Summary of the Status of *Juglans* Germplasm

Production Trends and Value of *Juglans*

The United States produces approximately 240,000 metric tons of Persian (English) walnuts annually, valued at about 300 million dollars. US production has increased steadily over the last several decades and is located almost entirely in California. The major portion of the US crop is sold as shelled nut meats but approximately 35% is marketed in-shell. Over 40% of the US crop is exported. China is the other major commercial producer and exporter with a crop size exceeding 250,000 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 14 metric tons annually. This tree is also highly prized for its timber and annual harvest exceeds 12 million cubic feet. Due to its high commercial value and the long period of time required to grow saw-timber grade trees, demand for this species has exceeded supply for several decades.

Crop Vulnerability

Most *Juglans* species are forest trees, valued for both nut production and timber quality. Several species have limited natural ranges and many are native to regions experiencing economic difficulties and population pressure, placing these species under threat from logging, land clearing, and grazing activities. Butternut, native to the eastern United States, is severely threatened by a new disease, butternut canker.

Germplasm Activity

Breeding programs are primarily in the public sector. The University of California – Davis program has emphasized improvements in Persian walnut yield and disease resistance. Several black walnut improvement programs in the eastern United States are directed toward both timber and nut production.

International collecting activities for Persian walnut 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, July 2001

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 North America 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*.

During 1999-2000, annual world Persian (English) walnut production totaled approximately 680,000 metric tons (all figures in-shell basis). Of this, the United States produced approximately 240,000 metric tons annually, which was valued at about 290 million dollars in 1999. Approximately 55% of the US crop is sold as shelled nut meats. Data from the last five years indicate that 50% of the US crop is typically exported. China is the other major contributor to the world walnut crop and produces about 300,000 metric tons annually, of which about 11% is typically exported.

Most (99%) of the Persian walnuts produced in the US are grown in California, which in 2000 had 193,000 bearing acres of the crop. Although there is an interest in growing walnuts in other parts of the US, commercially satisfactory varieties adapted to the different growing environments are not available.

From 1994 through 1998, 27 million pounds of in-shell *J. nigra* (Eastern black walnut) nuts were purchased annually for processing. Most of these nuts were 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 (Chuck Simon, Curator). Approximately 12 acres of the repository are devoted to walnuts. The collection at Davis now contains 399 accessions (1,477 trees) of *Juglans* representing 17 species. Related material includes 9 accessions from four *Pterocarya* species.

Table 1. Accessions in the Davis collection.

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

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

In addition, eleven 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.

Other smaller collections of *Juglans* spp. have been maintained elsewhere in the US.

The Davis NCGR walnut collection was "topped" with a commercial tree hedger during the winter of 1996 as recommended by a *Juglans* CGC subcommittee. The operation reduced tree height and should improve pest management and light distribution within and between trees. A microjet irrigation system was installed and is now used throughout the walnut collection.

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 over the last decade 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.

Most of the *J. major* and *J. mandshurica* accessions in the collection still have not been evaluated.

Table 2. Summary of germplasm exploration activities.

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

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, at the University of North Carolina, Chapel Hill. 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 are being 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 in collaboration with Potter. All of these activities should lead to the availability of reliable molecular markers for most species and cultivars within *Juglans* within the next several years.

D. Enhancement

1. Breeding programs – Persian walnut

a. US: Walnut Improvement Program, UC Davis

The Walnut Improvement Program at the University of California at 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 Walnut Marketing Board. The program is led by Dr. Gale McGranahan, Department of Pomology, University of California (UC) Davis and includes independent and cooperative work of several collaborators. The program emphasizes precocity, early harvest, late leafing, high yields, excellent quality and resistance to blackline. Cooperative and independent work 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 emphasizes late leafing, blight resistance, and lateral fruitfulness.

c. China: Ministry of Forestry

Walnut improvement in China emphasizes development of varieties with adaptation to China's growing conditions.

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 Fourth International Walnut Symposium published as Acta Horticulturae Number 544.

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 current season's 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. Resistance to soil-borne pests in rootstocks

The primary soil-born pest in the United States is *Phytophthora* spp. Selection of resistant or tolerant rootstock is high priority in the USDA walnut program at Davis.

2. Breeding programs – Black walnut

Breeding and selection programs for *J. nigra* are centered at the University of Missouri, Columbia and 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. Black Walnuts

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. 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. 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.

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. Few if any original stands remain. The nuts are sometimes collected for market 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.

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. This species has also been widely planted and hybridizes with *J. hindsii*.

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.

2. 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. It 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. The fungus is not known to be present in the western United States.

Although butternut is the only species that is killed, 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.

3. 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.

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, and blackline disease (caused by cherry leafroll virus, CLRV) continue as major sources of loss in the major walnut growing region of California.

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* is more widely distributed than these species, being found from Central through South America. 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. Some material has been collected from these areas and is in the current collections.

IV. Germplasm Needs

A. NCGR collection maintenance

There are a very limited number of *Juglans* accessions native to eastern North American at the NCGR, Davis. 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 evaluation has been performed it was not 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.

A repository location with a subtropical climate is needed for species with insufficient cold-hardiness to survive at Davis and consideration should be given to cryostorage of accessions as a backup for the existing collection.

Additional space is desirable 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 impedes evaluation of accessions.

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. *Juglans cinerea*

At the January, 2001, meeting of the *Juglans* CGC, the committee agreed that highest priority should be given to collection of germplasm of butternut (*Juglans cinerea*) in North America. 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, led to the conclusion that a proposal for this exploration should be put forward in 2001.

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, in folklore, 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.

2. 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.

3. 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 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.

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

At this time, the evaluation of *Juglans* germplasm is limited almost exclusively to an assessment of the phenotypic diversity present in the various collections. Descriptor lists for *Juglans* evaluation focus on phenology, flower and yield characteristics. While there is a need to continue this type of evaluation, there is a need for a DNA-based evaluation of the genetic diversity present both in wild populations and in collections. 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. The addition of data from analysis of

microsatellites and other DNA-based genetic markers will provide an important complement to the phenotypic data already available, assist in the characterization of accessions with uncertain identities, help resolve the parentage of species hybrids, and provide 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. Breeding efforts need to be 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.

V. Recommendations

Butternut

The collection and evaluation of butternut should receive a very 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 a critical first step 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 their 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.

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.

Evaluation of horticultural traits

Progress of walnut improvement programs depends heavily on evaluations that identify desirable traits. The *J. major* accessions in the Davis collection still need to be evaluated for phenology, yield and nut traits. Most of the *Juglans* accessions at Davis should be assessed more extensively and in more detail for insect and disease resistance traits. This information would be beneficial for improvement of both scions and rootstocks. The lack of adequate alternatives to methyl bromide has increased the

urgency of identifying resistance to soil-borne pests, including parasitic nematodes, *Phytophthora* spp., and *Armillaria* spp.

DNA marker evaluation

A core set of microsatellites should be identified for each species in the genus to characterize the diversity of accessions and wild germplasm. Microsatellite loci or alleles specific for each species in the genus would facilitate important studies of hybrids, hybridization zones and phylogeny. Such primers would also assist in the proper identification of mislabeled accessions. Additionally, a set of maximally informative microsatellite loci should be developed for each species. These loci could be used to characterize and evaluate the diversity of germplasm with a minimum of effort.

Importation protocols

The current guidelines for the importation of *Juglans* germplasm into the United States and especially to California need to be re-evaluated. The risk of pest and pathogen introduction via seed and scion wood should 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.

Support and monitor independent collections

We recommend that breeding efforts, especially those related to species native to the US, be better coordinated with the germplasm repository so that critical germplasm can be identified and maintained in suitable locations. *Ex situ* conservators should be provided with methods and standards for evaluation so that data from existing, non-NCGR collections can be integrated with more formal germplasm data banks. A method should be developed to identify and monitor the viability of these independent collections.

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.