EVALUATION OF WILD *JUGLANS* SPECIES FOR CROWN GALL RESISTANCE

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INTRODUCTION

Paradox, the most widely used rootstock in CA walnut production, is highly susceptible to the causal agent of crown gall (CG) *Agrobacterium tumefaciens*. The bacterial pathogen induces the formation of large tumors around the crown of the tree resulting in a reduction in both vigor and yield. If left untreated, tumors can completely girdle the tree leading to premature death. Currently, crown gall disease in mature orchards is managed using surgery to remove the gall and adjacent infected tissues.

Durable host resistance is the preferred form of resistance to all soil borne plant pathogens. This is important for crown gall disease given the fact that *Agrobacterium* spp. are found in all the walnut growing regions of California examined.

The wild relatives of cultivated species are often a rich source of genes coding for resistance to insect pests, microbial pathogens, and abiotic stresses. Identification of a durable source of resistance to crown gall in the *Juglans* germplasm collection, that could be utilized directly or introgressed into commercially viable rootstocks, is likely to be an effective strategy for controlling crown gall disease in walnut.

The walnut germplasm collection at the National Clonal Germplasm Repository, USDA-ARS in Davis, CA contains a wide range of intra- and interspecific diversity of the black walnuts and butternuts adapted to California conditions. The potentially useful black walnut species include *J. hindsii, J. nigra, J. microcarpa, J. major*, and some of their hybrids with cultivated species. Other members of the germplasm repository collection that could be used to develop both crown gall and nematode resistant rootstock include the Asian butternuts, *J. ailantifolia, J. mandshurica*, and *J. cathayensis*. In addition, wingnuts belonging to the genus *Pterocarya* have shown disease resistance characteristics that need to be exploited.

Although wild *Juglans* species have contributed to walnut rootstock development programs, the range of genetic variation for crown gall resistance within and between these wild species has not been characterized. It is anticipated that a systematic evaluation of the *Juglans* germplasm will reveal a source of resistance/tolerance to *A. tumefaciens* and other plant pathogens.

As a step towards development of crown gall resistant rootstocks, here we report on the identification of *Juglans* species exhibiting resistance to infection by *A. tumefaciens* EC1. Once identified, these novel sources of *Agrobacterium* resistance will be exploited in the ongoing U.C. Davis Walnut root stock breeding program to help reduce crown gall incidence in both nursery and production fields.

OBJECTIVE

Identify and characterize a novel source of crown gall (CG) resistance in the *Juglans* germplasm collection maintained in the USDA/ARS National Clonal Germplasm Repository in Davis, CA.

Anticipated Outcome

We anticipate the identification of a new source of crown gall resistance for use in development of crown gall resistant rootstocks in the UC Davis walnut breeding program. The germplasm thus identified will be shared with other pathologists and horticulturists to evaluate resistance to other diseases and examine horticultural characteristics.

PROCEDURES

Seedling germination and inoculation. Open pollinated seeds were collected from each of the black walnut and butternut accessions maintained at the Wolfskill Experimental Orchards in Winters, CA. Seeds were cold treated, germinated and grown under glasshouse conditions. Seedlings were inoculated at the crown with *A. tumefaciens* strain EC1 once they reached a trunk diameter of at least 0.5 cm. Depending on germination and growth rates, 6-9 trees from each accession were screened.

A "T-cut" into the cambium layer was made to open the outer layers of bark and 0.5 ml of a 10^9 cells/ml suspension of EC-1 was introduced inside the peeled back layers. After inoculation, the wound was wrapped with parafilm. Standard cultural practices were followed during the experiment and observations on tumor development were recorded at monthly intervals by noting first-appearance and then percent girdling (Gall rating: 1=no tumors, 2 = < 25% of trunk circumference galled, 3 = 25-50% trunk circumference galled, 4 = 50-100% trunk circumference galled). Selected germplasm was also inoculated using a stab technique described in C. Leslie et al. (2010) (this volume).

CG susceptible Paradox seedlings inoculated as described above, served as positive controls. To assess the wounding response in the absence of the pathogen, Paradox seedlings and a variety of accessions from the germplasm collection were inoculated as described above with sterile water. The efficacy of the *Agrobacterium* inoculum to cause tumor was confirmed by testing on the known susceptible host plant, Datura.

Evaluation of inoculated saplings. Tumor formation was monitored at two week intervals following inoculation. Differences in relative rates and trends of tumor formation among the different germplasm accessions were noted and recorded. Percent girdling of the stem was assessed and recorded for each seedling at 60 days post-inoculation. Photos of representative seedlings were taken at various intervals following inoculation. Seedlings were observed for three to six months after inoculation to monitor for late-forming or slow growing tumors. The durability of resistance in asymptomatic inoculated saplings was confirmed by monitoring for tumor formation during a second growing season. A select group of saplings which continued to show resistance or "reduced susceptibility", after two or more growing seasons, were propagated and retested as described above. Seedlings showing 25% or less of the stem girdled at 60 days

post inoculation were designated as a source of potential resistant germplasm and retained for further study. Mother trees associated with the seedlings showing resistance in 2006, 2007, and 2008 were identified and targeted for seed collection in 2009.

RESULTS AND DISCUSSION

O**bjective:** Identify a novel source of crown gall (CG) resistance in the *Juglans* germplasm collection maintained in the USDA/ARS National Clonal Germplasm Repository in Davis, CA.

During the 2009 NCGR germplasm screening season, we examined a total of ~300 seedlings from "new" genotypes for their resistance to *A. tumefaciens.* This consisted of seedlings from 89 mother trees representing *J. regia* and its conspecific taxon, *J. sinensis*, five species of black walnut (*J. hindsii, J. nigra, J. microcarpa, J. californica* and *J. major*), and three species of butternuts (*J. ailantifolia, J. mandshurica*, and *J. cathayensis*).(Table 1). As in our previous screening, the phenotype after *A. tumefaciens* inoculation ranged from total resistance to delayed gall development after dormancy to rapid gall formation 3 week post inoculation. Similar to 2008, we identified CG resistant *J. microcarpa, J. cathyensis, J. califonica, J. major*, and *Pteracarya* sp, individuals. This year we retained 43 individuals exhibiting a range of CG resistance (i.e. no tumors through limited tumor development (<25% girdling)) which represented 15% of the total number screened. Interestingly, the 43 individuals retained were harvested from 25 different mother trees in the NCGR collection.

Table 1: Juglans germplasm screened for Crown Gall resistance

Tree Classes	**Tested**	**Retained**	**% retained**
Juglans species	10	5	50%
Mother trees represented	89	25	28%
Individual trees	307	43	15%
***Juglans* species**	**Trees Tested**	**Trees Retained**	**% retained**
J. ailantifolia	17	0	0%
J. californica	18	4	22%
J. cathyensis	6	1	17%
J. hindsii	62	1	1.6%
J. major	104	10	10%
J. hopeinsis	4	0	0%
J. mandshurica	16	0	0%
J. microcarpa	61	27	44%
J. nigra	15	0	0%
J. sinensis	4	0	0%

Dormant cuttings were taken from selected seedlings shown to be CG resistant in the 2008 screening. The cuttings were rooted and cultivated under greenhouse conditions. As expected, we observed a wide range of rooting success (0% to 100%) across both, individuals within in a given genotype and across genotypes (Table 2).

Table 2. Rooting Success of dormant cuttings collected from CG resistant half sib progeny.

Species	# rooted	# propagated	% rooted
J. ailantifolia	70	239	29%
J. cathayensis	3	3	100%
J. hindsii	19	68	28%
J. hybrid	11	14	79%
J. major	73	299	24%
J. mandshurica	4	44	9%
J. microcarpa	8	101	8%
J. nigra	0	5	0%
J. regia	39	73	53%
Pteracarya sp.	45	63	71%

Some of the rooted individuals were screened again for CG resistance as described above. In Table 3 we present data illustrating the genetic stability of CG resistance in a small number of clonally propagated genotypes, i.e. *J. hindsii, J. major, J. microcarpa, J. ailantifolia*, in addition to a large number of *Pteracarya* sp cuttings. Interestingly, a large number of rooted cuttings from selections originally showing resistance developed tumors after *A. tumefaciens* inoculation. These observations demonstrate the need to examine numerous clones of individuals initially considered CG resistant. The rooted cuttings which continue to show CG resistance are being clonally propagated and multiplied for advanced CG screening under field conditions. In addition we are preparing to examine the horticultural characteristics of our most promising CG resistant genotypes.

Table 3. Screening results of rooted cuttings taken from CG resistant half-sib progeny seedlings.

Genus	Species	Year	Number Retested	Number Retained	Year	Number Retested	Number Retained
Juglans	*Hindsii*	2008	6	0	2009	15	2
Juglans	*major*	2008	8	4	2009	59	2
Juglans	*microcarpa*	2008	7	6	2009	6	2
Juglans	*regia*	2008	2	0	2009	5	0
Juglans	*ailantifolia*	2008	5	0	2009	57	3
Juglans	*cathayensis*	2008	0	0	2009	3	0
Juglans	*mandshurica*	2008	1	1	2009	3	0
Pteracarya	*pteracarya*	2008	15	9	2009	67	21
Totals	8		44	20		215	30

CONCLUSIONS

Crown gall resistant *Juglans* and *Pteracarya* genotypes have been identified in our screening assay conducted under greenhouse conditions. We have uncovered a high degree of variability in tumor formation rates among different host genotypes. Interestingly we also discovered the importance of monitoring putatively resistant selections through a dormancy cycle to confirm resistance. In addition, a limited number of rooted cuttings from CG resistant selections (i.e. open pollinated half sibs) continue to show CG resistance. Demonstration of the genetic stability of CG resistance is an essential prerequisite to incorporation of a given genotype into the walnut rootstock breeding program. We are examining why only a small percent of the original individuals selected as CG resistant remained resistant as rooting cuttings. The few rooted cuttings that appear to be remaining CG resistant are being cloned and multiplied for use in limited field studies.