Juglans Vulnerability Statement
October 2017
Juglans Crop Germplasm Committee

Production Trends and Value of *Juglans*

The United States produces approximately 569,640 metric tons of Persian (English) walnuts annually with a farm-gate value of $1.8 billion. US production has increased steadily over the last several decades and is located almost entirely in California. Approximately 60% of the US crop is exported. China is the other major commercial producer and exporter with a crop size of about 1.7 million 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. Annual timber 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., thousand cankers disease, husk fly, walnut blight, cherry leafroll virus, and insufficient chilling during some winters. Over 80% of the walnut industry in California is based on four cultivars, Chandler, Hartley, Howard, and Tulare. Dependence on only a few 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 in regions experiencing population pressure, and threatened by logging, grazing activities, and human activities.

Germplasm Activity

Germplasm is maintained by the National Clonal Germplasm Repository (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 emphasizes Persian walnut yield, harvest date, and disease resistance including use of diverse species for rootstock improvement. Black walnut programs are directed at timber (HTIRC) and nut (MU) production. Butternut accessions 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.

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. 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. Eastern black walnut
  - 3. Other black walnuts
  - 4. Butternut
- B. Foreign vulnerability
  - 1. Central and South America
  - 2. Central Asia
  - 3. Far East

IV Germplasm needs
- A. NCGR collection maintenance
- B. Exploration
  - 1. North, Central and South America
  - 2. *Juglans cinerea*
  - 3. *Juglans cathayensis*

4. Kyrgyzstan and Central Asian locations

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. Additional land sites
    B. Movement, export, and quarantine protocols
    C. Evaluation of horticultural traits
    D. Butternut
    E. Central and South American species
    F. 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 2014, annual world Persian walnut production approximated 3,230,000 metric tons (all figures in-shell basis). The United States produces approximately 518,000 metric tons annually, approximately two thirds of which is sold as shelled kernels, and more than 60% of the US crop is exported. In 2016, the US supplied 45.8% of worldwide commercial walnut production at a value of $1.3 billion. China (1,602,000 MT) and Iran (446,000 MT) are the other major producers (FAO, 2014).

Most (99%) Persian walnuts produced in the US are grown in California, which in 2015 had 300,000 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 USA, in 2017, there are 13,770 million cubic feet of walnut wood. Due to high commercial value and the long 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. Likewise, the primary commercial importance of Texas black walnut (*Juglans microcarpa*) is as a parent of hybrid ‘Paradox’ rootstock (*J. microcarpa x J. regia*).

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 702 accessions (1,700 trees) of *Juglans* representing 17 species. Related material includes 9 accessions from four *Pterocarya* species.

Table 1. Available accessions from the USDA-ARS collections in Davis and Corvallis.

Juglans spp.	# available	# clonal	# seedling
ailantifolia	20	0	20
australis	0	2	5
californica	16	0	16
cathayensis	0	1	1
*cinerea**	19	19	0
hindsii	18	2	16
hopeiensis	1	1	0
hybrid	8	7	1
major	18	0	18
mandshurica	8	1	7
microcarpa	7	1	6
mollis	1	0	1
neotropica	0	0	4
nigra	10	9	1
olanchana	2	0	2
regia	203	71	132
sigillata	0	0	3
sinensis	0	0	1
sp.	0	0	4
Total	274	138	214

Pterocarya spp.	# available	# clonal	# seedling
fraxinifolia	6	0	6
hupehensis	1	0	1
stenoptera	6	0	6
Total	13	0	13

* Juglans cinerea is held at NCGR-Corvallis.

In addition, 19 clonal *Juglans cinerea* (butternut) accessions are held at the NCGR facility in Corvallis, OR Any sub-tropical species are maintained as small, container-grown plants at Davis.

Collections of *Juglans* spp. maintained elsewhere in the US include a University of Missouri collection of 57 *J. nigra* cultivars selected for nut production that 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. There is also a collection of *Juglans* species at Southern Illinois University Carbondale.

The Davis NCGR walnut collection has been topped and hedged periodically with a commercial tree hedger to maintain vigorous scionwood. The operation is intended to regulate tree height, improve pest management and light distribution within and between trees.

There are trees in the collection at Davis that are severely stressed and threatened by a variety of problems including crown gall disease (*Agrobacterium tumefaciens*) and thousand cankers disease (Geosmithia spp.). *Juglans californica* trees in the A and B blocks have failed to thrive in the collection for many years, and over time, many have died, often from thousand cankers disease. Interestingly, most J. californica trees in the C block, and those that were repropagated and are in the S block, are growing well. Repropagation has been hampered by lack of available land upon which to relocate the National *Juglans* collection.

B. Exploration and acquisition

Seventeen exploration missions have been completed since 1983 (Table 2). These trips have resulted in acquisition of valuable wild *Juglans* species that have filled some of the critical genetic gaps in the NCGR collection. The newest material from the Republic of Georgia is grown in the nursery S (seedling) block at Wolfskill. Due to lack of land to accommodate a larger collection, no new collection trips are planned. This leaves important gaps in wild *Juglans* genetics in the collection.

Table 2. *Juglans* collection explorations.

1983 - Westwood, Japan, *J. ailantifolia*
1984 - Parfitt, New Mexico, Arizona, *J. major, J. microcarpa*
1987 - Parfitt, Mexico, *J. olanchana J. mollis J. pyriformis*
1989 - Dixon, Ecuador *J. neotropica*
1988 - Thompson, Pakistan, *J. regia*
1990 - McGranahan, Leslie, Barnett, China *J. re*gia
1990 - Millikan, USA, *J. cinerea*
1990 - Thompson, Sperling, Ramming, Uzbekistan, USSR, *J. regia*
1994 - McGranahan, Leslie, Kyrgyzstan, *J. regia*
1995 - McGranahan, Leslie, China *J. regia*
1999 - McGranahan, Argentina, *J. regia J. australis*

2000 - Simon, Potter, Ukraine, *J. regia*
2006 - Postman, Stover, Republic of Georgia, *J. regia*
2010 - Aradhya, Republic of Georgia, *J. regia, J. cathayensis*
2010 - Grauke, Texas, *J. microcarpa*
2011 - Preece, Postman, Albania, *J. regia*
2014 - Aradhya, Republic of Georgia, *J. regia*

*Located at Corvallis NCGR

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

Year	Total No.	No. Bearing Trees Evaluated	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. 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 (Fjellstrom et al, 1994; Fjellstrom 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.

Currently, the NCGR collaborates on walnut molecular research with Agriculture and Agri-Food Canada (You et al., 2012) on SNP discovery, with the UC Davis Plant Sciences Department (Wu et al., 2012; Chakraborty et al., 2015; Luo et al., 2015; Martinez-Garcia et al., 2016), on genome sequencing, transcriptome analysis, and physical mapping, and with the Genetics and Crop Pathology Unit, USDA-ARS, UC Davis on walnut rootstock breeding and susceptibility to pathogens (Dvorak et al., 2007; Kluepfel et al., 2016; Westphal et al., 2017).

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 is led by Dr. Pat Brown, Department of Plant Sciences, UC Davis, in collaboration with 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, resistance to blackline disease (CLRV) and walnut blight. 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, led by Eric Germain, from 1977 to 1995 emphasized late leafing, blight resistance, and lateral fruitfulness, using California cultivars as a source of lateral bearing, and released several cultivars. From 1996 to 2007, E. Germain and F. Delort continued the program using a large and diverse collection of material from the Mediterranean region, Iran, Japan, and Central Asia to avoid inbreeding issues. This work was discontinued in 2007 but recently INRA and CTIFL have cooperatively initiated a new breeding and genetics research program under Fabrice L'Heureux. Program goals include characterization of diverse material collected by Eric Germain, development of genomic information and tools for marker assisted selection, and release of cultivars with resistance to major walnut pathogens.

c. China: Ministry of Forestry

Walnut improvement in China emphasizes development of varieties with adaptation to China's growing conditions. They are also focusing on high yields per tree and a tree with short lateral branches for denser plantings and greater yields per hectare. Nearly all provinces that grow walnuts have selection programs; actual breeding is occurring at Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences.

d. Other Breeding programs

Many other countries have activities related to enhancement. Most notable are Spain, Iran, Turkey, India, 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 159, 284, 311, 442, 544, 705, 861, and 1050.

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. Another approach is to increase the number of fruit per cluster.

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.

v. Insect pests

The major insect pests affecting the fruit include husk fly, codling moth, and navel orange worm. The walnut twig beetle vectors the fungus responsible for thousand cankers disease. Resistant germplasm has not been identified, however factors involved in husk fly attraction and/or establishment in the hull and factors influencing twig beetle infestation are being investigated.

vi. Soil-borne pests in rootstocks

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, and *J. microcarpa* has been a promising source. Current genetic engineering approaches to the problem have resulted in complete resistance; however, they have not yet been released to the industry.

An important soil-borne pathogen 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. Again, *J. microcarpa* is a promising source of resistance.

One of the most serious soil borne problems is nematodes, particularly *Pratylenchus vulnus*. Once walnut orchards become infested with *P. vulnus* there is no known practical remedy. A fallow period of 10 years might be adequate. Potentially useful resistance to *P. vulnus* has been observed in *J. cathayensis* and *J. microcarpa*. 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. nig*ra 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 focused on selection for nut production and the use of black walnut in agroforestry plantings. The program at the HTIRC is focused on stem 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 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 57% 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 an insect-vectored fungus (Geosmithia spp.), has been identified in California orchards during the past decade. This disease represents a threat to not only *J. reg*ia scion cultivars, but also to *J. hindsii* and Paradox hybrids used as rootstock.

Other possible threats include butternut canker and witch's broom, which have not been found in California, and lack of adequate winter chilling unit accumulation associated with a warming climate.

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 *J. 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 and erratic bearing. Black walnut kernels have dark color and shrivel, which are the most limiting factors for marketable yield (Warmund, 2009).

Eastern black walnut is 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. Due to its high commercial value and the long time required to produce saw-timber grade trees, 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. TCD has been responsible for the decline and death of many *J. nigra* trees in urban and landscape settings throughout the western United States and also is present in several eastern states. The continued movement of the fungus east into additional areas of native *J. nigra* stands via wood transit or insect dispersal is a vulnerability of this species.

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 has since been widely planted as an orchard rootstock and street tree and is

common in riparian areas. Few, if any, original stands remain, but there is good genetic diversity within the current *J. hindsii* wild populations. 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 prized as sources of hybrid 'Paradox' seed used commercially as a rootstock. Additionally, *J. hindsii* is used directly as a seedling rootstock for commercial *J. regia* orchards. Thousand Cankers Disease is widespread in native *J. hindsii* stands and plantings.

Juglans californica is a shrubby tree native to the coastal ranges of 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. major and *J. microcarpa*, both native to the southwestern US and northern Mexico, are also harvested for timber, but without 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 have been of interest for rootstock breeding and development in California, with the better disease resistance coming from *J. microcarpa*. 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.

4. 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.

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 if 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 species. 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 and J. australis, which is used in Argentina as a rootstock, may have value as a source of Phytophthora resistance. Many of these species are endangered 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 mostly unknown. Most are not represented in the NCGR collection and they should be given high priority for exploration activities although most countries where these are native now severely restrict or prohibit germplasm exchange

2. Central Asia

Important sources of *J. regia* germplasm are being lost due to extensive logging and deforestation in Kyrgyzstan and other Central Asian areas of origin. 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, other root diseases, and TCD.

Many *Juglans* trees in this collection are in poor and declining condition, perhaps initiated by a variety of causes, but also show symptoms of TCD, which has likely exacerbated decline. Many are also impacted by crown gall disease.

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 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* clonal material difficult. Seeds can capture wild genetics and can come into California.

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. If vegetative propagules cannot be successfully cryopreserved, then attention should focus on cryopreservation of inbred seeds of wild walnuts and pollen.

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. jamaicens*is, and *J. pyriformis*), should be undertaken as a priority when and if there is access. 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 is difficult. *J. australis*, native to Argentina, is thought threatened by hybridization with introduced species. Most of these species are tropical or semi-tropical, so some of them, including *J. olanchana, J. pyriformis,* and *J. jamaicensis*, will probably need to be maintained in a greenhouse or a location that is not susceptible to freezes. *J. hirsuta* and *J. mollis* can be maintained at the more temperate Davis NCGR location.

2. *Juglans cinerea*

The Juglans CGC has given high priority to collection of germplasm of butternut (*J. cinerea*) in North America due to the immediate threat to many populations of this species posed by the butternut canker disease, 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, *J. 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 locations

Large-scale commercial logging of native walnut forests has occurred in Kyrgyzstan and other central Asian locations. These areas are important sources of genetic diversity for *J. regia*. There is concern regarding continued 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, particularly the more recent introductions.. There is a need for continued 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 and resistance to scion pests diseases, including husk fly, bacterial blight, and Botryosphaeria should be evaluated.

Genomes of major species have been sequenced, and a physical map and reference genome for Chandler *J. regia* have been published. Additional work to characterize the genetic diversity of accessions of several species in the collection has been initiated, and should be continued as a priority.

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. There has been a concerted effort to study the effects of *Juglans* wild relatives to produce disease resistant rootstock. Of the wild species tested, *J. microcarpa* has proven to be the best source of disease resistance for crown gall, Phytophthora, and nematodes. One commercially available rootstock, 'RX1' is a *J. microcarpa x J. regia* hybrid with the best resistance available for Phytophthora.

3. Microsatellite markers

Microsatellite libraries enriched for CA and GA dinucleotide repeats have been screened using a diversity panel of *J. nigra* from 12 provenances and from 3 *J. 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 are now providing an important complement to the phenotypic data already available, 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.

Additionally, SNPs have been discovered for walnut species as a part of the breeding program to develop disease resistant rootstock. A detailed genetic map of *J. regia* has also been developed at the University of California.

D. Enhancement

1. *Juglans regia*

a. Improved Persian walnut cultivars

To develop Persian (English) 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 underway 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. 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 interspecific 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 in the A and B blocks for many years and over time many have died. Losses are now extending to other species as well. Land is a limiting factor and there is not space to repropagate accessions threatened with loss. TCD has likely exacerbated decline. 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.

Careful consideration should be given to continue *ex situ* maintenance of *J. nigra* collections in the eastern United States. There are current plant quarantine obstacles and legitimate disease concerns regarding introductions of this species into California. The University of Missouri 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. The long-term maintenance of this collection and the *J. nigra* collection at the Hardwood Tree Improvement Center at Purdue, which is oriented towards timber, should be carefully monitored and ensured.

A method also needs 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. Movement, export, and quarantine protocols

The current guidelines for movement of *Juglans* germplasm and walnut wood within the United States include many restrictions. For example and special permit is required for the NCGR to send *Juglans* material to Indiana. Germplasm curated at either Davis, CA or Corvallis, OR currently cannot be shipped east into the native range of *J. nigra* without a special permit and vegetative germplasm from the eastern US should likely not be sent to the current repositories.

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. Pathogens of note that should be restricted from California orchards include bunch disease, butternut canker, and any new strains of cherry leafroll virus or *Geosmithia*.

Current US import permit rules have made importation of *Juglans* scionwood prohibitively difficult. Diameter restrictions on imported wood make grafting of walnuts nearly impossible and imported material, if grafted, must be held long-term in a screenhouse, a condition that makes evaluation of any imported walnut scionwood nearly impossible. In addition, APHIS import permits, which formerly were not geographically restricted, must now be applied for on an individual country basis. These restrictions, on top of the restrictions placed on exports by

other countries, have made importation of characterized walnut clonal germplasm (scionwood) very difficult to impossible. This is a serious impediment to US walnut breeding.

C. 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 accelerating commercialization of methods for producing clonal rootstocks all increase the need to evaluate a wider set of *Juglans* germplasm for rootstock-related traits, including 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. Further evaluation for traits related to scion breeding, including yield, harvest date, nut quality, oil composition, blight resistance, husk fly resistance, Botryosphaeria, and pistillate flower abscission, are needed t.

D. 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 poorly understood. Germplasm collection and evaluation is critical to preservation. Efforts to collect *J. cinerea* germplasm with resistance or tolerance to butternut canker should be in conjunction with a program to perform disease resistance screens on candidate genotypes. Genetic and phenotypic characterization of the germplasm can then be used in breeding and as a means to understand patterns of diversity within the species. This would require identification of a location 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 or tolerant to butternut canker, into state and private nurseries and seed orchards.

E. 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.

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 whenever possible. 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 are likely to have potential use in both scion and rootstock development.

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

The genetic diversity of the *Juglans* species native to North America is still insufficiently understood. We recommend further genetic and phenotypic evaluation of all the native *Juglans* with the goals of understanding the relationship between genetic and geographic distance, evaluating the relative importance of various threats, including global climate change, to the *in situ* germplasm, identifying threatened or critical populations, and contributing to crop enhancement by current breeding efforts. The value of additional in situ conservation efforts should be determined by characterizing diversity for the species in National Parks, wilderness areas and on other public and private lands.

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