crown gall incidence than the seedling rootstocks.

We continued our evaluation of our trial in San Joaquin County to evaluate resistance of rootstock RX1 to P. cinnamomi under orchard conditions. The trial compared performance of standard seedling Paradox rootstock and RX1 rootstock in a commercial orchard. In 2013 we confirmed P. cinnamomi was associated with death of trees in the trial. By July 2013, surviving trees on seedling Paradox and RX1 rootstocks had mean trunk circumferences of 15.1 and 27.8 cm, respectively. 48% of trees on Paradox seedling died, whereas no trees on RX1 died. The results validated greenhouse data indicating high Phytophthora resistance of 'RX1'.

#### **Emerging Diseases.**

In fall 2012, we identified commercial English walnut orchards exhibiting TCD. Seventeen orchards were surveyed for the presence of TCD and samples taken to confirm the disease. The majority of orchards tested positive for TCD. However, in most cases tree vigor was not affected even with disease in the Paradox rootstock or the English scion. We now have survey data from 3 to 6 orchards in each of 9 counties that include major walnut production areas in the state.

The majority of orchards surveyed for TCD tested positive. Paradox hybrids are the most susceptible to the TCD pathogen G. morbida, followed by J. microcarpa and other Juglans species. In field assays we found a consistent preference of the walnut twig beetle for eastern and California black walnuts (e.g., J. nigra and J. hindsii) over English walnut and the desert southwestern walnuts (J. major and J. microcarpa).

#### **What opportunities for training and professional development has the project provided?**

On multiple occasions, PI's Hasey, Seybold and Bostock provided training to UC farm advisors on the identification of Thousand Canker Disease under commercial orchard conditions.

PI Browne provided training to UC farm advisors on the identification of Lethal Paradox Canker under field conditions.

#### **How have the results been disseminated to communities of interest?**

Many of the SCRI PI's have presented their findings at the annual UC Davis Walnut Research Conference held in Bodega Bay, CA. This conference is attended by up to 100 walnut growers, nursery operators, farm advisors and researchers. In addition, SCRI rootstock information is also disseminated at the UC Walnut Work Group meeting and at two meetings of the Walnut Board of California Production Research Advisory Committee. Members of these groups then return with this information to their respective counties where their growers are informed of the latest research results.

SCRI PI's also have presented their information at County Wide growers meetings held by the local UC Farm advisors. Depending on the grower meeting, they can be attended by as many as 500 growers/nursery operators.

As itemized in the "other products" section we have presented and published in a variety of trade shows and journals.

**What do you plan to do during the next reporting period to accomplish the goals?**

We are making significant progress in the generation and assembly of Juglans germplasm. This includes everything from propagation of both open pollinated seeds from germplasm mother trees and seeds collected after making directed crosses between promising parental genotypes. In addition we have made significant progress in the invitro propagation of promising genotypes which are being cloned to provide large amounts of material for both greenhouse and advanced field trials where we are assessing disease resistance. The result of this first years efforts have lead to the identification of several genotypes in several different Juglans species which exhibit elevated resistance to CG, Phytophthora or lesion nematodes. These data were used to select parental genotypes which were used to make the directed crosses. Progeny from these crosses are starting to enter our disease resistance screening pipeline. Thesedisease resistance data will be used during this next reporting period to identify genetic loci which mediate the observed disease resistance phenotypes demonstrated in year one.

**Participants****Actual FTEs for this Reporting Period**

Role	Faculty and Non-Students	Students within Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	17	3	0	0	20
Professional	0	0	0	0	0
Technical	3	3	0	0	6
Administrative	0.5	0	0	0	0.5
Other	0	0	0	0	0
Computed Total	20.5	6	0	0	26.5

**Target Audience**Target Audience

The target audience consists primarily of walnut growers and nursery operators. Secondly, consultants associated with the production of these crops, e.g., pest control advisors, diagnosticians, farm advisors, along with government, industry and academic researchers have all been important targets for research results coming from this project. Communication with the target audience is facilitated by national, state, and regional grower organizations and by cooperative extension agents. Examples of specific grower organizations include; The Walnut Board of California, Nursery organizations, and California Association of Pest Control Advisors.

**Products**

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2012	NO

**Citation**

Leslie, C., Hackett, W. Robinson, R., McMahon, M., Grant, J., Lampinen, B., Anderson, K., Beede, B., Buchner, R., Caprile, J., DeBuse, C., Elkins, R., Hasey, J., Fazel, M., Manterola, N., Kluepfel, D., Browne, G., Evans, R. McKenry, M., Preece, J., Aradhya, M., Velasco, D. 2012. Clonal Propagation of walnut roostock genotypes for genetic improvement. UC Davis, FNIC, Walnut Research Reports. Pages 97-130. [http://walnutresearch.ucdavis.edu/2012/2012\\_97.pdf](http://walnutresearch.ucdavis.edu/2012/2012_97.pdf).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2012	NO

**Citation**

Browne, G., Bhat, R., Schmidt, L., Leslie, C., Michailides, T., Hackett, W., Robinson, R., Kluepfel, D., Arardhya, M., Beede, B., Buchner, R., Connell, J., Fichtner, E., Grant, J., Hasey, J., 2012. Etiology and management of crown and root rots of walnut. UC Davis, FNIC, Walnut Research Reports. Pages 395-409. [http://walnutresearch.ucdavis.edu/2012/2012\\_395.pdf](http://walnutresearch.ucdavis.edu/2012/2012_395.pdf).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2012	YES

**Citation**

Kluepfel, D., Aradhya, M., McClean, A., Moersfelder, J., Curiel, L., Hackett, W., 2012. IDENTIFICATION AND CHARACTERIZATION OF CROWN GALL RESISTANT JUGLANS GERMPLASM. UC Davis, FNIC, Walnut Research Reports. Pages 413-418. [http://walnutresearch.ucdavis.edu/2012/2012\\_413.pdf](http://walnutresearch.ucdavis.edu/2012/2012_413.pdf).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2012	NO

**Citation**

McKenry, M., and Buzo, T., 2012. Methyl bromide alternatives, focus on rootstocks. UC Davis, FNIC, Walnut Research Reports. Pages 427-434. [http://walnutresearch.ucdavis.edu/2012/2012\\_427.pdf](http://walnutresearch.ucdavis.edu/2012/2012_427.pdf).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2012	NO

**Citation**

Hasey, J., Browne, G., Bhat, R., Hackett, W., and Leslie, C., 2012. Field performance of RX1 clonal paradox rootstock. UC Davis, FNIC, Walnut Research Reports. Pages 89-91. [http://walnutresearch.ucdavis.edu/2012/2012\\_89.pdf](http://walnutresearch.ucdavis.edu/2012/2012_89.pdf).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2012	NO

**Citation**

Hasey, J., Hishinuma, S., Brocker, K., and Seybold, S. 2012. Thousand cankers disease and walnut twig beetle insecticide and fungicide spray study UC Davis, FNIC, Walnut Research Reports. Pages 347-351. [http://walnutresearch.ucdavis.edu/2012/2012\\_347.pdf](http://walnutresearch.ucdavis.edu/2012/2012_347.pdf).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2012	NO

**Citation**

Preece, J., Ibanez, A. M., Tran, Q., Bunawan, D., Leslie, C., Tricoli, D., Dandekar, A. 2012. Regeneration systems for pyramiding disease resistance into walnut rootstocks. UC Davis, FNIC, Walnut Research Reports. Pages 61-66. [http://walnutresearch.ucdavis.edu/2012/2012\\_61.pdf](http://walnutresearch.ucdavis.edu/2012/2012_61.pdf).

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Submitted	2013	YES

**Citation**

Resistance to Phytophthora identified among hybrid walnut rootstocks. Browne, G., Leslie, C., Grant, J., Bhat, R., Schmidt, L., Hackett, W., Kluepfel, D., Robinson, R., McGranahan, G. 2013. Resistance to Phytophthora identified among hybrid walnut rootstocks. HortScience (submitted)

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Under Review	2014	YES

**Citation**

Kluepfel, D., McClean, A., Aradhya, M., Moersfelder, J., Hackett, W. and Leslie, C. 2014. Identification of crown gall resistant Juglans spp germplasm. HortScience. (under review)

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2013	YES

**Citation**

Utlely, C., Nguyen, T., Roubtsova, T., Coggeshall, M., Ford, T. M., Grauke, L. J., Graves, A. D., Leslie, C. A., McKenna, J., Woeste, K., Yaghmour, M. A., Cranshaw, W., Seybold, S. J., Bostock, R. M., and Tisserat, N. 2013. Susceptibility of walnut and hickory species to *Geosmithia morbida*. *Plant Dis.* 97:601-607.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

**Citation**

Seybold, S.J., Dallara, P., Hishinuma, S., and Flint, M. 2013. Detecting and identifying the walnut twig beetle: Monitoring guidelines for the invasive vector of thousand cankers disease of walnut, University of California Agriculture and Natural Resources, Statewide Integrated Pest Management Program, Oakland, California, 13 pp., March 13, 2013,

<http://www.ipm.ucdavis.edu/PMG/menu.thousandcankers.html>

Refereed Outreach/Tech Transfer Document

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

**Citation**

Seybold, S.J., Dallara, P., Hishinuma, S., and Flint, M. 2013. Quick guide: Installing, maintaining, and servicing walnut twig beetle pheromone-baited traps, University of California Agriculture and Natural Resources, Statewide Integrated Pest Management Program, Oakland, California, 2 pp., March 13, 2013,

[http://www.ipm.ucdavis.edu/PDF/MISC/WTBtrapping\\_quickguide.pdf](http://www.ipm.ucdavis.edu/PDF/MISC/WTBtrapping_quickguide.pdf), <http://www.ipm.ucdavis.edu/thousandcankers>.

Refereed Outreach/Tech Transfer Document

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

**Citation**

Seybold, S.J., Haugen, D., O'Brien, J., and Graves, A. 2013. Thousand cankers disease. USDA Forest Service, Northeastern Area State and Private Forestry Pest Alert, NA-PR-02-10, originally published May, 2010, reprinted Aug. 2010, Oct. 2011, and Feb. 2013, 2 pp., <http://www.na.fs.fed.us/pubs/detail.cfm?id=5225>

Refereed Outreach/Tech Transfer Document

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2013	NO

**Citation**

Fichtner, E., Browne, G., Bhat, R. 2013. Lethal paradox canker continues to emerge in southern San Joaquin Valley orchards. *West Coast Nut Grower Guide*

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2013	NO

**Citation**

Fichtner, E., Browne, G., Bhat, R. 2013. Lethal paradox canker continues to emerge in southern San Joaquin Valley orchards. In: "In a Nutshell" newsletter ([http://cetulare.ucanr.edu/newletters\\_898819/In\\_a\\_Nutshell\\_84/](http://cetulare.ucanr.edu/newletters_898819/In_a_Nutshell_84/)), December 2013 Issue.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Awaiting Publication	2013	NO

**Citation**

Leslie, C.A. and McGranahan, G.H. 2013. The California Walnut Improvement Program: Scion Breeding and Rootstock Development. Acta Hort. (In Press).

**Other Products****Product Type**

Other

**Description**

Hasey, Seybold and Bostock. Provided a workshop/training to Univ. California farm advisors on the field identification of Thousand Cankers Disease. Tuesday, August 20, 2013. 10 Farm Advisors were in attendance.

**Product Type**

Other

**Description**

Seybold, S.J. Walnut twig beetle and thousand cankers disease. Thirty-minute presentation to approx. 50 growers and UC cooperative extension personnel at the 2013 Lake County Walnut Update sponsored by UC Cooperative Extension, March 8, 2013, Lakeport, California (oral presentation by SJS).

**Product Type**

Other

**Description**

Seybold, S.J. Walnut twig beetle detection techniques. Fifty-minute presentation to approx. 15 stakeholders with the Ohio Department of Agriculture, the Ohio State University, and USDA APHIS at the Columbus campus of the Ohio State University, January 29, 2013, Columbus, Ohio (oral presentation by SJS).

**Product Type**

Other

**Description**

Greg Browne; "Phytophthora and Lethal Paradox Canker: Long-Standing and Emerging Soilborne Diseases of Walnuts"; Fresno, CA; November 14, 2013; event was 2013 Grape, Raisin, and Nut Expo, Fresno

**Product Type**

Other

**Description**

Greg Browne; "Phytophthora and Lethal Paradox Canker: Long-Standing and Emerging Soilborne Diseases of Walnuts"; Turlock, CA; November 12, 2013; event was 2013 Tree & Vine Expo, Turlock

**Product Type**

Other

**Description**

Greg Browne; "Update on Crown and Root Diseases of Walnut"; Red Bluff, CA; February 1, 2013, event was Walnut grower meeting, UC Cooperative Extension

**Product Type**

Other

**Description**

Klupefel, Daniel, and Greg Browne; "SCRI Project: Development of Disease-Resistant Walnut Rootstocks: Integration of Conventional and Genomic Approaches"; Sacramento, CA; August 22, 2013; event was Walnut

PRAC industry meeting for genetic improvement.

**Product Type**

Other

**Description**

Leslie, C.A.. 2013. The California Walnut Improvement Program: Scion Breeding and Rootstock Development. Presented at: 7th International Walnut Symposium, July 21, 2013, Fengyang, China

**Changes/Problems**

{Nothing to report}