

## Summary of the Status of *Juglans* Germplasm

### Production Trends and Value of *Juglans*

The United States produces approximately 380,000 metric tons of Persian (English) walnuts annually with a farm-gate value of \$1.3 billion. US production has increased steadily over the last several decades and is located almost entirely in California. Approximately 40% of the US crop is exported. China is the other major commercial producer and exporter with a crop size of about 450,000 metric tons annually and increasing production.

Nut production of the native eastern black walnut is principally from natural stands in the eastern US and averages 17 metric tons annually. This tree is also highly prized for its timber and annual harvest exceeds 12 million cubic feet. The total standing volume is estimated to exceed 3.4 billion cubic feet with a value in excess of \$500 billion. Annual exports of walnut wood products are estimated at \$325 million.

### Crop Vulnerability

The major problems facing the walnut industry are crown gall, nematodes, *Phytophthora spp.*, walnut blight, cherry leafroll virus, codling moth, and potentially insufficient chilling under anticipated climate change. Over 50% of the walnut industry in California is based on two cultivars, Chandler and Hartley. Dependence on two clonally propagated cultivars results in a high degree of genetic vulnerability and there is a relatively narrow germplasm base in reserve to combat these problems.

Thousand cankers disease, a fungus (*Geosmithia*) vectored by the walnut twig beetle, presents a new and potentially serious threat to the California nut industry, black walnut forests of the eastern US, and current germplasm collections. The few remaining stands of native butternut are severely threatened by both butternut canker and hybridization with introduced heart nut. Most other *Juglans* species are forest trees valued for their wood and nuts, often with limited natural ranges, native to regions experiencing population pressure, and threatened by logging and grazing activities.

### Germplasm Activity

Germplasm is maintained by the NCGR at Davis CA, the NCGR at Corvallis OR, the University of Missouri (MU), and the USFS Hardwood Tree Improvement and Regeneration Center (HTIRC) at Purdue. Breeding programs are primarily in the public sector. The University of California-Davis breeding program has emphasized improvements in Persian walnut yield and disease resistance. Use of diverse species for rootstock improvement is an increasing component. Black walnut improvement programs in the eastern United States are directed toward timber (HTIRC) and nut (MU) production. Butternut cultivars and selections are kept at NCGR-Corvallis.

International collecting activities have emphasized broadening the narrow germplasm base found in existing cultivars and identifying sources of disease resistance. Most Latin American walnut species have been sparsely collected and poorly characterized. The rapid decline of butternut warrants accelerated efforts to identify and collect disease resistant genotypes.

## ***Juglans* CGC Report to NGRL, January, 2010**

### Summary

#### I Introduction

#### II Present germplasm activities

A. Collection and maintenance, NCGR, Davis

B. Exploration and acquisition

C. Evaluation

1. Description of NCGR accessions

2. Isozymes

3. Molecular markers

D. Enhancement

1. Breeding Programs – Persian walnut

a. USA

b. France

c. China

d. Other

e. Goals of Persian walnut breeding

i. Lateral bud fruitfulness

ii. Shell and kernel quality

iii. Phenology

iv. Diseases

v. Insect pests

vi. Soil-borne pests in rootstocks

2. Breeding programs – Black walnut

#### III Status of crop vulnerability

A. Domestic vulnerability

1. Persian walnut

2. Butternut

3. Black walnut

B. Foreign vulnerability

1. Central and South America

2. Central Asia

3. Far East

#### IV Germplasm needs

A. NCGR collection maintenance

B. Exploration

1. *Juglans cinerea*

2. Central and South America

3. Kyrgyzstan and central Asia republics

C. Evaluation

1. Description of NCGR collection
2. Species hybrids for rootstocks
3. Microsatellite markers

D. Enhancement

1. *Juglans regia*
  - a. Improved Persian walnut cultivars
  - b. Rootstock improvement
2. *Juglans nigra*
  - a. Nut production
  - b. Timber production
  - c. Rootstock development
3. *Juglans cinerea*

E. Importation protocols

V. Recommendations

- A. Butternut
- B. Central and South American species
- C. Evaluation of horticultural traits
- D. DNA marker evaluation
- E. Importation protocols
- F. Support and monitor independent collections
- G. Evaluate diversity of *J. nigra* and other native *Juglans*

## I. Introduction

The genus *Juglans* includes about 21 species of trees and large shrubs whose natural distributions range, in the Old World, from southeastern Europe to eastern Asia and Japan, and, in the New World, from the eastern half of the US, California, and the southwestern states south through Mexico and Central America to South America and the West Indies. The most economically important species is *J. regia*, the English or Persian walnut, cultivated for its edible nuts; second in economic importance is *J. nigra*, the eastern black walnut, grown primarily for its timber. Several other species and hybrids, notably *J. hindsii* (northern California black walnut) and Paradox (hybrids of *J. hindsii* and *J. regia*, sometimes with contributions from other species), have considerable commercial importance as rootstocks for cultivars of *J. regia*.

In 2007, annual world Persian (English) walnut production totaled approximately 1,600,000 metric tons (all figures in-shell basis). The United States produces approximately 380,000 metric tons, annually with a value of about 550 million dollars. Approximately two thirds of the US crop is sold as shelled nut meats. About 50% of the US crop is typically exported and in 2002 the US supplied 55% of total walnuts exported worldwide. China is the other major contributor to the world walnut crop and produces over 600,000 metric tons annually and supplied 18% of the world's exports in 2002.

Most (99%) of the Persian walnuts produced in the US are grown in California, which in 2009 had 223,000 bearing acres of the crop. Although there is an interest in growing walnuts in other parts of the US, acceptable cultivars adapted to the different growing environments are not available.

*J. nigra* (eastern black walnut) yields in excess of 11,000 tons of in-shell nuts annually but demand for black walnut kernels continues to exceed supply. Most of these nuts are collected from wild trees in Missouri, Illinois, Indiana and Iowa. Eastern black walnut is also one of the most highly valued hardwood species. The USDA Forest Service Forest Inventory Analysis (FIA) indicates that more than 15.4 million acres of timberland in 30 states contain black walnut. The vast majority of this resource is in natural stands, with a small percentage grown in plantations. In the North Central Region an estimated 7 million cubic feet of black walnut growing stock and 5.3 million cubic feet of black walnut non-growing stock are harvested annually. Because of its high commercial value and the long period of time required to produce saw-timber grade trees, the demand for this species has exceeded supply for several decades.

The primary commercial importance of the Northern California black walnut (*J. hindsii*) is as a rootstock for commercial Persian walnut (*J. regia*) orchards or as parent of the widely used hybrid rootstock 'Paradox' (*J. hindsii* x *J. regia*). This species is also a producer of high quality burl wood.

## II. Present Germplasm Activities

### A. Collection and maintenance, NCGR, Davis

Walnuts are assigned to the National Clonal Germplasm Repository (NCGR) in Davis, California. Approximately 12 acres of the repository are devoted to walnuts. The collection at Davis now contains 700 accessions (1,700 trees) of *Juglans* representing 17 species. Related material includes 9 accessions from four *Pterocarya* species.

**Table 1. Accessions in the USDA-ARS collections in Davis and Corvallis.**

<i>Juglans</i> spp.	# available	# total	# clonal	# seedling
<i>ailantifolia</i>	13	14	0	14
<i>australis</i>	0	7	2	5
<i>californica</i>	16	16	0	16
<i>cathayensis</i>	2	2	1	1
<i>cinerea*</i>	25	25	25	0
<i>hindsii</i>	18	18	2	16
<i>hopeiensis</i>	0	1	1	0
<i>hybrid</i>	10	16	11	5
<i>major</i>	19	19	0	19
<i>mandshurica</i>	5	10	1	9
<i>microcarpa</i>	6	7	1	6
<i>mollis</i>	1	1	0	1
<i>neotropica</i>	1	4	0	4
<i>nigra</i>	16	17	16	1
<i>olanchana</i>	2	2	0	2
<i>regia</i>	164	210	103	107
<i>sigillata</i>	0	3	0	3
<i>sinensis</i>	1	1	0	1
sp.	0	4	0	4
Total	274	352	138	214

<i>Pterocarya</i> spp.	# available	# total	# clonal	# seedling
<i>caucasica</i>	1	1	0	1
<i>fraxinifolia</i>	1	1	0	1
<i>pterocarpa</i>	2	2	0	2
<i>stenoptera</i>	5	5	0	5
Total	9	9	0	9

\* *Juglans cinerea* is held at NCGR-Corvallis.

In addition, 25 clonal *Juglans cinerea* (butternut) accessions are held at the NCGR facility in Corvallis, OR and two accessions of *J. nigra* are currently located at Ames, IA. Any sub-tropical species insufficiently cold-hardy for survival at Davis are to be held at Riverside, CA.

Collections of *Juglans* spp. maintained elsewhere in the US include a University of Missouri collection of 57 *J. nigra* cultivars selected for nut production which have been recently characterized using microsatellites and seven phenological descriptors (Coggeshall and Woeste 2009). In addition, the largest collection of black walnut genotypes suitable for wood production is located at the US Forest Service Hardwood Tree Improvement and Regeneration Center, West Lafayette, IN.

The Davis NCGR walnut collection has been topped and hedged annually with a commercial tree hedger on one side during the dormant season and on the other during mid-summer as recommended by the *Juglans* CGC. The operation is intended to regulate tree height, improve pest management and light distribution within and between trees, and develop canopies suitable for both wood and nut collection and for yield evaluation. A microjet irrigation system was installed and is now used throughout the walnut collection.

Many trees in the collection are severely stressed and threatened by a variety of problems including crown gall disease (*Agrobacterium tumefaciens*) and the recently identified fungus responsible for thousand canker disease (*Geosmithia* spp.). *Juglans californica* trees have failed to thrive in the collection for many years and over time many have died. Losses are now extending to other species as well. An effort has been made to repropagate as many accessions as possible but losses have increased recently and land is a limiting factor.

## **B. Exploration and acquisition**

Eleven exploration missions have been completed since 1983 (Table 2). These trips have resulted in acquisition of 152 new accessions of *Juglans* spp.

## **C. Evaluation.**

### **1. Description of NCGR accessions**

Characterization of the collection has been a high priority of the *Juglans* Crop Germplasm Committee. *Juglans* accessions in the NCGR collection have been described using the standard descriptors published in the International Plant Genetic Resources Institute (IPGRI) guideline *Descriptors for Walnut* (*Juglans* spp.), (McGranahan et al., 1994).

For five consecutive years (1988-92), data on phenology, flowering, and yield characteristics were obtained from 524 trees of *Juglans* spp. at the NCGR, Davis and entered into GRIN. Most of the *J. cathayensis*, *J. californica*, *J. ailantifolia*, *J. microcarpa*, and *J. hindsii* accessions were evaluated during that period.

During the years 1995-2000, evaluation efforts concentrated on the *J. regia* accessions (Table 3.) Data collected included both field characteristics (phenology, bearing habit, yield, incidence of insect and disease) and seventeen descriptors of nut traits (kernel weight, percent kernel, kernel color, shell seal and strength etc.). Nut traits were evaluated only for trees with ten or more nuts. Only field data was collected on *J. nigra* and *J. mollis* accessions. This data has been entered into GRIN as it was collected.

More recently, all accessions in the collection have been evaluated for disease resistance traits, particularly those of interest for rootstock breeding including nematode and crown gall resistance. *J. microcarpa*, *J. major* and *J. mandshurica* are among species that have shown promise and are likely to be used in breeding efforts.

Material in the collection has also been evaluated for variation in chilling requirements and susceptibility to pistillate flower abscission.

**Table 2.** Summary of germplasm exploration activities.

Year	Location of collections	Participants	Species collected	No. of accessions at NCGR, Davis	
				No. received	No. alive (no. of seedlings)
1983	Japan	Westwood	<i>J. ailantifolia</i>	11	10 (106)
1984	N Mex, Ariz	Parfitt	<i>J. major</i> <i>J. microcarpa</i>	20 3	17 (127) 3 (9)
1987	Mexico	Parfitt	<i>J. olanchana</i> <i>J. mollis</i> <i>J. pyriformis</i>	2 6 1	0 1 (4) 0
1989	Ecuador	Dixon	<i>J. neotropica</i>	2	2 (7)
1988	Pakistan	Thompson	<i>J. regia</i>	45	33 (120)
1990	China	McGranahan Leslie Barnett	<i>J. regia</i>	55	43 (158)
1990	USA	Millikan	<i>J. cinerea</i>	14*	11*
1994	Kyrgyzstan	McGranahan Leslie	<i>J. regia</i>	74**	6 (64)**
1995	China	McGranahan Leslie	<i>J. regia</i>	16	13 (66)
1999	Argentina	McGranahan	<i>J. regia</i> <i>J. australis</i>	16	13 (80)
2000	Ukraine	Simon Potter	<i>J. regia</i>	43	

\*Located at Corvallis NCGR.

\*\*Accessions fumigated in Kyrgyzstan did not survive.

**Table 3.** *Juglans regia* trees under evaluation 1995-2000

Year	Total No. Evaluated	No. Bearing Trees	No. Evaluated for Nut Traits
1995	489	270	80
1996	494	292	96
1997	558	302	95
1998	433	262	70
1999	409	373	165
2000	306	281	135

## 2. Isozymes

In 1992-93, eight isozyme systems were used to characterize 396 accessions of *Juglans* spp. Eleven species and five hybrids of *Juglans* were represented, but accessions of *J. regia* comprised 88% of the selections tested. Thirty-one accessions were identified to maximize isozyme diversity in a subset of selections to be tested for hypersensitivity to CLRV.

## 3. Molecular markers

In the early 1990s RFLP markers were developed for walnut in D. Parfitt's lab at UC Davis and used to characterize genetic relationships with *J. regia* (Fejellstrom et al, 1994; Fejellstrom and Parfitt, 1994, 1995) and establish the parentage of walnut somatic embryos (Aly et al., 1992.) A molecular phylogenetic study of *Juglans*, based on nuclear and chloroplast DNA sequences, was published in 2000 by A. Stanford, R. Harden, and C. Parks. Chloroplast and nuclear genome sequence markers for North American black walnut species and hybrids, and inter-simple sequence repeat (ISSR) markers for some *J. regia* cultivars, have been developed in D. Potter's lab at U. C. Davis, in conjunction with the Paradox Diversity Study. Concurrently, microsatellite, or simple sequence repeat (SSR), markers were developed for *J. nigra* in K. Woeste's lab at Purdue and for *J. regia* at the Davis NCGR, under the direction of C. Simon and M. Aradhya in collaboration with Potter. More recently molecular markers useful for determining hybridization of butternut with other walnut species were published by Hoban, et al., (2009). These activities have produced reliable molecular markers for most *Juglans* species and cultivars.

## **D. Enhancement**

### **1. Breeding programs – Persian walnut**

#### **a. US: Walnut Improvement Program, UC Davis**

The Walnut Improvement Program at the University of California - Davis is a comprehensive program incorporating both classical breeding and genetic engineering to develop new Persian walnut cultivars. It is a cooperative effort between UC Davis, USDA, and the California Walnut Board. The program was led by Dr. Gale McGranahan, Department of Plant Sciences, UC Davis, and is being continued by Chuck Leslie, Specialist in the same department. The program also includes the independent and cooperative work of several collaborators and emphasizes precocity, early harvest date, late leafing, high yields, excellent quality and resistance to blackline (CLR/V). Cooperative and independent work on rootstocks emphasizes selection and development of genetic resistance to *Phytophthora* root and crown rots, parasitic nematodes, and crown gall disease.

#### **b. France: INRA**

Walnut improvement at INRA has emphasized late leafing, blight resistance, and lateral fruitfulness. The breeding program in France was discontinued in 2007.

#### **c. China: Ministry of Forestry**

Walnut improvement in China emphasizes development of varieties with adaptation to China's growing conditions. Nearly all provinces that grow walnuts have selection programs; actual breeding is less common.

#### **d. Other Breeding programs**

Many other countries have activities related to enhancement. Most notable are Turkey, Morocco, India, Greece, Hungary, Romania, Ukraine and New Zealand. Descriptions of activities can be found in the Proceedings of the International Walnut Symposium published as Acta Horticulturae Numbers 544 and 705.

#### **e. Goals of Persian walnut breeding programs**

##### **i. Lateral bud fruitfulness**

The most significant component of yield that can be manipulated through breeding is lateral bud fruitfulness, a bearing habit in which the lateral buds produce flowers. Lateral fruitfulness is also associated with precocity. Old cultivars and the preponderance of germplasm from Europe is terminal bearing. Incorporation of this trait into new cultivars is high priority in all breeding programs.

## **ii. Shell and kernel quality**

Improved walnut cultivars require a well-sealed shell with a light-colored kernel, free of off flavors, comprising about 50% of the nut weight. Oil quality may be a concern in the future.

## **iii. Phenology**

Phenology is of major concern in many breeding programs. Late leafing is especially important in France and other areas with late spring frosts. Late leafing cultivars also tend to escape blight in areas with spring rains and dry summers. A recent emphasis in the UC Davis program has been on breeding for an earlier harvest than is typical of late leafing cultivars.

## **iv. Diseases**

Disease resistance is a goal in several breeding programs. In the US and France resistance to blight is of primary importance, but blight resistant germplasm has not been identified. Resistance to blackline disease caused by the cherry leafroll virus is a goal in the UC Davis program. Crown gall (*Agrobacterium tumefaciens*) is primarily a rootstock problem and susceptibility appears to depend on the species. Efforts are underway to identify Paradox rootstocks with some resistance, but current genetic engineering approaches to the problem appear more promising.

## **v. Insect pests**

The major insect pest in the US is the codling moth, *Cydia pomonella*. Resistant germplasm has not been identified, however factors involved in codling moth establishment in the hull are being investigated.

## **vi. Soil-borne pests in rootstocks**

The primary soil-borne disease in the United States is *Phytophthora* spp. Selection of resistant or tolerant rootstock is a high priority of the walnut breeding program at Davis. *Phytophthora* problems are accentuated by seasonal floods that occur in low-lying fields that are slow to drain.

One of the most serious soil borne problems is nematodes, particularly *Pratylenchus vulnus*, *Mesocriconema xenoplax* and *Meloidogyne* spp. These three different nematode genera cause three different forms of root damage but the general result is to limit the root system development. Once walnut orchards become infested with nematodes, such as *P. vulnus*, there is no known remedy, although a fallow period of 10 years might be adequate. There is resistance to *Meloidogyne* spp. in black walnut; however a newly described root knot species reported from Texas is known to circumvent root knot nematode resistance. Potentially useful resistance to *P. vulnus* has been observed in *J. cathayensis*. The search for additional sources of resistance is

continuing and success is critical in view of the current absence of post-plant nematicides and the phase out of methyl bromide.

## **2. Breeding programs – Black walnut**

Breeding and selection programs for *J. nigra* are centered at the University of Missouri, Columbia and at the Hardwood Tree Improvement and Regeneration Center (HTIRC), a work unit of the US Forest Service housed within the department of Forestry and Natural Resources, Purdue University, West Lafayette, IN. The program at the University of Missouri, is led by Mark Coggeshall, includes several collaborators, and is focused on selection for nut production and the use of black walnut in agroforestry plantings. The program at the HTIRC is focused on straightness, diameter growth and other traits important to the hardwood and veneer industries. The HTIRC also performs research related to basic genetics, walnut seed orchards, wood quality, tissue culture, rooting, and nursery practices.

### **III. Status of crop vulnerability**

#### **A. Domestic vulnerability**

##### **1. Persian or English Walnut**

*J. regia* is an introduced species in the United States. Except for NCGR collections, the gene pool in the US is largely limited to US cultivars and their relatives, which represent very little of the species' variation. Most domestic commercial walnut varieties are derived from the same gene pool of a few progenitors. Two cultivars, Hartley and Chandler, make up over 50% of the bearing acreage.

Over 10% of potential walnut production is lost to pests and diseases annually. For many of the major diseases, chemical forms of control are either unavailable or ineffective. Codling moth, walnut blight, *Phytophthora* root and crown rots, nematodes, and blackline disease (caused by cherry leafroll virus, CLRV) continue as major sources of loss in the major walnut growing region of California.

Thousand cankers disease (TCD), caused by a newly identified and potentially lethal insect-vectored fungus (*Geosmithia* spp.), has recently been identified in California orchards. This disease represents a threat to not only *J. regia* scion cultivars, but also to *J. hindsii* and Paradox hybrids used as rootstock. The degree of susceptibility of these *Juglans* species, particularly under field conditions, is not yet known. Other possible threats include butternut canker and witch's broom, which have not been found in California, and loss of winter chilling under anticipated climate change.

##### **2. Eastern Black Walnut**

Of the black walnut species native to the US (*J. nigra*, *J. hindsii*, *J. californica*, *J. microcarpa* and *J. major*), only *Juglans nigra* has been commercialized to any great extent for nut or wood production.

Most of the commercial harvest of eastern black walnuts is collected from wild trees in Missouri, Illinois, Indiana and Iowa. Currently demand for black walnut kernels exceeds supply which is limited by insect pests, erratic bearing, and ambers. Of these, ambers, characterized by dark kernel color and shriveling of the kernel tissue, is the most limiting factor in marketable yield (Warmund, 2009).

Eastern black walnut is also one of the most highly valued hardwood species. It is found throughout the eastern half of the United States, concentrated in stands on suitable sites. The USDA Forest Service Forest Inventory Analysis (FIA) indicates that more than 15.4 million acres of timberland in 30 States contain black walnut. The vast majority of this resource is in natural stands, with a small percentage grown in plantations. In the North Central Region an estimated 7 million cubic feet of black walnut growing stock and 5.3 million cubic feet of black walnut non-growing stock are harvested annually. Because of its high commercial value and the long period of time required to produce saw-timber grade trees, the demand for this species has exceeded supply for several decades.

Midwestern landowners prize eastern black walnut as a multipurpose species: it provides valuable timber, is regionally adapted, and attractive to wildlife. During the first 5 years of the 1990's more than 3 million black walnut seedlings were distributed annually by State nurseries.

*J. nigra* faces a potentially very serious threat from thousand cankers disease (TCD), caused by an insect-vectored (*Pityophthorus juglandis*) fungus (*Geosmithia spp.*). TCD has been responsible for the decline and death of many *J. nigra* trees in urban and landscape settings throughout the western United States. *J. nigra* trees affected to date have all been outside their native range and the identified insect vector has not been collected within the native range of *J. nigra* – but movement of the fungus east into areas of native *J. nigra* stands via wood transit or insect dispersal is distinctly possible and the potential for damage is a serious concern.

### **3. Other Black Walnuts**

Two *Juglans* species are native to California. These are *J. hindsii*, the Northern California black walnut and *J. californica*, the Southern California black walnut.

At the time of European settlement, *J. hindsii* was found in only a few isolated sites in Northern California but it has since been widely planted as an orchard rootstock and street tree and is common in riparian areas. Few if any original stands remain. The nuts are sometimes collected for marketing and trees with burls or desirable grain are extremely valuable. Individual trees capable of hybridizing with *J. regia* are infrequent

and are prized as sources of hybrid ‘Paradox’ seed used commercially as a rootstock. *Geosmithia* fungus, associated with thousand cankers disease, has recently been isolated from numerous dying and declining *J. hindsii* roadside and urban landscape trees, as well as nursery trees used for ‘Paradox’ seed production. The potential impact of TCD on *J. hindsii* resources is a current concern which has commercial walnut production, landscape, and wildlife implications.

*Juglans californica* is a shrubby tree native to riparian areas of coastal Southern California. Its small original range has been further reduced by agriculture and urban encroachment. *J. californica* appears to be particularly susceptible to thousand cankers disease, which may put additional pressure on the species in remaining native stands. *J. californica* has also been widely planted and hybridizes with *J. hindsii*.

*J. major* and *J. microcarpa*, both native to the southwestern US and northern Mexico, are also harvested for timber, but so far this does not appear to have had a large impact on the germplasm. Timber theft, always a problem in *J. nigra*, is also an increasingly important issue for *J. major*, since this species commonly forms valuable burls at maturity.

There are no concerted efforts to plant *J. major* or *J. microcarpa* in the United States, but China has been buying seeds of *J. microcarpa* for use as a rootstock in alkaline soils. Both species are of interest for rootstock breeding and development in California. They are also native to areas where thousand cankers disease may have originated and are thus of interest in understanding the development of that disease.

The impact of selective harvest, habitat fragmentation, urbanization, and other environmental changes on populations of North American black walnut species is not clear. Areas of local or unique genetic diversity have not been identified for any of these species.

### **3. Butternut**

Butternut (*Juglans cinerea*), also called white walnut, grows on rich loamy soils along stream banks in mixed hardwood forests and on well-drained, rocky soils of limestone origin. Its native range is similar to eastern black walnut, extending farther north but not as far south. Its native range is from eastern Canada west to Minnesota and as far south as Arkansas, Alabama, Georgia, and Mississippi. Butternut has been planted widely outside of its native range.

Butternut has similar insect pests to black walnut. Butternut curculio (*Conotrachelus juglandis*), the most serious of these, injures young stems and fruit.

The most serious threat to butternut throughout its range is butternut canker, caused by what is believed to be an introduced pathogen *Sirococcus clavigignenti-juglandacearum*. The sticky spores of the pathogen are spread locally by rain splash and long distance on seed and most likely by insects and birds. Multiple branch and stem cankers often girdle and kill infected trees of all ages. Stump sprouts, if they develop at all, are quickly infected and killed as well.

The disease, first observed in Wisconsin in 1967, has since killed up to 80% of the butternut in some states and is threatening its survival as a viable species throughout North America. The fungus is not known to be present in the western United States.

Butternut is listed as a sensitive species or a species of special concern in many states and the harvest of healthy butternut on Federal lands and on land managed by several states is restricted. Butternut is now listed as endangered in Canada and conservation and restoration efforts are underway in Ontario. Even in the absence of the disease, butternut is dying from old age. Decline in regeneration is attributed to insufficient site disturbance needed to create optimum seedbeds (light, bare soil) and to seed predation.

Although butternut is the only species that is killed by the pathogen, eastern black walnut (*J. nigra*) and heartnut (*J. ailantifolia* var. *cordiformis*) have been found infected in plantings where the fungus causes a twig blight but not stem cankers on these species. Other hardwood species such as pecan, hickories and *J. regia* have been shown to be susceptible in inoculation experiments. However, it is not known if the fungus is naturally present on these other species or whether it could threaten walnut plantations in the west if it was accidentally introduced.

Efforts are underway to identify, screen and propagate putatively tolerant and resistant genotypes. *J. cinerea* is harvested for timber on an occasional basis as suitable trees are identified. This practice tends to remove the larger and potentially more tolerant genotypes, placing the species at even greater risk from the disease. Nut growers in the Eastern United States plant hybrids between butternut and heartnut, and the effect of heartnut gene flow into the wild butternut populations is not known.

## **B. Foreign vulnerability**

### **1. Central and South America**

The status of most of the species of *Juglans* occurring in Mexico, Central and South America, and the Caribbean is uncertain. Based on observations by Dan Parfitt during his exploration in Mexico in 1987, however, it is probable that at least *J. pyriformis*, *J. olanchana*, and *J. mollis* are endangered. Other species occurring in these regions include: *J. hirsuta* (Mexico), *J. steyermarkii* (Guatemala), *J. jamaicensis* (West Indies), *J. soratensis* (Bolivia), *J. venezuelensis* (Venezuela), and *J. australis* (Argentina). *Juglans neotropica*, native to South America, is more widely distributed. All of these are

potentially important timber species; in fact, the endangered status of many of them is due to their value for timber combined with their limited geographic distributions. The potential of these species as sources of genes for disease resistance and/or valuable secondary compounds is unknown. Most of these species are not represented in the NCGR collection and they should be given high priority for future exploration activities (see below).

## **2. Central Asia**

Important sources of *Juglans regia* germplasm are being lost due to extensive logging in Kyrgyzstan. Populations in other parts of central Asia are thought to be under similar threat from deforestation. Several characterized collections of material from this region were established in the past within the former Soviet Union but these collections are also at risk.

## **3. Far East**

The current status of germplasm resources of species native to Japan, Korea, Manchuria, coastal China and southern China are not well known. As elsewhere, logging and population pressure are likely threats to forest populations. Limited material has been collected from these areas and is in the current collections.

# **IV. Germplasm Needs**

## **A. NCGR collection maintenance**

Additional space is urgently needed at the NCGR, Davis. Limited space availability for collections has required close planting of the *Juglans* accessions. While this is adequate for production and distribution of vegetative material, tight spacing increases annual management costs considerably, prevents normal canopy development and cropping, and severely impedes evaluation of accessions. In addition there is not space to repropagate current accessions threatened with loss from crown gall and other root diseases.

Both the fungus (*Geosmithia spp.*) implicated in thousand cankers disease and the vectoring bark beetle (*Pityophthorus juglandis*) have been identified during the last year within the National Clonal Germplasm Repository's *Juglans* collection at Winters, CA. Many *Juglans* trees in this collection are in poor and declining condition, perhaps initiated by a variety of other causes, but now show symptoms of TCD which has likely exacerbated decline and may seriously threaten the collection. Attention needs to be given to protecting the collection and repropagating impacted trees.

There are currently a very limited number of accessions of *Juglans* species native to eastern North American in the NCGR, Davis collection. Restrictions on the

importation of *Juglans* germplasm into California due to bunch disease and concerns about butternut canker make expansion of the collections of native North American *Juglans* species difficult.

An additional location or locations are needed for the *ex situ* maintenance of valuable germplasm of the native *Juglans* species, especially *J. nigra* and *J. cinerea*. Currently, collections of these two species are scattered in as many as eight states. Many of these collections are inadequately documented and catalogued and where evaluations have been performed, work was not always based on standard descriptors. The relatedness of the selections is not known. As far as possible the valuable material from these collections needs to be identified, consolidated and planted in a suitable location where standardized evaluations can be performed. Additionally, it would be advantageous to have a single location where new valuable germplasm can be grown, evaluated and distributed. Currently, the largest collection of *J. nigra* nut cultivars is located at the University of Missouri. A total of 500 *J. cinerea* accessions and related hybrids are maintained by the HTIRC at Purdue University.

A repository location with a subtropical climate is needed, and has long been requested, for species with insufficient cold-hardiness to survive at Davis.

Experimental cryostorage work has been initiated for both *J. regia* and *J. nigra*. Consideration should be given to cryostorage of accessions when feasible as a backup for the existing collection.

## **B. Exploration**

Exploration locations have been prioritized according to genetic diversity that is potentially available and according to the stability of the germplasm sources.

### **1. North, Central and South America**

Approximately 11 species of black walnut are native to areas of Central and South America. Development of a representative collection of these species is a high priority of the committee.

Exploration for species native to Mexico, the Caribbean, South and Central America, most of which are not currently represented in the collection (e.g., *J. hirsuta*, *J. jamaicensis*, and *J. pyriformis*), should be undertaken within the next 2-3 years. Exploration in Mexico would also increase the geographic representation and genetic diversity of accessions of *J. major* in the collection. Collection of *J. pyriformis* is likely to be difficult since any remaining stands will be located in a few remote locations in southeastern Mexico, to which access may be restricted. *J. australis*, native to Argentina, is thought threatened by hybridization with introduced species

These species are for the most part tropical and will not tolerate hard freezes so some of them, especially *J. olanchana*, *J. pyriformis*, *J. mollis*, and *J. jamaicensis*, will probably need to be maintained at a semitropical location, or a location that is not susceptible to freezes. *J. hirsuta* and *J. mollis* can be maintained at the more temperate Davis NCGR location.

Exploration for additional accessions of *J. microcarpa* and *J. major* would be useful for several purposes. Seedlings of some *J. microcarpa* and *J. major* accessions currently in the NCGR collection have shown promise in evaluations for resistance to crown gall disease (*Agrobacterium tumefaciens*), *Phytophthora* spp., and nematodes. Both species are native to areas with high soil pH, high salts, and drought and in limited testing have shown promise as sources of rootstock resistance to these problems. More recently, it has been suggested that germplasm from this area may provide a source of resistance to thousand cankers disease.

## **2. *Juglans cinerea***

The *Juglans* CGC has given high priority to collection of germplasm of butternut (*Juglans cinerea*) in North America due to the immediate threat to many populations of this species posed by the butternut canker disease (*Sirococcus clavigignenti-juglandacearum*) combined with the relative ease of arranging collections within the U.S. where this species occurs.

*Juglans cinerea* is probably the most threatened North American species in the genus. The species was once widely distributed, and formerly had some commercial importance both as a nut tree and as a source of timber. As a native species, butternut also enjoys a place in Native-American cultures, folklore, ethnobotany for medicinal purposes, and in folk art. Since there have been very few scientific evaluations of the genetic or phenotypic diversity within butternut, the location of unique and/or unusual and valuable genotypes are poorly understood. In some cases, local experts (e.g., foresters, landowners, timber buyers, conservation biologists) have identified areas where butternut is or was an important part of the hardwood forest.

## **3. *Juglans cathayensis***

In evaluations of NCGR accessions and other germplasm sources, *Juglans cathayensis*, a species native to eastern China, has shown promising resistance to lesion nematodes (*Pratylenchus vulnus*), the key nematode pest of walnut. The NCGR collection currently contains only 2 accessions of this species. We recommend inclusion and evaluation of additional sources of *J. cathayensis*.

#### **4. Kyrgyzstan and central Asian republics**

Large-scale commercial logging of native walnut forests is occurring in Kyrgyzstan and central Asian republics. These areas are important sources of genetic diversity for *J. regia*. There is concern that political and economic instability in these areas will result in continued and accelerated loss of *Juglans* genetic diversity. Deterioration of established and characterized Soviet-era collections from this region is an additional concern.

### **C. Evaluation**

#### **1. Description of NCGR *Juglans* collection**

There is need for continuing description of the phenology, flower, yield, and nut characteristics in existing accessions of *J. major*, *J. nigra*, and *J. mandshurica*. There is a need for intensified evaluations of resistance to soilborne pests (plant parasitic nematodes, *Phytophthora* spp., and *Armillaria* spp.) among accessions of many species represented in the collection. In addition, the chilling requirements of walnut cultivars and accessions should be documented.

#### **2. Species hybrids for rootstocks**

Data available on performance of hybrids as rootstocks is limited. NC Paradox hybrid (a hybrid from *J. hindsii* x *J. regia*) is widely used in California due to its superior vigor and documented resistance and tolerance to several soilborne pests. However, negligible information is available on horticultural traits or genetic resistance to soilborne pests among hybrids of other *Juglans* spp. that are represented in the NCGR collection. Evaluation of these traits may be critical as the walnut industry is forced to rely less on methyl bromide fumigation as a form of pest control.

#### **3. Microsatellite markers**

Microsatellite libraries enriched for CA and GA dinucleotide repeats have been screened using a diversity panel of *Juglans nigra* from 12 provenances and from three *Juglans regia* cultivars. At least 250 loci were polymorphic within *J. nigra* and 82 loci were polymorphic within *J. regia*. Chloroplast microsatellites were also screened using the same diversity panel: two of the chloroplast microsatellites were monomorphic for both *J. nigra* and *J. regia*, one was apparently monomorphic within species but polymorphic between species and three were polymorphic within *J. nigra*. Additional microsatellites are being developed and screened.

Microsatellites of this type are excellent tools for evaluating the diversity and relatedness of germplasm. They can also be important tools for clone identification and breeding. Data from analysis of microsatellites and other DNA-based genetic markers is

now providing an important complement to the phenotypic data already available, is assisting in the characterization of accessions with uncertain identities, helping resolve the parentage of species hybrids, and providing a means for the identification of divergent populations or populations with a high frequency of rare or unique alleles.

## **D. Enhancement**

### **1. *Juglans regia***

#### **a. Improved Persian walnut cultivars**

To develop Persian walnut cultivars with improved precocity, lateral bearing, and short-season crop development, the Walnut Improvement Program must continue its main approach, which includes hybridization between English cultivars and individuals with desired traits followed by backcrossing. Continued introgression is also required for development of English cultivars with tolerance or resistance to walnut blight and hypersensitivity to CLRV.

#### **b. Rootstock improvement**

Intensified interdisciplinary efforts are needed for continued development of improved rootstocks. Improvements that are especially needed in walnut rootstocks include tolerance to CLRV, resistance or tolerance to *Phytophthora* spp., *Armillaria* spp., and parasitic nematodes and resistance to crown gall (*Agrobacterium tumefaciens*). Improvements in responses to pests must be accompanied by horticultural acceptability.

### **2. *Juglans nigra***

#### **a. Nut Production**

The bulk of current black walnut nut production is from unimproved natural stands. Selections for improved yield, annual bearing and more desirable nut traits exist and have been characterized. The University of Missouri breeding program is directed towards improved tree yield, precocity, lateral and annual bearing habits, anthracnose resistance and greater kernel yield per nut.

#### **b. Timber production**

Landowners typically have several objectives when they plant *J. nigra* for timber production. The two most important objectives are forest regeneration and plantation establishment. These objectives require distinct management schemes and distinct genetic stocks. Forest regeneration requires improved seed of relatively low cost that will produce trees that grow well with little maintenance. Traditional seed orchards containing a large number of genetically diverse but select progeny are well suited to meet the large demand for improved seed used in forest regeneration.

Plantation establishment or clonal forestry requires genotypes that respond well to management. These genotypes are usually produced by intercrossing a few elite individuals followed by stringent selection and extensive testing. This approach is also used to create populations with unusual and valuable wood quality traits such as figured wood. The HTIRC is selecting and evaluating *J. nigra* genotypes with both forest regeneration and plantation establishment objectives in mind.

### **c. Rootstock development**

Numerous investigators have commented on the apparent hybrid vigor for vegetative growth found in inter-specific crosses of *Juglans*. Paradox hybrids are often the rootstock of choice for *J. regia* in California. Seedling *J. nigra* rootstocks are the only option currently available for propagating black walnut scions. There is a need for vigorous, adapted rootstocks that can be propagated by rooting. The potential of inter-specific hybrids as rootstocks for *J. nigra* needs further investigation.

### **3. *Juglans cinerea*:**

There is not yet a formal breeding program for *J. cinerea* (butternut), but there is an ongoing effort to identify and propagate historically important selections and to identify new selections that appear to be resistant to, or tolerant of, butternut canker.

### **E. Importation Protocols**

Current guidelines for germplasm importation were developed by the relevant State and Federal regulators. Most significant are the Animal Plant Health Inspection Service (APHIS) and the California Department of Food and Agriculture (CDFA). Imported bud or graft wood is subject to APHIS inspection on entry.

The California Plant Quarantine Manual states:

- (1) All species of *Juglans* (walnut, butternut) trees and parts capable of propagation, except nuts, are:
  - (a) Prohibited entry into California from any state east of the eastern borders of Idaho, Utah, and Arizona.
  - (b) Admissible into California from Idaho, Nevada, Oregon, Utah and Washington provided each lot is accompanied by a certificate issued by the Department of Agriculture of the state of origin affirming (1) The material was grown in the state of origin, (2) Brooming disease is unknown in the state of origin, and (3) The amount and kind of commodities covered.

There are no current limitations on the importation of seeds although these may potentially harbor important diseases and pests and caution is strongly advised. It is known that the pathogen causing butternut canker, *Sirococcus clavigignenti-juglandacearum*, can be seed borne in butternut and potentially in other *Juglans* species.

## V. Recommendations

### A. Additional land and sites

Additional space is urgently needed for the NCGR-Davis collections. Limited space availability has required close planting of the *Juglans* accessions. While this is adequate for production and distribution of vegetative material, tight spacing increases annual management costs considerably, prevents normal canopy development and cropping, and severely impedes evaluation of accessions. Many *Juglans* trees in this collection are in poor and declining condition, severely stressed, and threatened by a variety of problems including crown gall disease. *Juglans californica* trees have failed to thrive in the collection for many years and over time many have died. Losses are now extending to other species as well. An effort has been made to repropagate as many accessions as possible but losses have increased recently. Land is a limiting factor and there is not space to repropagate accessions threatened with loss. TCD has likely exacerbated decline of trees and may seriously threaten the collection itself. Attention needs to be given to protecting the current germplasm and repropagating impacted trees, finding land for re-establishing the collection on reasonable spacing, developing methods of alternate storage, and examining approaches to curating the collection that reduce space requirements.

A repository location with a subtropical climate is needed, and has long been requested, for *Juglans* species with insufficient cold-hardiness to survive at Davis.

An additional location is needed for the *ex situ* maintenance of *J. nigra*. There are current plant quarantine obstacles and legitimate disease concerns that preclude future introductions of this species into California. Specifically, we recommend that a *J. nigra* repository be designated at the University of Missouri. The MU collection is the largest assemblage of *J. nigra* nut cultivars currently existing and has been well characterized using both phenological descriptors and microsatellite markers to form the basis of an active nut breeding program. Perhaps most importantly, the available land base and infrastructure will allow for future expansion at this location. We recommend that breeding efforts, especially those related to species native to the US, be better coordinated with the germplasm repository and given financial support so that critical germplasm can be identified, maintained and evaluated in suitable locations under suitable conditions. A method also needs to be developed to better identify and monitor the viability of independent collections.

Experimental cryostorage work has been initiated and consideration should be given to cryostorage of accessions when feasible as a backup for the existing collection.

## **B. Thousand cankers disease**

Thousand cankers disease poses a new and potentially serious threat to commercial walnut production in California and to black walnut resources in the eastern United States. To date, little information is available on species susceptibility, the degree of variation within species, differences in symptoms between species, or differences in ability to host the vector. Evaluation of *Juglans* germplasm for these and other traits related to the TCD insect-disease complex should have very high priority. Collection of additional material which may have resistance, particularly *Juglans* species native to the southwestern US and northern Mexico where resistance is thought likely to occur, should be emphasized.

## **C. Movement, export, and quarantine protocols**

The current guidelines for movement of *Juglans* germplasm and walnut wood within the United States, as well as procedures for exporting germplasm overseas, need to be evaluated in view of the identification of thousand cankers disease in the western US in general and in the NCGR collection at Davis, CA in particular. The presence of thousand cankers disease means shipment of *Juglans* germplasm between California or Oregon and the eastern US in either direction is either prohibited or inadvisable at this time. Germplasm curated either at Davis, CA or Corvallis, OR currently cannot be shipped east into the native range of *J. nigra* and germplasm from the eastern US should likely not be sent to the current repositories. Even if sent, it cannot be returned for further use in the eastern states.

In addition, the general risk of pest and pathogen introduction into California via seed and scion wood should continue to be assessed based on the most current information from pathologists and those experienced with germplasm acquisition. Importation guidelines should be updated to minimize the risks to existing germplasm resources. Diseases of note that should be restricted from California orchards include bunch disease, butternut canker, and any new strains of cherry leafroll virus or *Geosmithia*.

## **D. Evaluation of horticultural traits**

The lack of adequate alternatives to methyl bromide has increased the urgency of identifying resistance to soil-borne pests, including parasitic nematodes, *Phytophthora* spp., *Agrobacterium tumefaciens*, and *Armillaria* spp. Absence of adequate fumigants, changes in water availability and use, and the recent development and commercialization of methods for producing clonal rootstocks have all increased the need to evaluate a wider set of *Juglans* germplasm for rootstock-related traits that include disease and pest resistance, drought and salt tolerance, and height control. Most of the *Juglans* accessions at Davis should be evaluated more extensively and in greater detail for these traits.

Expected global warming and the recent frequency of low-chill winters indicate a need to evaluate the chilling requirements of *J. regia* accessions. Evaluations for traits related to scion breeding, including yield, harvest date, nut quality, oil composition, blight resistance, and pistillate flower abscission need to be continued.

### **E. Butternut**

The collection and evaluation of butternut should continue to receive a high priority, since this species may be extremely endangered and many of the necessary resources for collection and evaluation are already in place. Identifying the most threatened *J. cinerea* populations and determining the best conservation strategies for these populations is critical in preventing the complete loss of this species' commercial potential.

The germplasm of *J. cinerea* is very poorly understood. Germplasm collection and evaluation is critical to preservation. An aggressive effort to collect *J. cinerea* germplasm with potential resistance to, or tolerance of, butternut canker should be undertaken in conjunction with a program to perform disease screens on the candidate genotypes.

The genetic and phenotypic characterization of the germplasm can proceed at the same time. The resulting genetic data can then be used as part of any subsequent breeding effort and as a means to understand patterns of diversity within the species. This will require the identification of new locations for *ex situ* conservation and evaluation of *J. cinerea* germplasm within its natural range.

The best long-term strategy for species enhancement will be based on the introduction of genotypes that are resistant to, or tolerant of, butternut canker into state and private nurseries and seed orchards.

### **F. Central and South American species**

Approximately 11 species of black walnut are native to areas of Central and South America. Development of a representative collection of these species is a high priority of the committee. Exploration for species native to Mexico, the Caribbean, South and Central America, most of which are not currently represented in the collection, should be undertaken within the next 2-3 years. Remaining stands of many of these are thought to be remote and access is likely to be difficult and restricted. Many of these species are thought to be endangered and have potential use in rootstock development.

**G. Evaluate diversity of *J. nigra* and other native *Juglans*.**

The genetic diversity of the *Juglans* species native to North America is poorly understood. We recommend enhanced evaluation of all the native *Juglans* with the goals of understanding the relationship between genetic and geographic distance, evaluating the relative importance of the various threats to the germplasm, and identifying threatened or critical populations. To support these objectives, in view of quarantine restrictions in California, a new site or sites for maintenance of collections of species native to the eastern US should be identified and developed. The value of *in situ* conservation should be determined by characterizing diversity for the species in National Parks, wilderness areas and on other public and private lands.