# EVALUATION OF WILD *JUGLANS* SPECIES FOR CROWN GALL RESISTANCE

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## ABSTRACT

The soilborne bacterium *Agrobacterium tumefaciens* is the causal agent of crown gall disease of walnut. Large tumors located near the crown of the tree are hallmark symptoms induced by the bacterial pathogen. Untreated tumors can have an adverse effect on tree health resulting in reduced nut yield and tree vigor. At present, crown gall disease is managed using surgical removal the gall and infected tissues or complete excavation of the diseased tree. This is a costly and time consuming endeavor especially if a large number of trees are infected. The presence of *Agrobacterium* spp. in all walnut growing regions surveyed indicates the likelihood of a crown gall outbreak in any area or county is possible. This makes improved host resistance in commercial walnut rootstock genotypes a requirement for better crown gall management.

Wild species are often the best source for identifying durable resistance to pests and pathogens. The walnut germplasm collection at the National Clonal Germplasm Repository (NCGR), USDA-ARS in Davis, CA contains a large and diverse array of black walnuts and butternuts adapted to California conditions including *J. hindsii, J. nigra, J. microcarpa, J. ailantifolia, J. major*, and additional wingnut selections belonging to the genus *Pterocarya*. Members of the collection from multiple species have been screened to characterize their resistance to *Agrobacterium tumefaciens* within the NCGR collection. *Juglans* species exhibiting increased tolerance to infection by *A. tumefaciens* strain EC1 have been identified. Further characterization of these novel sources of *Agrobacterium* resistance will be exploited in the ongoing U.C. Davis Walnut root stock breeding program to develop suitable rootstocks with improved resistance to crown gall.

## OBJECTIVE

Identify and characterize a novel source of crown gall (CG) resistance in the *Juglans* germplasm collection maintained in the NCGR collection at Davis, CA**.**

### Anticipated Outcome

The aim of this research is 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.***
Seedlings derived from open pollinated seeds 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, 5-9 trees from each accession were screened.
Trees were inoculated at two areas on the main trunk by stabbing with a twin (2 blades) wood chisel blade dipped in EC1 suspension. The bacterial inoculum contained $10^9$ colony forming units (CFU)/ ml of EC1. After inoculation, the wounds were wrapped with parafilm. Standard cultural practices were followed to maintain trees growing in the greenhouse. 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 by scoring tumor development 60 days post inoculation 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%). Differences in relative rates and trends of tumor formation among the different germplasm accessions were noted and recorded. Photos of representative seedlings were taken at various intervals following inoculation. 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" were propagated (i.e. rooted dormant cuttings) and retested as described above. Seedlings showing 25% or less of the stem girdled at 60 days post inoculation were retained and designated as a source of potential resistant germplasm and retained for further study. Mother trees associated with the seedlings showing resistance were targeted for large scale seed collection in 2010 and again in subsequent years.

## RESULTS AND DISCUSSION

| **2009 Seedlings Screened in 2010** | **Tested** | **Retained** | **% retained** |
|---|---|---|---|
| *Juglans* species | 4 | 1 | 25 |
| Individual trees | 155 | 22 | 14 |
| | | | |
| Individual species | Trees Tested | Trees Retained | |
| *J. ailantifolia* | 3 | 0 | 0 |
| *J. major* | 12 | 0 | 0 |
| *J. microcarpa* | 134 | 22 | 16 |
| *Juglans* sp. | 6 | 0 | 0 |
| | | | |

**Table 1a. 2009 Juglans seedlings screened for crown gall resistance in 2010.**

| 2010 Seedlings Screened in 2010 | Tested | Retained | % retained |
|---|---|---|---|
| *Juglans* and *Pterocarya* species | 6 | 6 | 100 |
| Individual trees | 1063 | 215 | 20 |
| | | | |
| Individual species | Trees Tested | Trees Retained | % retained |
| *J. ailantifolia* | 47 | 4 | 9 |
| *J. cathyensis* | 32 | 4 | 13 |
| *J. major* | 84 | 1 | 1 |
| *J. microcarpa* | 797 | 169 | 21 |
| *J. mandshurica* | 22 | 3 | 14 |
| *Pterocarya* species | 76 | 34 | 45 |
| *Juglans* species | 4 | 0 | 0 |

**Table 1b. 2010 Juglans seedlings screened for crown gall resistance in 2010.**

During the 2010 NCGR germplasm screening season, we examined a total of ~1218 seedlings from "new" host genotypes for their resistance to *A. tumefaciens*. This consisted of seedlings from two species of black walnut (*J. microcarpa* and *J. major*), three species of butternuts (*J. ailantifolia, J. mandshurica*, and *J. cathayensis*), and wingnut or *Pterocarya* species (Table 1a, Table 1b). A wide range in size and rate of tumor development in response to inoculation was observed among the seedlings. Some tumors took twice as long (4 months) to develop as others (less than 2 months). Other tumors on different trees continued to grow at a rapid rate. Still several interesting candidates were discovered with no or limited tumor development (<25% girdling). CG resistant *J. microcarpa, J. cathyensis, J. mandshurica, J. major*, and *Pteracarya* sp, individuals were identified but the majority were *J. microcarpa* and *Pterocarya* species with 21% and 45% retained respectively. The trees will be kept for an additional growing season to assess the durability of each seedling's crown gall resistance.

| seedling source | species | total cuttings | rooted cuttings | rooting efficiency | year source plant tested | 2010 retest 1 | | 2010 retest 2 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | # cuttings | # retained | # cuttings | # retained |
| 68.17 | *J. major* | 8 | 2 | 25% | 2009 | 2 | 1 | 1 | 0 |
| 31.8 | *J. microcarpa* | 24 | 1 | 4% | 2009 | 1 | 1 | 1 | 1 |
| 53.2 | *J. microcarpa* | 8 | 3 | 38% | 2009 | 3 | 3 | 3 | 0 |
| unlabeled | *Juglans sp.* | 8 | 1 | 13% | 2009 | 1 | 1 | 1 | 1 |
| 31.5 | *J. microcarpa* | 28 | 2 | 7% | 2009 | 2 | 2 | 2 | 0 |
| 29.8 | *J. microcarpa* | 11 | 1 | 9% | 2009 | 1 | 1 | 1 | 0 |
| 31.6 | *J. microcarpa* | 8 | 1 | 13% | 2009 | 1 | 1 | 1 | 0 |
| 31.10 | *J. microcarpa* | 21 | 7 | 33% | 2009 | 7 | 7 | 7 | 2 |
| 31.1 | *J. microcarpa* | 32 | 7 | 22% | 2009 | 7 | 7 | 7 | 4 |
| 18.2 | *J. microcarpa* | 7 | 0 | 0% | 2009 | 0 | 0 | 0 | 0 |
| 70.6 | *J. major* | 9 | 0 | 0% | 2009 | 0 | 0 | 0 | 0 |
| 69.14 | *J. major* | 3 | 0 | 0% | 2009 | 0 | 0 | 0 | 0 |
| 17.12 | *J. microcarpa* | 7 | 0 | 0% | 2009 | 0 | 0 | 0 | 0 |
| 90.2 | *J. major* | 5 | 0 | 0% | 2009 | 0 | 0 | 0 | 0 |
| 80.6 | *J. major* | 9 | 0 | 0% | 2009 | 0 | 0 | 0 | 0 |
| 29.2 | *J. microcarpa* | 9 | 0 | 0% | 2009 | 0 | 0 | 0 | 0 |
| 31.9 | *J. microcarpa* | 7 | 0 | 0% | 2009 | 0 | 0 | 0 | 0 |
| 29.6 | *J. microcarpa* | 7 | 2 | 29% | 2009 | 2 | 2 | 2 | 1 |
| total | | 223 | 28 | 13% | | 28 | 27 | 27 | 10 |

**Table 2. Rooted cuttings from previously resistant *Juglans* and *Pterocarya* trees re-screened for crown gall resistance.**

Dormant cuttings were taken from 18 selected seedlings shown to be CG resistant in the 2009 screening. The cuttings were rooted and cultivated under greenhouse conditions. As expected, we observed a wide range of rooting success (0% to 38%) across genotypes The rooted cuttings were tested for crown gall resistance twice as described in procedures (Table 2). Only *J. microcarpa* sp. were retained with limited tumor development (<25% girdling) after the second inoculation test. One of the samples, 31.7, comes from a mother tree that has been identified as producing “resistant” seedlings in a previous screen.

## CONCLUSIONS

Additional crown gall tolerant *Juglans* and *Pteracarya* genotypes have been identified in a screening assay conducted under greenhouse conditions. Great variability in tumor formation rates among different host genotypes continue to be observed especially after seedlings cycle through dormancy and begin pushing in the spring. Rooted cuttings from CG resistant selections (i.e. open pollinated half sibs) were successfully prepared and continue to show CG resistance. Demonstration of the stability of CG resistance is an absolute requirement to introduction of a given genotype into the walnut rootstock breeding program. Efforts to investigate the variability of CG resistance in rooted cuttings of the same genotype are ongoing. The remaining cuttings that appear CG resistant are being further characterized and prepared for additional greenhouse analyses.